Professor Leonard Fabiano
Mr. Stephen M. Tieri
Professor Daniel A. Hammer
Department of Chemical and Biomolecular Engineering
Room 311A Towne Building
220 South 33rd Street
University of Pennsylvania
Philadelphia, PA 19104

April 12, 2011

Dear Professor Fabiano, Mr. Tieri, and Professor Hammer,

The enclosed report contains our renewable process to generate 1,4-butanediol (BDO) from a renewable sugarcane feedstock via anaerobic fermentation of a genetically engineered strain of *Escherichia coli* (*E. coli*). We designed our plant to produce approximately 50 million pounds per year of 99% pure BDO using a combination of continuous and batch processing.

Our plant will be built adjacent to a sugar and ethanol sister facility in Brazil, which will provide us with our renewable molasses feedstock. This feedstock will be fed into a continuous fermenter along with corn steep liquor media, water, and *E. coli* cells. The BDO excreted by the *E. coli*, along with remnants of the feed, will subsequently be sent to a continuous centrifuge. The impurities will then be removed through distillation, carried out in the bottoms using oleic acid, and recovered with a decanter. The resulting mineral-water mixture, called vinasse, will be sold back to our sister plant as fertilizer. The BDO product from the top of the distillation tower will be sold for use as a solvent and in the manufacturing of plastics, elastic fibers, and polyurethanes.

The plant design has a positive economic forecast, assuming no significant shocks in the demand for BDO. Moreover, since our process is based on sugar cane, we would not expect our costs to co-vary with the rising price of oil as much as that of our competitors, who use petroleum-based feeds. The net present value (NPV) of the project 15 years after construction is $283 million. The total permanent investment required is about $13.5 million and the internal rate of return (IRR) is 157%. Due to the high profit margins attainable by this process, licensing our technology will capture additional revenues. We would expect to negotiate licensing fees of about $1700 per ton BDO produced, based on a selling price of $2,420 per ton BDO. Future research may need to be conducted to find out if additional equipment is needed in the actual plant, or if we were too optimistic on our pricing for the raw materials and utilities.

If there are any questions, comments, or concerns regarding this report, please do not hesitate to contact us. Thank you for your time, support, and guidance throughout the duration of the project and for your current consideration.

Sincerely,

Erinn R. Bibolet

Gabriel E. Fernando

Somil M. Shah

Renewable 1,4-Butanediol

CBE Senior Design Project 2011

Erinn R. Bibolet

Gabriel E. Fernando

Somil M. Shah

April 12, 2011

Professor Leonard A. Fabiano

Professor Daniel A. Hammer

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Abstract

The purpose of this project is to design a commercial-scale facility to produce 50 million pounds per year of 1,4-butanediol (BDO) from a renewable feedstock. A genetically engineered strain of *Escherichia coli* developed by Genomatica, Inc. will metabolize a molasses feed, delivered from an adjacent sugar and ethanol facility, into BDO. The BDO product purity and quality must meet or exceed current commercial requirements for polymer-grade material to be acceptable to prospective customers. The innovative technology to produce environmentally-friendly BDO will convert biomass-derived and renewable feedstocks in fewer steps than traditional petrochemical routes, with no toxic byproducts and minimal greenhouse gas emissions.

Our BDO plant will be built in São Paulo, Brazil. This location was chosen due to its proximity to our sister sugar and ethanol facility. Despite the need to stop production for three months in mid-December through mid-March during the rainy season, when our sister plant will cease its molasses production, we determined that the low cost per amount of sugar from the Brazilian molasses will outweigh the ability to run year-round in a corn-based facility in the Midwestern United States. To account for the downtime associated with the rainy season, our facility has included extra molasses storage capacity to extend production for an additional month after our sister facility shuts down. We also anticipate 10 days of downtime due to maintenance and cleaning, which will result in about 290 days of full-scale facility operation.

An economic analysis of our design demonstrated profitability after the first year of operation. Our feed materials, corn steep liquor, oleic acid, process water, and molasses, will cost us a total of about $200 per ton of BDO produced. But our vinasse co-product, which will be sold back to our sister sugar and ethanol plant for fertilizer, will result in additional revenues of $190 per ton of BDO produced. The selling price of the vinasse is discounted by 70% of the current fertilizer market price

since we are selling it back to our sister facility, in return for discounted molasses and electricity. The direct permanent investment of the plant will be about $10.5 million and startup costs will be about $1.5 million, which results in a total permanent investment of $13.5 million. The net present value (NPV) of our facility with 15 years of production is $283 million and the internal rate of return (IRR) is 157%. We intend to sell our 99% pure BDO at $2,420 per ton produced, which will result in revenues of $72.5 million per year based on our commercial-scale production of 8,600 pounds of BDO per hour. Due to the profitability of our design, we will be able to sell our BDO at the low end of the U.S. market price range of $2,420 – $2,840 per ton, as was reported in the third quarter of 2010. Future research may need to be conducted to find out if additional equipment is needed in the actual plant, or if we were too optimistic on our pricing for the raw materials and utilities.

Introduction

1,4-Butanediol (BDO) is an organic compound commonly used as a solvent in industrial cleaners and glue removers, like THF. It is also used in the manufacturing of engineering plastics (e.g. PBT), fibers (e.g. Spandex), and polyurethanes (e.g. car bumpers) (Kuwana, 2005). Current industrial BDO manufacturers, such as BASF, DuPont, Linde, and LyondellBasell, use a variety of non-renewable, petrochemical-based processes to produce BDO. The most common is the acetylene-based Reppe process, named after German chemist Walter Reppe and developed in the 1930s, in which one mole acetylene reacts with two moles of formaldehyde to produce 1,4-butynediol. The 1,4-butynediol is then hydrogenated to yield BDO (LookChem). In 1990, LyondellBasell first commercialized a proprietary, multi-step method to produce 1,4-butanediol without the use of acetylene (LyondellBassell). The process begins with propylene oxide and converts it to allyl alcohol. Hydroformylation then converts the allyl alcohol to 4-hydroxybutyraldehyde, which is hydrogenated to form BDO (ACS). Other routes have been derived with BDO being produced from compounds such as maleic anhydride, butadiene, allyl acetate, and succinic acid, but many of these involve toxic compounds, expensive catalysts, and non-environmentally friendly byproducts.

The historical dependence of BDO manufacturing on petroleum-based feeds has fostered the development of renewable BDO production processes due to the dwindling availability of fossil fuels. One such method, developed by Genomatica, a San Diego-based company, utilizes genetically engineered *E. coli* cells to metabolize a sugar feed into BDO. The specific strain of *E. coli* has been modified to be able to survive in high concentrations of BDO and to secrete solely BDO in order to survive, as it is the only available metabolic pathway. This method not only reduces the dependence on non-renewable feeds, but is also much less energy intensive than the comparable petroleum-based processes (Wilson, 2008).

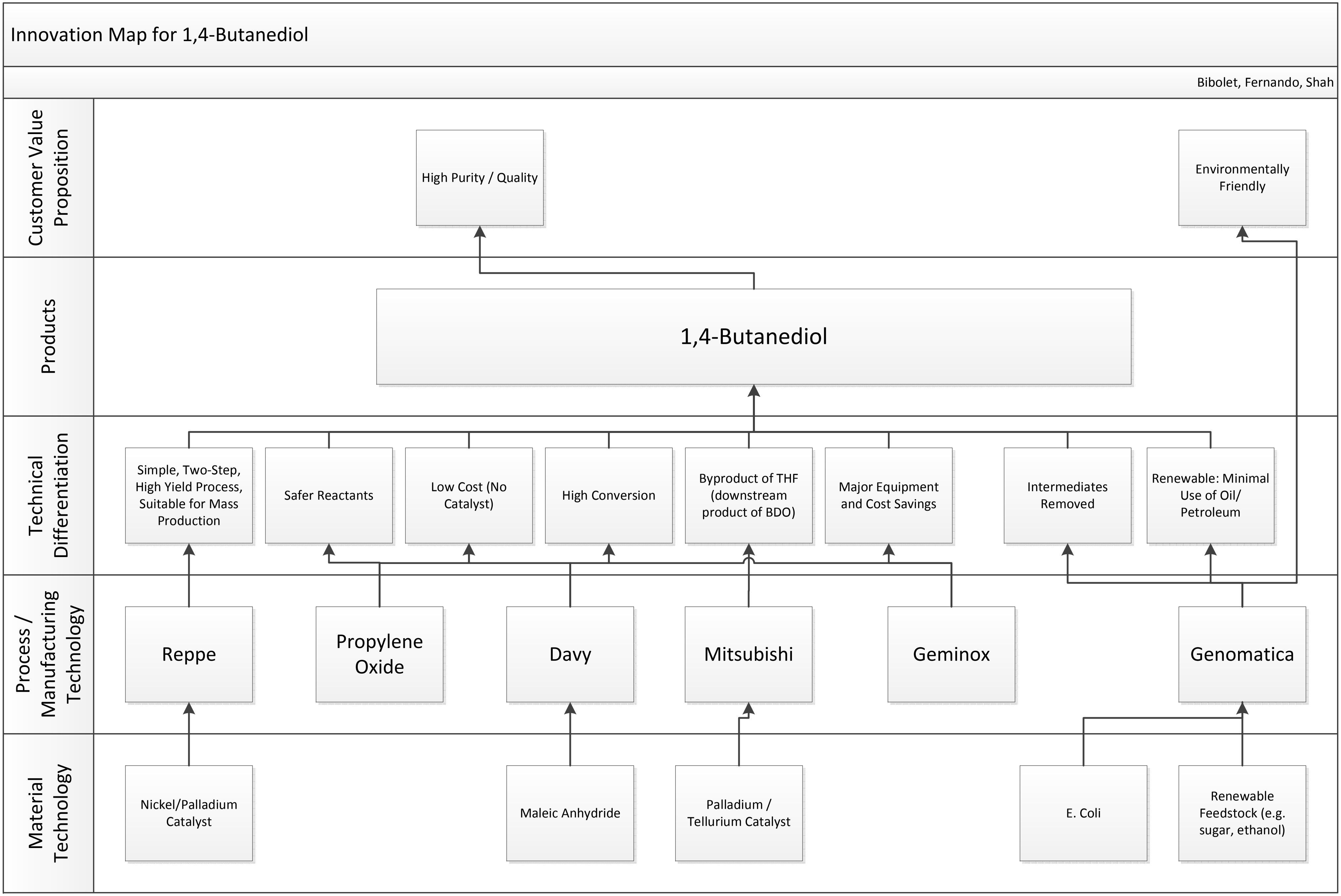
Our plant will produce 50 million pounds per year of BDO from a renewable molasses feedstock. We will operate 290 days a year and will be co-located in São Paulo, Brazil with a sugar and ethanol production facility. We will be continuously running two fermenters with the Genomatica *E. coli* cells and will separate the solids from the outlet stream using a continuous reciprocating pusher centrifuge. The majority of the solids, which are primarily *E. coli* cells and ash, will be recycled in order to maintain a constant concentration of cells and nutrients in the fermenter. The pH of the fermenter will also be continuously adjusted by used of a concentrated HCl stream to ensure the fermenter remains below a pH of 6. At this acidity, replication of the *E. coli* is severely inhibited, so cell buildup in the fermenter will not be an issue. The batch of *E. coli* cells will also have to be replaced after one month with a fresh supply from a seed fermenter, but continuous monitoring of the cell and BDO concentration in the large-scale fermenters will also take place to ensure the cells are robust and generating sufficient BDO yields.

We will separate the BDO product from the centrifuge liquid stream with a distillation tower and a high boiling point oleic acid stream. The mineral impurities from the feed will be removed through the oleic acid in the bottoms stream, recovered with a decanter, and sold back to our sister sugar and ethanol facility as a mineral-water fertilizer stream called vinasse. The BDO will be separated from water in the top of the distillation column through a partial condenser. The vapor stream will be primarily water, whereas the liquid reflux will be 99% BDO. We will also have two day tanks after the distillation tower to ensure proper BDO yield and purity.

Project Charter

Project Name	Renewable 1,4-Butanediol
Project Champions	Stephen M. Tieri, DuPont
Project Leader	Erinn Bibolet, Gabe Fernando, Somil Shah
Specific Goals	To design a commercial facility to competitively produce 50MM lb/yr of 1,4-Butanediol using bio-mass derived and renewable feedstocks.
Project Scope	**In-Scope:** ● Safety ● Byproducts ● Feed-Stock Choice (and Location) ● Equipment and Process Design ○ Meet BDO Process Requirements ● Licensing (valuation) ● Economic Feasibility ● Consumers (delivery method, consider already existing facility) ● Marketing Strategy ● Production of Microorganisms **Out of Scope:** ● Protection and security of facility ● Production process of the actual feedstock ● Initial Pilot Testing ● R&D ● Design of Microorganisms
Deliverables	1. Plant Location Analysis 2. Competitive Analysis (incl. environmental factors) 3. Flow Diagrams 4. Market Forecast 5. Economic Analysis 6. Product Life-Cycle Assessment 7. Toxicity/Safety Data 8. Reaction Kinetics/Thermophysical Property Data 9. Marketing Analysis 10. Licensing Analysis
Time Line	Process Design by February 22 Equipment Design by March 22 Financial Analysis by March 29 Written Report by April 5 Presentation by April 21 Poster by April 29

Figure 1: Project Charter



Innovation Map for 1,4-Butanediol

Bibolet, Fernando, Shah

Concept Stage

Market and Competitive Analysis

Market Analysis of 1,4-Butanediol

Current Market Pricing: The majority of BDO is manufactured from petroleum-based products, such as formaldehyde and propylene oxide. As a result, the price of BDO is closely tied with petroleum products. In 2004 to 2005, as crude oil prices increased 33% (Capital Professional Services, LLC, 2011), BDO prices increased 50%. In 2010 Q3, BDO market prices ranged from $2,420 per ton to $2,840 per ton. The American market commands a slight premium over the European and Asian markets. (ICIS, 2010)

The world consumption of BDO for 2009 by region is depicted below. The biggest consumers are Western Europe, China, the United States, Other Asia (primarily Taiwan), and Japan (Davis, Kälin, & Kumamoto, 2010).

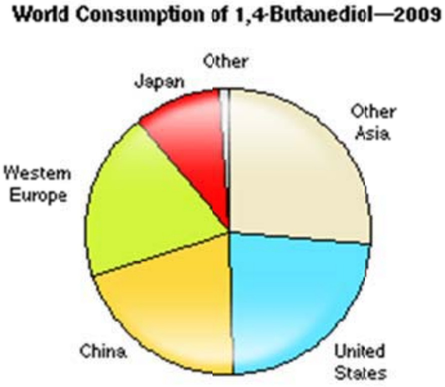


Figure 3: World Consumption of BDO in 2009

Market Growth: BDO use in the US increased 3% in 2004 to 2009. From 2009 to 2012, US BDO demand is expected to grow 2% per year, increasing from 392,000 tonnes in 2008 to 424,000 tonnes in 2012. The American market currently has no plans to expand BDO production. As a result, the forecast expects that US imports of BDO will increase during this period. The European market expects to see 4% growth per year (ICIS, 2009). Asian markets expect to see growth of 7-9% per year. Moreover, analysts estimate China's BDO market to grow 10% per year (ICIS, 2011).

China's BDO market has also become self-sufficient, and as a result, it is expected that they will become a significant exporter to European and US markets.

Other Uses of BDO: BDO has also been used as a recreational drug (Satta, Dimitrijevic, & Manev, 2003). Therefore, in the future, BDO may become a controlled substance, and further regulation in this industry may occur.

Competitive Analysis of 1,4-Butanediol Industry

Customers*:* The customers of this market are primarily other downstream chemical companies making other intermediate products. There are several uses for BDO, and therefore, multiple customers downstream, making the power of customers significant, but not too constraining.

Suppliers*:* Most BDO processes use a petrochemical based route of production. The most common processes are the Reppe, Propylene Oxide, Davy, Mitsubishi, and Geminox processes. These traditional methods use formaldehyde, propylene oxide, and n-butane as their feedstock. These feedstocks are all petroleum-based with few alternative methods, and, therefore, the feedstock manufacturers have significant power. However, the new bio-based methods for BDO production will mitigate this problem, giving the industry more options for the production of BDO.

New Entrants*:* As with any commoditized chemical, there is not much room for specialization, and therefore economies of scale are required to gain advantages. In order to achieve economies of scale, significant initial capital investment is required. Therefore, the industry is dominated by a few large companies. Most processes used to make BDO are also patented. As a result, potential market entrants must either develop a new process, or must license the technology from an incumbent or outside source.

Substitutes*:* BDO is a specialized chemical intermediate, so while future innovations of downstream processes may avoid using BDO, most current processes and infrastructure are designed for use with BDO. Moreover, since BDO is not significantly toxic, there is not much of a reason for processes to try to move away from BDO.

Internal Competition*:* Average plant sizes range from 55,000 – 75,000 tonnes/yr (ICIS, 2009). The major players in this industry are BASF, Dairen Chemical, LyondellBasell, and ISP. In 2010, these four companies had 58% of the market share (Davis, Kälin, & Kumamoto, 2010).

Customer Requirements

Downstream customers of 1,4-butanediol primarily desire a low cost and high purity product to feed into their processes. Customers of our specific process desire an environmentally-friendly way to produce BDO, which means not using traditional petroleum-based routes and minimizing energy. Moreover, since the process is not petroleum-based, it will provide a lower-cost product whose cost co-varies less with oil prices.

Feedstock and Location Choice

Our plant can be co-located with one of two sister facilities, one in the Midwestern United States, and one in Brazil. The two locations, apart from having different cultural and business environments, provide two different feedstocks for our process.

Option 1: United States

The plant in the Midwest United States operates an ethanol dry mill using a corn feedstock. Corn is readily available all year, allowing our plant to operate year-round using sugar from the corn as our feed. The dry milling process produces energy and steam which our plant can purchase at a discounted rate, rather than purchasing energy from outside sources. Corn only contains four to six percent sugar, and costs $230 per ton. In order to produce 50 million pounds of BDO per year, the amount of corn needed to produce the BDO was the colossal amount of 91.3 tons per hour, or $21,000 per hour. Furthermore, the sugar obtained from the sister plant would still contain plant material and would require intensive filtering and solids treatment before entering the fermenter. This mash could then be dehydrated and sold as supplemental feedstock to farms, but the dehydration would incur further costs.

In addition, the corn dry milling process produces obstacles that may affect our plant in the long term. First, the community has started to perceive the conversion of corn to ethanol as energy inefficient (Alfano, 2005). As a result, ethanol plants may be shut down. Since these plants provide the primary feed to this process, as well as some utilities, our plant would also be shut down. Another obstacle is the recent increase in the cost of corn due to its dual nature as a food source. Further increases in feed costs would cause this method to become economically unviable. Moreover, in the U.S., there have been some protests against using a food source as an energy source (Sauser, 2007). These factors affecting the feed have led us to move away from locating our plant in the United States.

Option 2: Brazil

The other potential plant location is Brazil, where sugar and ethanol facilities use sugarcane as their feed. The plants' byproducts are raw sugar and blackstrap molasses containing 54% sugar. The blackstrap molasses is the most cost-effective feed to our process, at $70 per ton, compared to $628 per ton of raw sugar. The sister plant, similar to the corn mill, will produce energy and steam that our plant can purchase at a discounted rate, instead of purchasing electricity from the grid and producing steam continuously at our plant.. Though molasses contains some solid material and minerals, the material and minerals can be fed into the fermenter directly and later removed and sold as fertilizer to the sister facility at minimal cost.

The Brazil plant also presents obstacles to the viability of our plant. Molasses is very viscous, which may cause the pipes, pumps, and valves to clog up if not maintained properly. Also, microorganisms require minerals and amino acids, which are found in corn steep liquor, a product obtained from wet corn mills, which are uncommon in Brazil. The corn steep liquor would need to be transported for a long distance, which increases costs. The largest obstacle, however, is the rainy season in Brazil that lasts for three months and prevents the sister plant from operating. For those months, our plant would either need to stop production, or operate using external utilities.

Decision

We chose to locate the plant in Brazil, utilizing blackstrap molasses due to the cost of feed versus the percentage of sugar found in the feed. Despite the location obstacles unique to Brazil, the feedstock in Brazil is more appealing. Not only is blackstrap molasses cheaper than corn, but it also contains a higher percentage of sugar, leading to a cheaper feedstock overall.

Preliminary Process Synthesis

Design History and Logic

Our initial attempts included a process that required a liquid-liquid extractor followed by a distillation tower, a distillation tower preceded by a flash drum, two distillation towers, a process that required one distillation tower followed by a flash drum, and another process that required two flash drums. These processes all tried to solve the same problem using different methods: separating the BDO from the water and the rest of the other impurities. The final optimized solution requires one distillation tower with a partial condenser along with another flash vessel.

Liquid-Liquid Extraction

The original patent noted a generic separation process to be followed after the fermentation procedure, simply stating that standard liquid-liquid extraction methods with toluene could be used. This method worked well in bench-scale tests (Burgard, Van Dien, Burk, & Niu). However, after scaling up, it yielded inefficient separation. Due to BDO's high miscibility with water, the solvent would extract some of the BDO, but also a high portion of the water and minerals. Since toluene was not yielding promising results in simulations, we analyzed other solvents, including hexane, hexene, methyl ethyl ketone, and benzene, to determine the feasibility of a liquid-liquid extraction. With a large amount of solvent, the best extraction was found with methyl ethyl ketone, yielding a 50% recovery of BDO, with impurities. However, regardless of the BDO purity, a 50% recovery is extremely low, and results in tremendous waste, especially for a commercial-scale production plant. We decided to deviate from the recommendations of the patent and try to find other efficient methods to obtain a better yield and purity of BDO.

Distillation Preceded by a Flash Drum

The main failure of the liquid-liquid extraction technique was the miscibility of water and BDO. We decided to try flashing off the majority of water from the overflow of the centrifuge. Since their boiling points differ by around 200°F, this would provide an easy method to separate out the BDO. However, the purity acquired from this single separation was not high enough. Therefore, we fed it to a distillation column to further separate the water from BDO. In later runs, we optimized this into a single distillation column.

This process, however, did not take into account all the minerals present in the system. We found that after water was vaporized using either a flash or distillation column, dissolved impurities would not boil with the water, and other undissolved impurities would also remain, leaving the bottoms BDO product with significant impurities. This effectively resulted in the same problem as before, except this time, without water. This is also a very expensive and energy inefficient process to evaporate a large amount of water, especially since the low temperature of the steam could not be used to heat any other streams in our process.

Two Distillation Towers

Since the BDO could not be separated from the minerals by evaporating off the water, we decided to vaporize both the BDO and water, and the leave the minerals behind. In order to achieve this we would need a mineral sink, or a chemical with a higher boiling point than BDO, which would not vaporize and would provide a liquid stream to remove the minerals.

Our initial chemical choice was ethyl vanillin, which has a boiling point of 545°F (ScienceLab), which is higher than the 455°F boiling point of BDO (BASF, 1997). Ethyl vanillin would be added to the liquid product of the centrifuge, and then be sent to a distillation tower. The BDO and water would boil off as the distillate, and the ethyl vanillin would remain as a liquid carrying out the impurities. The water and BDO mixture would then be condensed and sent to a second distillation tower to provide a high purity separation between the BDO and water. A revised version of this plan fed the water and BDO distillate to the second tower directly as a vapor, to save on unneeded cooling expenses. However, vanillin costs $15 per kg when produced petrochemically and $700 per kg when produced synthetically. Compared to the $0.59 per kg cost of BDO, this would be quite an expensive solution, and so we decided to search for another option. Moreover, since we are an environmentally friendly plant, using a petrochemical product would negate our 'green' claim.

Oleic Acid Mineral Extraction

The separating agent we need to carry out the extraction of the impurities from the BDO stream must have a higher boiling point than BDO (455°F), as well as provide an easy way to extract the mineral impurities from the agent. We will put the minerals back into water via liquid-liquid separation, in order to sell the impurities to our sister sugar and ethanol facility as vinasse and recycle the separating agent. Oleic acid provides the ideal compound as it is a non-polar, hydrophobic, high boiling point, environmentally friendly chemical that will efficiently transfer the mineral impurities back into the water phase when sent through a single decanter unit. It is a monounsaturated omega-9 fatty acid with a composition of primarily olive oil. This leads to its insolubility in water and high boiling point of 547°F (ScienceLab).

One Vacuum Distillation with Side Stream Draw

One possible idea to isolate BDO was to have a liquid side stream draw-off on the second tray from the top. The distillate stream would be a water vapor that would then be sent to a total condenser, whose liquid would partially be sent back as a reflux. The main problem with this set up was the total condenser at the top of the distillation tower. The carbon dioxide that was dissolved in the water vaporizes. In order to condense it, the condenser would need to run at around -77°F. This was determined to be unfeasible, so we instead tried a partial condenser with the side stream draw-off. In both cases, the side stream draw-off has a 7.0% loss of BDO (which ends up in the distillate). This is a significant loss of revenue, and in the long run, will not justify the amount saved to purchase a flash vessel and condenser. Moreover, the use of a side stream will result in a significant amount of BDO in the distillate stream. After partially condensing the distillate, the water, containing BDO, will be used to extract the minerals from the oleic acid and mix with the solid centrifuge waste, and will then be placed into the environment as vinasse. However, this stream is 14% BDO, and would, therefore, still require an additional separation process, before the water can be released to the environment.

Pre-Distillation Feed Heater

In order to decrease the heating costs associated with the primary distillation tower, we considered pre-heating our feed using the oleic acid from the reboiler as the hot stream. The oleic acid leaves the reboiler at the bottom of the tower at 439°F. This is significantly higher than the distillation feed stream temperature of 88°F. A heat exchanger was placed immediately before the tower to transfer heat across the two streams, resulting in a transfer of about 277,000 BTU per hour and an increase of 32°F in the distillation feed stream. The addition of the distillation feed heat exchanger saves us $14,000 per year.

Centrifuge

In order to separate the solids coming out of the continuous fermenters from the liquid product, we had multiple options. Initially, we tried to implement a screen inside the fermenters to separate the cells from the liquid. The idea was that the cells would be held above the bottom of the tank and the liquid would flow through the screen and exit the reactor from the bottom. The difficulty with this is that the screen would get clogged very easily and would have to be cleaned and replaced frequently. In addition, if the screen is clogged, the liquid may not flow through the cells to deliver the necessary media. To counteract the clogging, the speed of the fermenter agitator could be increased, but if the revolutions per minute were set to too high, the shear stress on the cells may cause widespread cell rupturing.

Determining Water Content: Concentration vs. Viscosity

In order to calculate the amount of water needed in the reactor, we initially decided to pump enough water to reduce the viscosity of the feed blackstrap molasses to the same viscosity of the sugar-water mixture in the bench-scale lab testing as in the patent. We used the Refutas Equation (King, 2011) to calculate the sufficient amount of water:

$$\mathrm{VBN} = 14.534 \times \ln\left[\ln(v + 0.8)\right] + 10.975$$

(Equation 1)

where *VBN* is the Viscosity Blending Number of each component of the mixture and *v* is the kinematic viscosity in centistokes. It is important to note that the kinematic viscosity of each component of the blend be obtained at the same temperature.

$$\mathrm{VBN}_{\mathrm{Blend}} = [x_A \times \mathrm{VBN}_A] + [x_B \times \mathrm{VBN}_B] + \cdots + [x_N \times \mathrm{VBN}_N]$$

(Equation 2)

where x_x is the mass fraction of each component of the blend

$$v = \exp\left(\exp\left(\frac{\mathrm{VBN}_{\mathrm{Blend}} - 10.975}{14.534}\right)\right) - 0.8,$$

(Equation 3)

The calculated amount of water is about 1.03 grams of water per gram of molasses fed, assuming pure glucose was added in the bench-scale test and molasses is 54% sugar. This results in a blended mixture viscosity of 8.2 cP, which is about eight times more viscous than water.

After discussion with our industry consultants, we decided to compare the equated viscosity water amount with the amount of water needed to equate the concentration of the molasses sugar to the concentration of the glucose in the bench-scale test. The amount of water needed to equate the concentrations was 0.40 grams of water per gram of molasses fed. This is 62% less than the amount needed to equate the viscosities. The blended viscosity of the resultant mixture was the same as that in the equated viscosity calculation. Therefore, we decided to use the equated concentration calculation, which results in less water required to feed into our process.

Assembly of Database

Chemical Kinetics

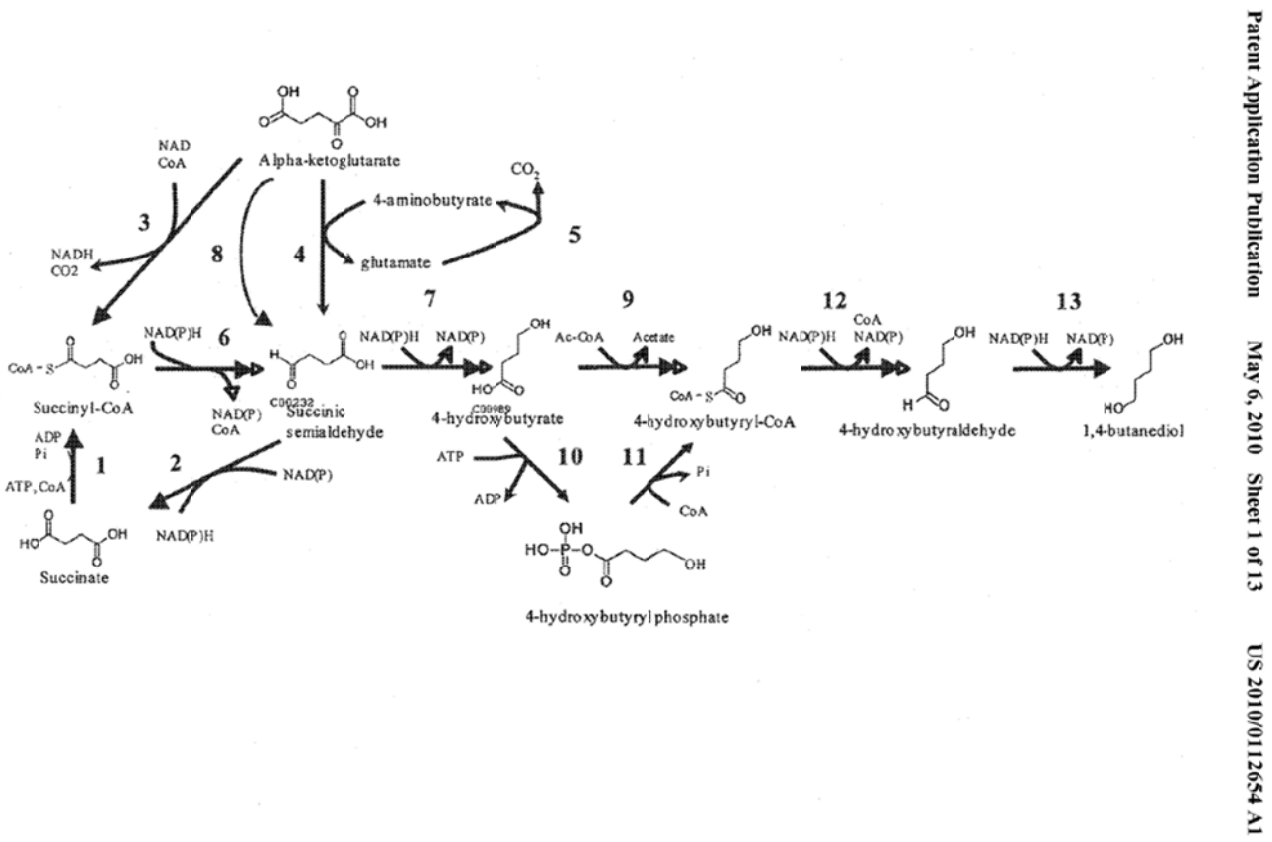


Figure 4: Metabolic Pathway for the Production of BDO

Toxicity

1,4-Butanediol

BDO is a nonvolatile liquid that may cause respiratory tract irritation if inhaled or ingested at high concentrations. It may also have a narcotic effect with symptoms similar to drunkenness. This is due to the enzymes present in the human body converting the 1,4-BDO to Gamma-Hydroxybutyric acid (GHB). Excessive ingestion or prolonged exposure may lead to a dizziness, headache, drowsiness, nausea, confusion, damage to the nervous system and kidneys, or death. Women who are pregnant should not have prolonged expose as it may harm or kill the fetus.

Skin contact does not cause any adverse health effects, but eye contact may cause mild irritation. If contact is made with skin or eyes, immediately flush with plenty of water for 15 minutes. BDO is not OSHA regulated, and not carcinogenic. Wear standard skin, eye, and body protective gear when coming in contact with BDO. See MSDS in Appendix D for further information.

Blackstrap Molasses

Blackstrap molasses is the viscous remains of the sugar syrup after the third boiling of the sugar. It contains sugar (54%), water (20%), carbohydrates (4%), acids (5%), nitrogenous compounds (4.5%), ash (12%) and other (5%). Blackstrap molasses is FDA approved and is commonly used for human consumption. See MSDS in Appendix D for further information.

Corn Steep Liquor

Corn steep liquor is an organic by product from the wet corn milling process. It contains ash (17%), crude protein (47%), corn mash, minerals, amino acids, and water. It is a safe substance, commonly used in animal feed that contains no toxins. See MSDS in Appendix D for further information.

Microorganisms

The genetically engineered *E. coli* were obtained from Genomatica, Inc. to metabolize sugar and produce BDO with no secondary metabolites. The cells are not harmful to the environment when released with vinasse to be used as fertilizer. The cells must be kept under sterile conditions in order to prevent contamination in the fermenter.

Oleic Acid

Oleic acid is a low health hazard risk that can cause irritation when in contact with the skin or eyes. At high temperatures, it is a highly corrosive acid. At room temperature it is a stable organic acid that is not flammable in cases of shacks or tremors. However, it may be combustible at temperatures over 685.4°F. See MSDS in Appendix D for further information.

Vinasse

Vinasse is an acidic mixture of ash, minerals, and water that is moderately corrosive. It is a non-toxic, non-flammable substance that requires standard protective gear when handling. See MSDS in Appendix D for further information.

Water

Standard procedures should be used for the handling of water. See MSDS in Appendix D for further information.

Safety

The molasses sterilizer, distillation feed pre-heater, and distillation reboiler will be running at temperatures exceeding 350°F. Operators must use caution and proper protection when handling the equipment.

The pumps, fermenters, centrifuge, compressors, blowers, and water sterilization skid have moving parts. Operators must not insert objects or body parts in to the machinery while running.

Operators must not ingest or mishandle any chemicals or organisms in the process. At all times, operators must follow safety procedures and wear goggles, lab coats, hard hats, etc. in designated areas.

Pricing

Table 1: Primary Material Prices

Principal Material	**US Dollars Per Ton**
BDO	$2420
Blackstrap Molasses	$70
Corn Steep Liquor	$50
Oleic Acid	$1270
Vinasse	$145

Thermophysical and Transport Properties

With the exception of molasses's high viscosity, most materials used in this process do not exhibit any special thermophysical and transport properties. For specific property data, please see Appendix A.

Bench-Scale Laboratory Work

The initial bench-scale testing of Genomatica's genetically engineered *E. coli* was performed in a 10-L bioreactor as a batch and continuous setup. The batch design was initially performed with 5 L of broth containing 25 g K_3PO_4, 12.5 g NH_4Cl, 2.5 g $MgSO_4$, 150 g CSL, and 100 g glucose. As the cells grew and consumed the initial glucose feed, a total of 70 g of additional glucose was steadily added throughout the process in order to keep the glucose concentration constant at about 20 g/L. The batch bioreactor was maintained at 30°C and pH 4.5 (using concentrated NaOH and HCl) for a batch time of about 24 hours. The resulting BDO concentration ranged from 20 to 200 g/L.

The 10-L continuous bioreactor setup required *E. coli* to be initially built up in the batch bioreactor using the above glucose and broth concentrations. The cells were then supplied to the continuous bioreactor to maintain a constant concentration of 3 – 5 g/L. Media was fed at the same concentrations as in the batch reactor at a feed rate of 0.5 – 1 L/hr. The resulting BDO concentration ranged from 30 to 40 g/L.

According to Mr. Stephen M. Tieri, our industry consultant from DuPont, when these bench scale tests were successful, Genomatica began small scale pilot tests to determine the feasibility of a commercial-scale facility. These trials were run in a 3,000-gallon fermenter, reaching BDO concentrations over 80 g/L. The BDO was then purified to greater than 99% using standard separation methods. Genomatica performed an economic feasibility analysis on the pilot tests, and the yield, *E. coli* productivity, separation and purity were all on target to deliver profitable results at the commercial scale (Burgard, Van Dien, Burk, & Niu).

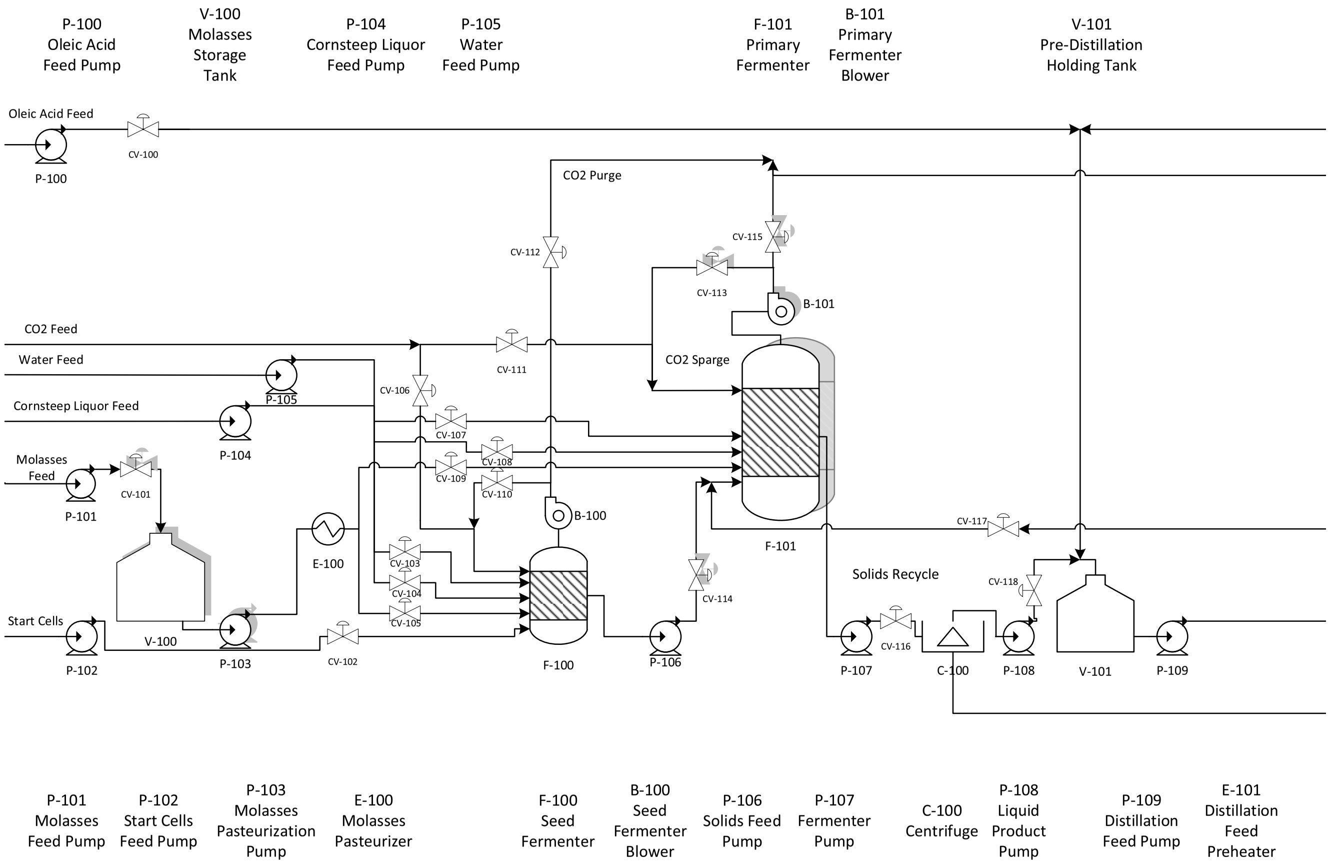
Feasibility, Development, Manufacturing, and Product-Introduction Stages

Process Flow Diagrams and Material Balances

The following pages illustrate our process and include details on the streams.

Renewable 1,4-Butanediol from Molasses

(Part 1)



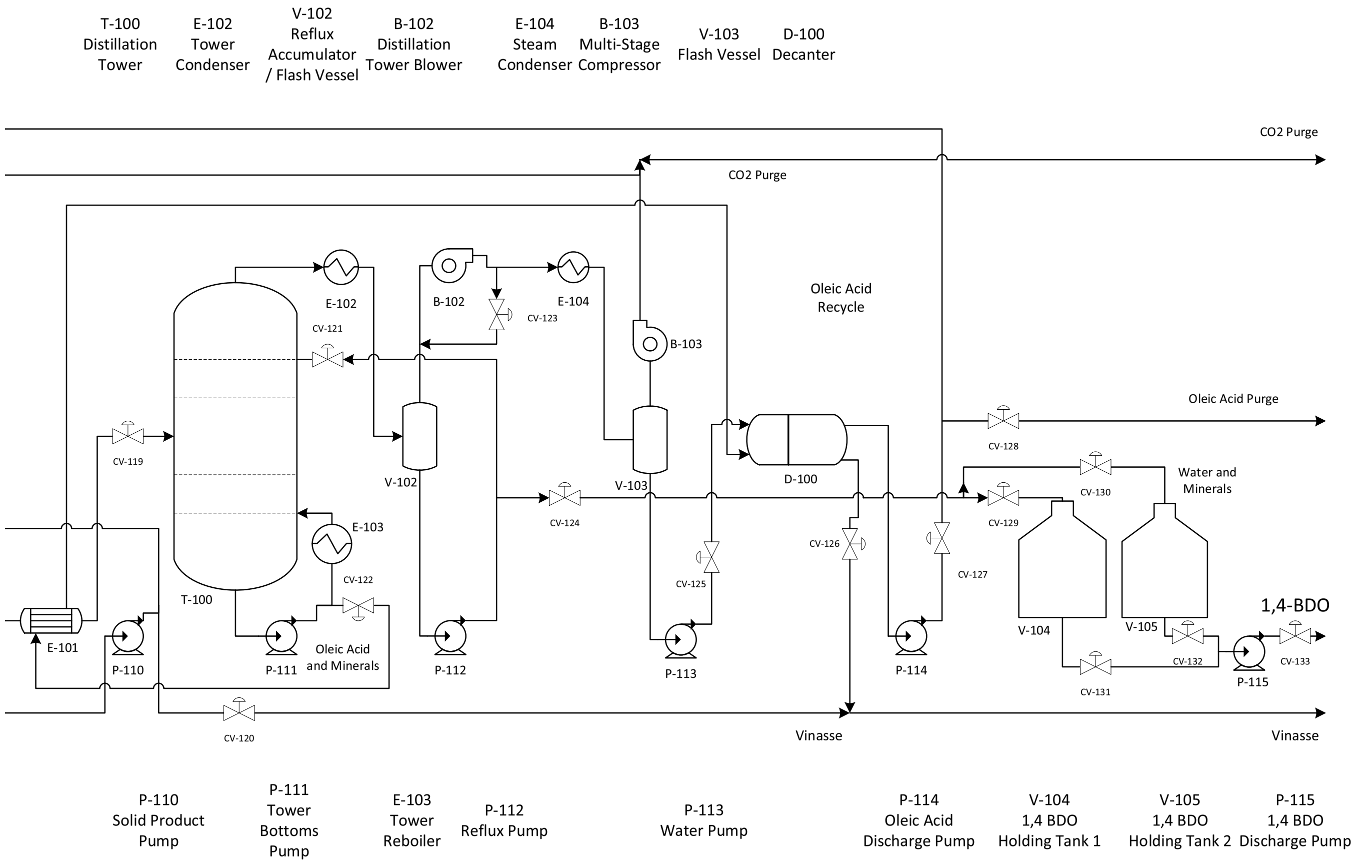
Control Valves

CV-100	Flow Control for P-100
CV-101	Flow Control for P-101
CV-102	On/Off Control for F-100 Cell Feed
CV-103	On/Off Control for F-100 Water Feed
CV-104	On/Off Control for F-100 Cornsteep Liquor Feed
CV-105	On/Off Control for F-100 Molasses Feed
CV-106	On/Off Control for F-100 CO2 Feed
CV-107	Flow Control for F-101 Water Feed
CV-108	Flow Control for F-101 Cornsteep Liquor Feed
CV-109	Flow Control for F-101 Molasses Feed
CV-110	Flow Control for F-100 CO2 Sparge Recycle
CV-111	Flow Control for F-101 CO2 Feed
CV-112	Pressure Control for F-100
CV-113	Flow Control for F-101 CO2 Sparge Recycle
CV-114	Flow Control for F-100 Product
CV-115	Pressure Control for F-101
CV-116	Flow Control for F-101 Product
CV-117	Flow Control for C-100 Recycle

version 5d03
"Mao"

Renewable 1,4-Butanediol from Molasses

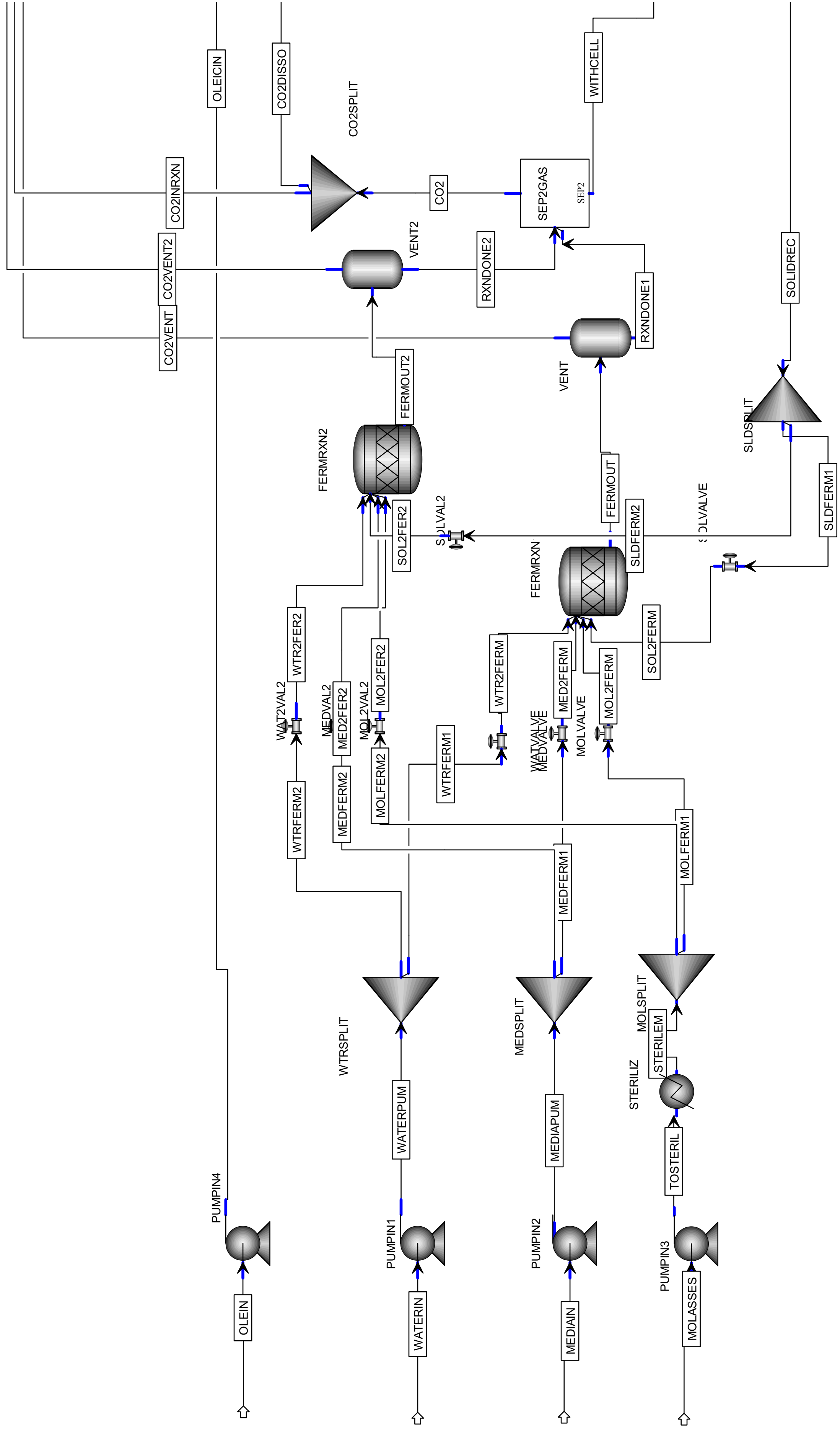
(Part 2)



Control Valves

CV-118	Inside Level Control for C-100 (Liquid)
CV-119	Flow Control for V-101
CV-120	Outside Level Control for C-100 (Solid)
CV-121	Flow Control for T-100 Reflux
CV-122	Level Control for T-100 Tower
CV-123	Pressure Control for T-100 Tower
CV-124	Level Control for V-102 Vessel
CV-125	Level Control for V-103 Vessel
CV-126	Level Control for D-100 Bottom Phase
CV-127	Level Control for D-100 Top Phase
CV-128	Flow Control for Oleic Acid Purge
CV-129	On/Off Control for V-104 Quality Control Tank 1 Feed
CV-130	On/Off Control for V-105 Quality Control Tank 2 Feed
CV-131	On/Off Control for V-104 Quality Control Tank 1 Product
CV-132	On/Off Control for V-105 Quality Control Tank 2 Product
CV-133	Flow Control for 1,4 BDO Product

Bibolet, Fernando, Shah
05 April 2011

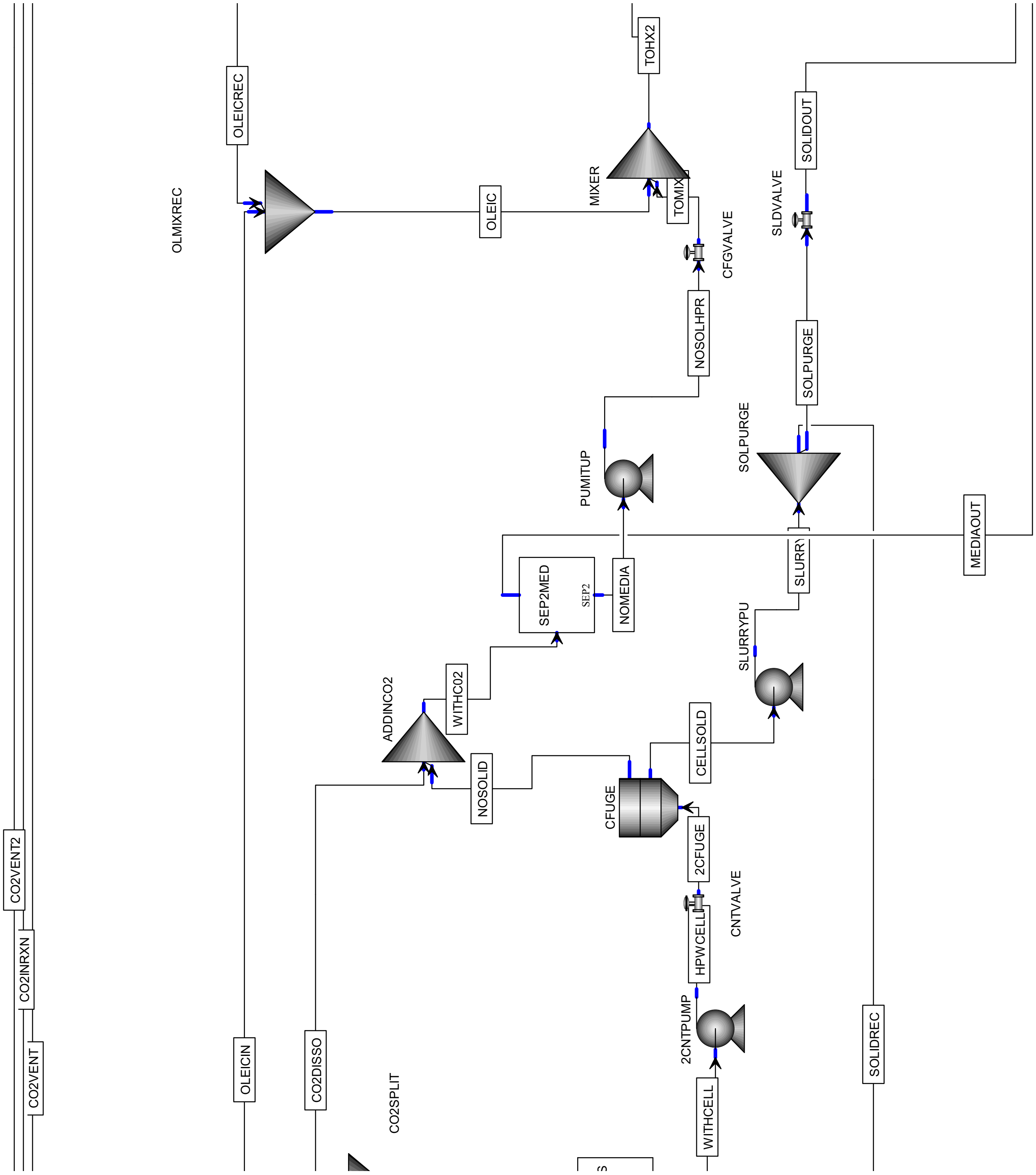

OLEICIN
CO2DISSO
CO2SPLIT
WITHCELL
CO2INRXN
CO2
SEP2GAS
SEP2
VENT2
CO2VENT2
RXNDONE2
CO2VENT
RXNDONE1
SOLIDREC
VENT
FERMOUT2
FERMRXN2
SLDSPLIT
FERMOUT
SOLVAL2
SOL2FER2
SLDFERM2
SOLVALVE
SLDFERM1
FERMRXN
SOL2FERM
WTR2FER2
WAT2VAL2
MEDVAL2
MED2FER2
MOL2VAL2
MOL2FER2
WTR2FERM
MED2FERM
MOL2FERM
WATVALVE
MEDVALVE
MOLVALVE
WTRFERM1
WTRFERM2
MEDFERM2
MOLFERM2
MOLFERM1
MEDFERM1
MOLSPLIT
STERILEM
STERILIZ
WTRSPLIT
MEDSPLIT
WATERPUM
MEDIAPUM
TOSTERIL
PUMPIN4
PUMPIN1
PUMPIN2
PUMPIN3
OLEIN
WATERIN
MEDIAIN
MOLASSES

	CO2	CO2DISSO	CO2INRXN	CO2VENT	CO2VENT2	FERMOUT	FERMOUT2	MED2FER2	MED2FERM	MEDFERM1	MEDFERM2	MEDIAIN
*** VAPOR PHASE ***												
Density lb/cuft	0.132	0.132	0.132									
Viscosity cP	0.015	0.015	0.015									
*** LIQUID PHASE ***												
Density lb/cuft						60.883	60.883	54.681	54.68	54.684	54.684	54.69
Viscosity cP						1.201	1.201	0.539	0.538	0.539	0.539	0.539
Surface Ten dyne/cm						45.002	45.002	35.037	35.035	35.042	35.042	35.052
Temperature F	86	86	86			86	86	86.2	86.3	86.2	86.2	86
Pressure psia	17.405	17.405	17.405			17.405	17.405	25	17.4	42.4	42.4	14.696
Mass VFrac	1	1	1			0	0	0	0	0	0	0
Mass SFrac	0	0	0			0.541	0.541	0	0	0	0	0
*** ALL PHASES ***												
Mass Flow lb/hr	4242.411	11.03	4231.381	0	0	30546.625	30546.625	7527.481	7527.481	7527.481	7527.481	15054.961
Volume Flow cuft/hr	32250.929	83.852	32167.077			274.275	274.275	137.662	137.665	137.654	137.654	275.279
Enthalpy Btu/hr	-1.63E+07	-42385.911	-1.63E+07	0	0	-4.43E+07	-4.43E+07	-1.68E+07	-1.68E+07	-1.68E+07	-1.68E+07	-3.36E+07
Density lb/cuft	0.132	0.132	0.132			111.372	111.372	54.681	54.68	54.684	54.684	54.69
Mass Flow lb/hr												
1:4-B-01	0	0	0			4420.309	4420.309	0	0	0	0	0
WATER	0	0	0			1742.509	1742.509	0	0	0	0	0
DEXTR-01	0	0	0			228.509	228.509	0	0	0	0	0
CARBO-01	4242.411	11.03	4231.381			2121.206	2121.206	0	0	0	0	0
OLEIC-01	0	0	0			0	0	0	0	0	0	0
LYSIN-01	0	0	0			20.91	20.91	20.548	20.548	20.548	20.548	41.096
GLYCI-01	0	0	0			20.91	20.91	20.548	20.548	20.548	20.548	41.096
ISOLE-01	0	0	0			20.91	20.91	20.548	20.548	20.548	20.548	41.096
LEUCI-01	0	0	0			20.91	20.91	20.548	20.548	20.548	20.548	41.096
METHI-01	0	0	0			20.91	20.91	20.548	20.548	20.548	20.548	41.096
L-PHE-01	0	0	0			20.91	20.91	20.548	20.548	20.548	20.548	41.096
THREO-01	0	0	0			20.91	20.91	20.548	20.548	20.548	20.548	41.096
TRYPT-01	0	0	0			20.91	20.91	20.548	20.548	20.548	20.548	41.096
TYROS-01	0	0	0			20.91	20.91	20.548	20.548	20.548	20.548	41.096
VALIN-01	0	0	0			20.91	20.91	20.548	20.548	20.548	20.548	41.096
INOSI-01	0	0	0			14.522	14.522	14.27	14.27	14.27	14.27	28.541
NIACI-01	0	0	0			3.739	3.739	3.674	3.674	3.674	3.674	7.348
POTAS-01	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
MAGNE-01	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
CALCI-01	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
SULFU-01	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
SODIU-01	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
IRON	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
ZINC	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
MANGA-01	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
COPPE-01	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
CHROM-01	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
MOLYB-01	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
COBAL-01	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
HYDRO-01	0	0	0			15.198	15.198	14.935	14.935	14.935	14.935	29.869
ETHYL-01	0	0	0			5074.498	5074.498	7109.908	7109.908	7109.908	7109.908	14219.816
CELLU-01	0	0	0			16534.663	16534.663	0	0	0	0	0

	MEDIAPUM	MOL2FER2	MOL2FERM	MOLASSES	MOLFERM1	MOLFERM2	OLEICIN	OLEIN	RXNDONE1	RXNDONE2	SLDFERM1
*** VAPOR PHASE ***											
Density lb/cuft											
Viscosity cP											
*** LIQUID PHASE ***											
Density lb/cuft	54.684	1006.254	1006.254	1177.439	1006.256	1006.256	55.069	55.097	60.883	60.883	60.486
Viscosity cP	0.539	1612.209	1612.223	5.61E+13	1612.366	1612.366	27.132	27.758	1.201	1.201	2.407
Surface Ten dyne/cm	35.042	16.23	16.23	20.557	16.23	16.23	32.155	32.202	45.002	45.002	55.254
Temperature F	86.2	266	266	86	266	266	81.5	80.3	86	86	86.2
Pressure psia	42.4	25	27.5	30	52.5	52.5	74.7	14.7	17.405	17.405	42.4
Mass VFrac	0	0	0	0	0	0	0	0	0	0	0
Mass SFrac	0	0.42	0.42	0.42	0.42	0.42	0	0	0.541	0.541	0.985
*** ALL PHASES ***											
Mass Flow lb/hr	15054.961	7873.148	7873.148	15746.297	7873.148	7873.148	186	186	30546.625	30546.625	13433.666
Volume Flow cuft/hr	275.308	13.364	13.364	25.408	13.364	13.364	3.378	3.376	274.275	274.275	38.708
Enthalpy Btu/hr	-3.36E+07	-1.32E+07	-1.32E+07	-2.74E+07	-1.32E+07	-1.32E+07	-2.32E+05	-2.32E+05	-4.43E+07	-4.43E+07	-5.99E+05
Density lb/cuft	54.684	589.147	589.147	619.742	589.147	589.147	55.069	55.097	111.372	111.372	347.052
Mass Flow lb/hr											
1:4-B-01	0	0	0	0	0	0	0	0	4420.309	4420.309	76.556
WATER	0	0	0	0	0	0	0	0	1742.509	1742.509	30.179
DEXTR-01	0	4566.214	4566.214	9132.429	4566.214	4566.214	0	0	228.509	228.509	3.958
CARBO-01	0	0	0	0	0	0	0	0	2121.206	2121.206	0
OLEIC-01	0	0	0	0	0	0	186	186	0	0	0
LYSIN-01	41.096	0	0	0	0	0	0	0	20.91	20.91	0.362
GLYCI-01	41.096	0	0	0	0	0	0	0	20.91	20.91	0.362
ISOLE-01	41.096	0	0	0	0	0	0	0	20.91	20.91	0.362
LEUCI-01	41.096	0	0	0	0	0	0	0	20.91	20.91	0.362
METHI-01	41.096	0	0	0	0	0	0	0	20.91	20.91	0.362
L-PHE-01	41.096	0	0	0	0	0	0	0	20.91	20.91	0.362
THREO-01	41.096	0	0	0	0	0	0	0	20.91	20.91	0.362
TRYPT-01	41.096	0	0	0	0	0	0	0	20.91	20.91	0.362
TYROS-01	41.096	0	0	0	0	0	0	0	20.91	20.91	0.362
VALIN-01	41.096	0	0	0	0	0	0	0	20.91	20.91	0.362
INOSI-01	28.541	0	0	0	0	0	0	0	14.522	14.522	0.252
NIACI-01	7.348	0	0	0	0	0	0	0	3.739	3.739	0.065
POTAS-01	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
MAGNE-01	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
CALCI-01	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
SULFU-01	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
SODIU-01	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
IRON	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
ZINC	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
MANGA-01	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
COPPE-01	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
CHROM-01	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
MOLYB-01	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
COBAL-01	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
HYDRO-01	29.869	0	0	0	0	0	0	0	15.198	15.198	0.263
ETHYL-01	14219.816	0	0	0	0	0	0	0	5074.498	5074.498	87.885
CELLU-01	0	3306.934	3306.934	6613.868	3306.934	3306.934	0	0	16534.663	16534.663	13227.729

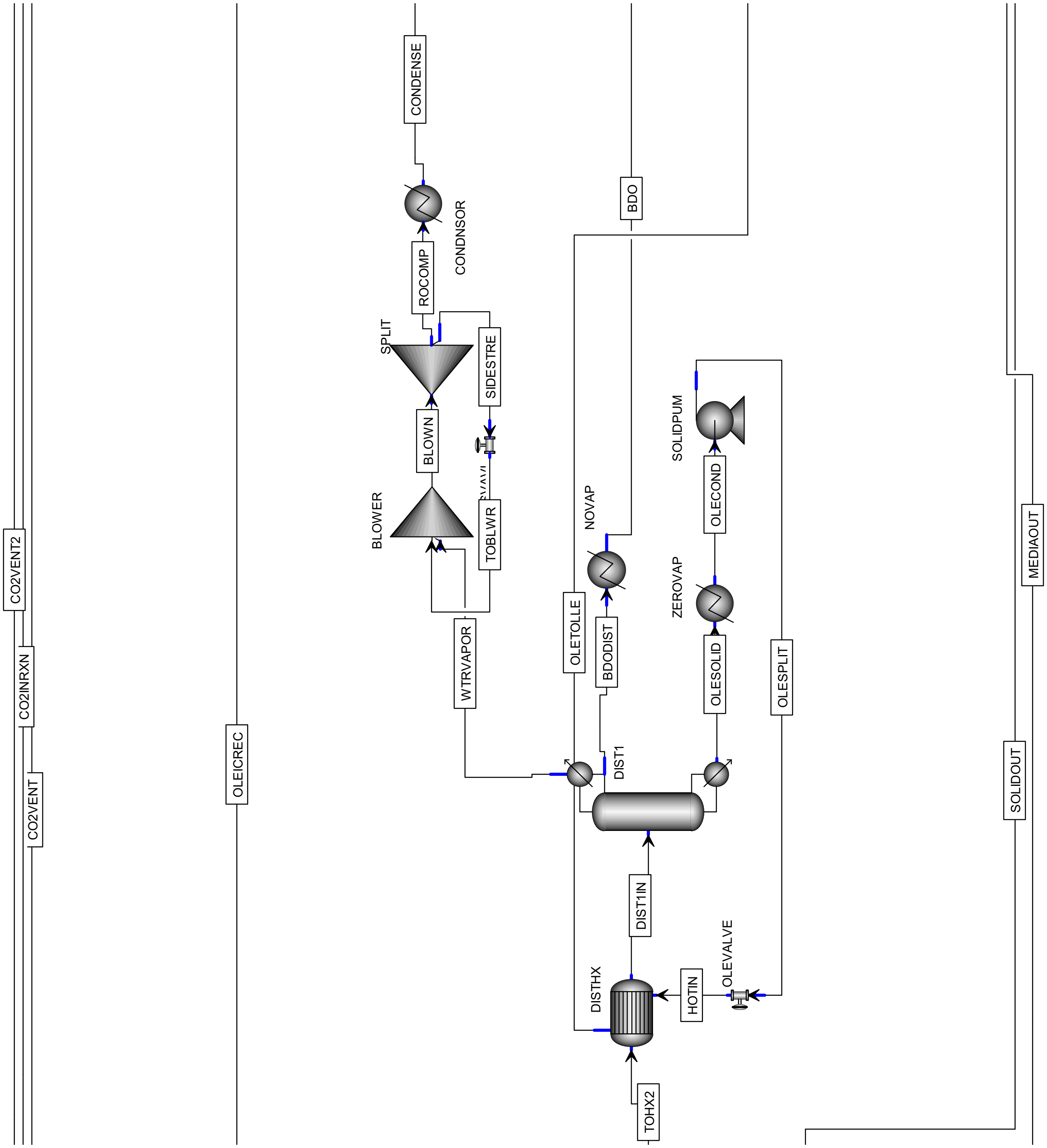
	SLDFERM2	SOL2FER2	SOL2FERM	SOLIDREC	STERILEM	TOSTERIL	WATERIN	WATERPUM	WITHCELL	WTR2FER2	WTR2FERM
*** VAPOR PHASE ***											
Density lb/cuft											
Viscosity cP											
*** LIQUID PHASE ***											
Density lb/cuft	60.486	60.486	60.486	60.486	1006.256	1177.433	61.749	61.741	60.492	61.74	61.739
Viscosity cP	2.407	2.407	2.407	2.407	1612.366	5.60E+13	0.82	0.817	2.413	0.817	0.817
Surface Ten dyne/cm	55.254	55.254	55.253	55.254	16.23	20.557	71.817	71.792	55.268	71.787	71.784
Temperature F	86.2	86.2	86.2	86.2	266	86	86	86.2	86	86.3	86.3
Pressure psia	42.4	25	17.4	42.4	52.5	52.5	14.7	42.4	17.405	25	17.4
Mass VFrac	0	0	0	0	0	0	0	0	0	0	0
Mass SFrac	0.985	0.985	0.985	0.985	0.42	0.42	0	0	0.582	0	0
*** ALL PHASES ***											
Mass Flow lb/hr	13433.666	13433.666	13433.666	26867.332	15746.297	15746.297	3424.661	3424.661	56850.836	1712.33	1712.33
Volume Flow cuft/hr	38.708	38.708	38.708	77.416	26.727	25.408	55.461	55.468	481.393	27.735	27.735
Enthalpy Btu/hr	-5.99E+05	-5.99E+05	-5.99E+05	-1.20E+06	-2.64E+07	-2.74E+07	-2.33E+07	-2.33E+07	-7.02E+07	-1.17E+07	-1.17E+07
Density lb/cuft	347.052	347.052	347.052	347.052	589.147	619.742	61.749	61.741	118.097	61.74	61.739
Mass Flow lb/hr											
1:4-B-01	76.556	76.556	76.556	153.111	0	0	0	0	8840.618	0	0
WATER	30.179	30.179	30.179	60.357	0	0	3424.661	3424.661	3485.018	1712.33	1712.33
DEXTR-01	3.958	3.958	3.958	7.915	9132.429	9132.429	0	0	457.017	0	0
CARBO-01	0	0	0	0	0	0	0	0	0	0	0
OLEIC-01	0	0	0	0	0	0	0	0	0	0	0
LYSIN-01	0.362	0.362	0.362	0.724	0	0	0	0	41.82	0	0
GLYCI-01	0.362	0.362	0.362	0.724	0	0	0	0	41.82	0	0
ISOLE-01	0.362	0.362	0.362	0.724	0	0	0	0	41.82	0	0
LEUCI-01	0.362	0.362	0.362	0.724	0	0	0	0	41.82	0	0
METHI-01	0.362	0.362	0.362	0.724	0	0	0	0	41.82	0	0
L-PHE-01	0.362	0.362	0.362	0.724	0	0	0	0	41.82	0	0
THREO-01	0.362	0.362	0.362	0.724	0	0	0	0	41.82	0	0
TRYPT-01	0.362	0.362	0.362	0.724	0	0	0	0	41.82	0	0
TYROS-01	0.362	0.362	0.362	0.724	0	0	0	0	41.82	0	0
VALIN-01	0.362	0.362	0.362	0.724	0	0	0	0	41.82	0	0
INOSI-01	0.252	0.252	0.252	0.503	0	0	0	0	29.044	0	0
NIACI-01	0.065	0.065	0.065	0.13	0	0	0	0	7.477	0	0
POTAS-01	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
MAGNE-01	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
CALCI-01	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
SULFU-01	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
SODIU-01	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
IRON	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
ZINC	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
MANGA-01	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
COPPE-01	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
CHROM-01	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
MOLYB-01	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
COBAL-01	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
HYDRO-01	0.263	0.263	0.263	0.526	0	0	0	0	30.395	0	0
ETHYL-01	87.885	87.885	87.885	175.771	0	0	0	0	10148.996	0	0
CELLU-01	13227.729	13227.729	13227.729	26455.459	6613.868	6613.868	0	0	33069.323	0	0

	WTRFERM1	WTRFERM2
*** VAPOR PHASE ***		
Density lb/cuft		
Viscosity cP		
*** LIQUID PHASE ***		
Density lb/cuft	61.741	61.741
Viscosity cP	0.817	0.817
Surface Ten dyne/cm	71.792	71.792
Temperature F	86.2	86.2
Pressure psia	42.4	42.4
Mass VFrac	0	0
Mass SFrac	0	0
*** ALL PHASES ***		
Mass Flow lb/hr	1712.33	1712.33
Volume Flow cuft/hr	27.734	27.734
Enthalpy Btu/hr	-1.17E+07	-1.17E+07
Density lb/cuft	61.741	61.741
Mass Flow lb/hr		
1:4-B-01	0	0
WATER	1712.33	1712.33
DEXTR-01	0	0
CARBO-01	0	0
OLEIC-01	0	0
LYSIN-01	0	0
GLYCI-01	0	0
ISOLE-01	0	0
LEUCI-01	0	0
METHI-01	0	0
L-PHE-01	0	0
THREO-01	0	0
TRYPT-01	0	0
TYROS-01	0	0
VALIN-01	0	0
INOSI-01	0	0
NIACI-01	0	0
POTAS-01	0	0
MAGNE-01	0	0
CALCI-01	0	0
SULFU-01	0	0
SODIU-01	0	0
IRON	0	0
ZINC	0	0
MANGA-01	0	0
COPPE-01	0	0
CHROM-01	0	0
MOLYB-01	0	0
COBAL-01	0	0
HYDRO-01	0	0
ETHYL-01	0	0
CELLU-01	0	0


CO2VENT2
CO2INRXN
CO2VENT
OLEICREC
OLMIXREC
OLEICIN
CO2DISSO
CO2SPLIT
OLEIC
MIXER
TOMIX
TOHX2
CFGVALVE
NOSOLHPR
PUMITUP
SEP2MED
SEP2
NOMEDIA
WITHC02
ADDINCO2
NOSOLID
CFUGE
CELLSOLD
2CFUGE
CNTVALVE
HPWCELL
2CNTPUMP
WITHCELL
SLURRYPU
SLURRY
SOLPURGE
SOLPURGE
SLDVALVE
SOLIDOUT
MEDIAOUT
SOLIDREC

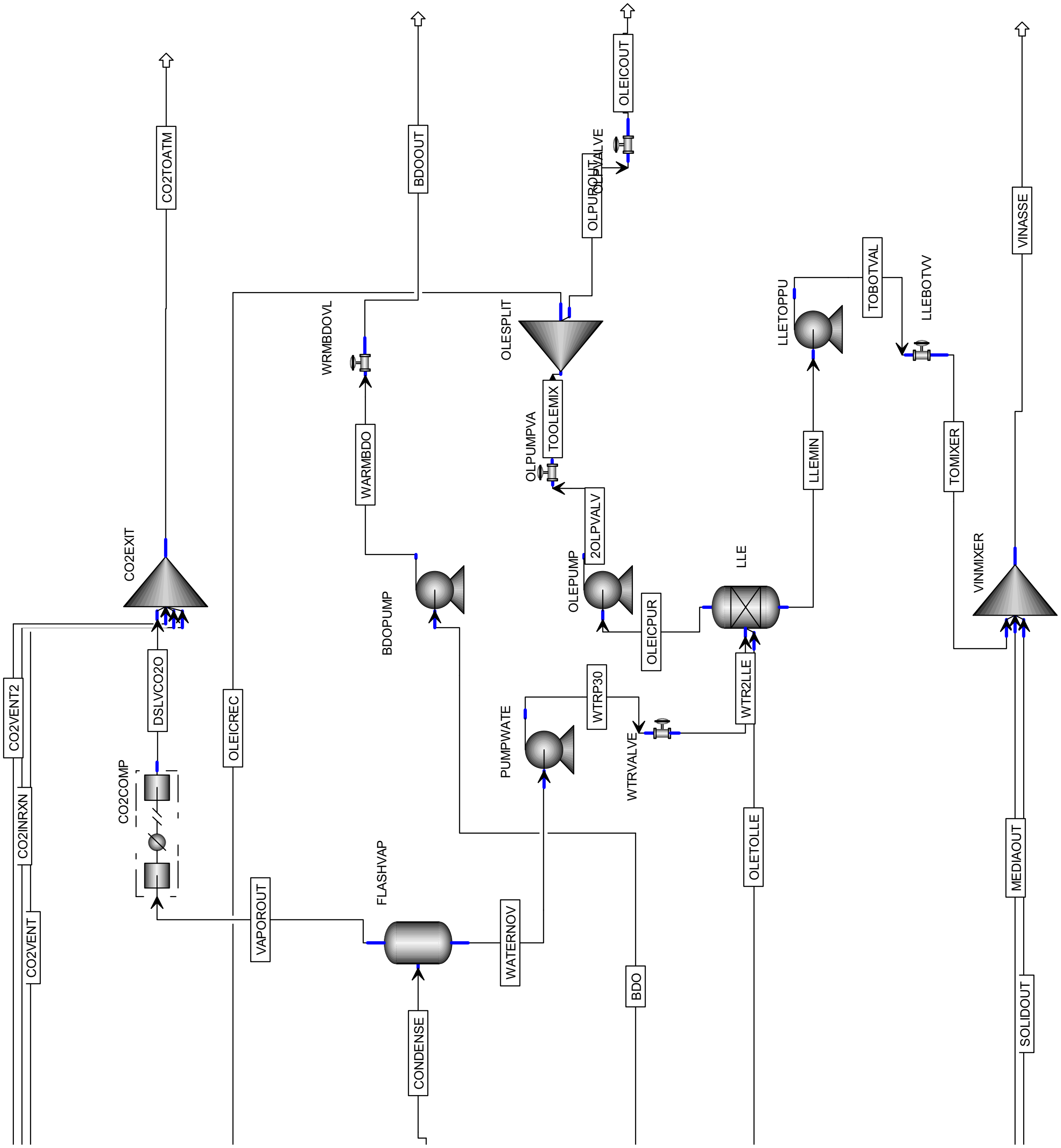
	2CFUGE	CELLSOLD	CO2DISSO	CO2INRXN	CO2VENT	CO2VENT2	HPWCELL	MEDIAOUT	NOMEDIA	NOSOLHPR	NOSOLID	OLEIC
*** VAPOR PHASE ***												
Density lb/cuft			0.132	0.132								
Viscosity cP			0.015	0.015								
*** LIQUID PHASE ***												
Density lb/cuft	60.486	60.486					60.49	55.572	62.525	62.498	60.486	55.347
Viscosity cP	2.407	2.407					2.411	0.405	4.614	4.538	2.407	34.382
Surface Ten dyne/cm	55.255	55.255					55.262	22.608	67.378	67.299	55.255	32.628
Temperature F	86.2	86.2	86	86			86.1	86.8	86.8	87.7	86.2	69.3
Pressure psia	14.7	14.692	17.405	17.405			39.7	14.692	14.692	99.7	14.692	30
Mass VFrac	0	0	1	1			0	0	0	0	0	0
Mass SFrac	0.582	0.985	0	0			0.582	0	0	0	0	0
*** ALL PHASES ***												
Mass Flow lb/hr	56850.836	33584.165	11.03	4231.381	0	0	56850.836	9929.282	13348.42	13348.42	23266.672	929.442
Volume Flow cuft/hr	481.429	96.77	83.852	32167.077			481.409	178.675	213.489	213.583	384.659	16.793
Enthalpy Btu/hr	-7.02E+07	-1.50E+06	-42385.911	-1.63E+07	0	0	-7.02E+07	-2.32E+07	-4.57E+07	-4.57E+07	-6.87E+07	-1.16E+06
Density lb/cuft	118.088	347.052	0.132	0.132			118.093	55.572	62.525	62.498	60.486	55.347
Mass Flow lb/hr												
1:4-B-01	8840.618	191.389	0	0			8840.618	0	8649.229	8649.229	8649.229	0
WATER	3485.018	75.446	0	0			3485.018	0	3409.572	3409.572	3409.572	0
DEXTR-01	457.017	9.894	0	0			457.017	0	447.123	447.123	447.123	0
CARBO-01	0	0	11.03	4231.381			0	0	11.03	11.03	0	0
OLEIC-01	0	0	0	0			0	0	0	0	0	929.442
LYSIN-01	41.82	0.905	0	0			41.82	0	40.915	40.915	40.915	0
GLYCI-01	41.82	0.905	0	0			41.82	0	40.915	40.915	40.915	0
ISOLE-01	41.82	0.905	0	0			41.82	0	40.915	40.915	40.915	0
LEUCI-01	41.82	0.905	0	0			41.82	0	40.915	40.915	40.915	0
METHI-01	41.82	0.905	0	0			41.82	0	40.915	40.915	40.915	0
L-PHE-01	41.82	0.905	0	0			41.82	0	40.915	40.915	40.915	0
THREO-01	41.82	0.905	0	0			41.82	0	40.915	40.915	40.915	0
TRYPT-01	41.82	0.905	0	0			41.82	0	40.915	40.915	40.915	0
TYROS-01	41.82	0.905	0	0			41.82	0	40.915	40.915	40.915	0
VALIN-01	41.82	0.905	0	0			41.82	0	40.915	40.915	40.915	0
INOSI-01	29.044	0.629	0	0			29.044	0	28.415	28.415	28.415	0
NIACI-01	7.477	0.162	0	0			7.477	0	7.316	7.316	7.316	0
POTAS-01	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
MAGNE-01	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
CALCI-01	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
SULFU-01	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
SODIU-01	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
IRON	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
ZINC	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
MANGA-01	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
COPPE-01	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
CHROM-01	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
MOLYB-01	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
COBAL-01	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
HYDRO-01	30.395	0.658	0	0			30.395	0	29.737	29.737	29.737	0
ETHYL-01	10148.996	219.714	0	0			10148.996	9929.282	0	0	9929.282	0
CELLU-01	33069.323	33069.323	0	0			33069.323	0	0	0	0	0

	OLEICIN	OLEICREC	SLURRY	SOLIDOUT	SOLIDREC	SOLPURGE	TOHX2	TOMIX	WITHC02	WITHCELL
*** VAPOR PHASE ***										
Density lb/cuft										
Viscosity cP										
*** LIQUID PHASE ***										
Density lb/cuft	55.069	55.421	60.486	60.486	60.486	60.486	61.554	62.494	60.463	60.492
Viscosity cP	27.132	36.723	2.407	2.407	2.407	2.407	4.623	4.529	2.376	2.413
Surface Ten dyne/cm	32.155	32.755	55.254	55.253	55.254	55.254	66.918	67.29	55.165	55.268
Temperature F	81.5	66	86.2	86.2	86.2	86.2	87.7	87.8	86.8	86
Pressure psia	74.7	49.7	42.4	17.4	42.4	42.4	30	74.7	14.692	17.405
Mass VFrac	0	0	0	0	0	0	0	0	0	0
Mass SFrac	0	0	0.985	0.985	0.985	0.985	0	0	0	0.582
*** ALL PHASES ***										
Mass Flow lb/hr	186	743.442	33584.165	6716.833	26867.332	6716.833	14277.862	13348.42	23277.702	56850.836
Volume Flow cuft/hr	3.378	13.414	96.77	19.354	77.416	19.354	231.957	213.594	384.994	481.393
Enthalpy Btu/hr	-2.32E+05	-9.32E+05	-1.50E+06	-3.00E+05	-1.20E+06	-3.00E+05	-4.68E+07	-4.57E+07	-6.87E+07	-7.02E+07
Density lb/cuft	55.069	55.421	347.052	347.052	347.052	347.052	61.554	62.494	60.463	118.097
Mass Flow lb/hr										
1:4-B-01	0	0	191.389	38.278	153.111	38.278	8649.229	8649.229	8649.229	8840.618
WATER	0	0	75.446	15.089	60.357	15.089	3409.572	3409.572	3409.572	3485.018
DEXTR-01	0	0	9.894	1.979	7.915	1.979	447.123	447.123	447.123	457.017
CARBO-01	0	0	0	0	0	0	11.03	11.03	11.03	0
OLEIC-01	186	743.442	0	0	0	0	929.442	0	0	0
LYSIN-01	0	0	0.905	0.181	0.724	0.181	40.915	40.915	40.915	41.82
GLYCI-01	0	0	0.905	0.181	0.724	0.181	40.915	40.915	40.915	41.82
ISOLE-01	0	0	0.905	0.181	0.724	0.181	40.915	40.915	40.915	41.82
LEUCI-01	0	0	0.905	0.181	0.724	0.181	40.915	40.915	40.915	41.82
METHI-01	0	0	0.905	0.181	0.724	0.181	40.915	40.915	40.915	41.82
L-PHE-01	0	0	0.905	0.181	0.724	0.181	40.915	40.915	40.915	41.82
THREO-01	0	0	0.905	0.181	0.724	0.181	40.915	40.915	40.915	41.82
TRYPT-01	0	0	0.905	0.181	0.724	0.181	40.915	40.915	40.915	41.82
TYROS-01	0	0	0.905	0.181	0.724	0.181	40.915	40.915	40.915	41.82
VALIN-01	0	0	0.905	0.181	0.724	0.181	40.915	40.915	40.915	41.82
INOSI-01	0	0	0.629	0.126	0.503	0.126	28.415	28.415	28.415	29.044
NIACI-01	0	0	0.162	0.032	0.13	0.032	7.316	7.316	7.316	7.477
POTAS-01	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
MAGNE-01	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
CALCI-01	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
SULFU-01	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
SODIU-01	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
IRON	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
ZINC	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
MANGA-01	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
COPPE-01	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
CHROM-01	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
MOLYB-01	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
COBAL-01	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
HYDRO-01	0	0	0.658	0.132	0.526	0.132	29.737	29.737	29.737	30.395
ETHYL-01	0	0	219.714	43.943	175.771	43.943	0	0	9929.282	10148.996
CELLU-01	0	0	33069.323	6613.865	26455.459	6613.865	0	0	0	33069.323


CO2VENT2
CO2INRXN
CO2VENT
OLEICREC
CONDENSE
CONDNSOR
ROCOMP
SPLIT
SIDESTRE
BLOWN
BLOWER
TOBLWR
WTRVAPOR
BDO
OLETOLLE
NOVAP
BDODIST
DIST1
DIST1IN
DISTHX
TOHX2
HOTIN
OLEVALVE
SOLIDPUM
OLECOND
ZEROVAP
OLESOLID
OLESPLIT
MEDIAOUT
SOLIDOUT

	BDO	BDODIST	BLOWN	CO2INRXN	CO2VENT	CO2VENT2	CONDENSE	DIST1IN	HOTIN	MEDIAOUT	OLECOND	OLEICREC
*** VAPOR PHASE ***												
Density lb/cuft			0	0.132			0.001					
Viscosity cP			0.01	0.015			0.01					
*** LIQUID PHASE ***												
Density lb/cuft	64.461	63.25					63.264	60.537	56.373	55.572	56.381	55.421
Viscosity cP	129.02	45.332					1.487	2.665	3.192	0.405	3.201	36.723
Surface Ten dyne/cm	46.912	44.705					76.808	64.065	128.354	22.608	128.37	32.755
Temperature F	49.9	87.8	87.8	86			41	119.9	392.7	86.8	392.3	66
Pressure psia	0.074	0.175	0.15	17.405			0.15	30	24.7	14.692	0.522	49.7
Mass VFrac	0	0	1	1			0.037	0	0	0	0	0
Mass SFrac	0	0	0	0			0	0	0	0	0	0
*** ALL PHASES ***												
Mass Flow lb/hr	8601.312	8601.312	3396.629	4231.381	0	0	3393.232	14277.862	2283.137	9929.282	2283.137	743.442
Volume Flow cuft/hr	133.435	135.99	7.31E+06	32167.077			215430.498	235.854	40.5	178.675	40.494	13.414
Enthalpy Btu/hr	-2.11E+07	-2.09E+07	-1.94E+07	-1.63E+07	0	0	-2.29E+07	-4.66E+07	-2.80E+06	-2.32E+07	-2.80E+06	-9.32E+05
Density lb/cuft	64.461	63.25	0	0.132			0.016	60.537	56.373	55.572	56.381	55.421
Mass Flow lb/hr												
1:4-B-01	8523.251	8523.251	13.572	0			13.558	8649.229	112.376	0	112.376	0
WATER	70.671	70.671	3342.276	0			3338.933	3409.572	0	0	0	0
DEXTR-01	0	0	0	0			0	447.123	447.09	0	447.09	0
CARBO-01	0.144	0.144	10.898	4231.381			10.887	11.03	0	0	0	0
OLEIC-01	0.067	0.067	0	0			0	929.442	929.299	0	929.299	743.442
LYSIN-01	0	0	0	0			0	40.915	40.912	0	40.912	0
GLYCI-01	0	0	0	0			0	40.915	40.912	0	40.912	0
ISOLE-01	0	0	0	0			0	40.915	40.912	0	40.912	0
LEUCI-01	0	0	0	0			0	40.915	40.912	0	40.912	0
METHI-01	0	0	0	0			0	40.915	40.912	0	40.912	0
L-PHE-01	0	0	0	0			0	40.915	40.912	0	40.912	0
THREO-01	0	0	0	0			0	40.915	40.912	0	40.912	0
TRYPT-01	0	0	0	0			0	40.915	40.912	0	40.912	0
TYROS-01	0	0	0	0			0	40.915	40.912	0	40.912	0
VALIN-01	0	0	0	0			0	40.915	40.912	0	40.912	0
INOSI-01	0	0	0	0			0	28.415	28.413	0	28.413	0
NIACI-01	7.171	7.171	0.125	0			0.125	7.316	0.019	0	0.019	0
POTAS-01	0	0	0	0			0	29.737	29.735	0	29.735	0
MAGNE-01	0	0	0	0			0	29.737	29.735	0	29.735	0
CALCI-01	0	0	0	0			0	29.737	29.735	0	29.735	0
SULFU-01	0	0	0	0			0	29.737	29.735	0	29.735	0
SODIU-01	0	0	0	0			0	29.737	29.735	0	29.735	0
IRON	0	0	0	0			0	29.737	29.735	0	29.735	0
ZINC	0	0	0	0			0	29.737	29.735	0	29.735	0
MANGA-01	0	0	0	0			0	29.737	29.735	0	29.735	0
COPPE-01	0	0	0	0			0	29.737	29.735	0	29.735	0
CHROM-01	0	0	0	0			0	29.737	29.735	0	29.735	0
MOLYB-01	0	0	0	0			0	29.737	29.735	0	29.735	0
COBAL-01	0	0	0	0			0	29.737	29.735	0	29.735	0
HYDRO-01	0.009	0.009	29.758	0			29.729	29.737	0	0	0	0
ETHYL-01	0	0	0	0			0	0	0	9929.282	0	0
CELLU-01	0	0	0	0			0	0	0	0	0	0

	OLESOLID	OLESPLIT	OLETOLLE	ROCOMP	SIDESTRE	SOLIDOUT	TOBLWR	TOHX2	WTRVAPOR
*** VAPOR PHASE ***									
Density lb/cuft	0.008			0	0		0		0.001
Viscosity cP	0.011			0.01	0.01		0.01		0.01
*** LIQUID PHASE ***									
Density lb/cuft	55.573	56.375	62.25			60.486		61.554	
Viscosity cP	2.395	3.194	247.567			2.407		4.623	
Surface Ten dyne/cm	130.042	128.358	140.147			55.253		66.918	
Temperature F	437.5	392.6	120	87.8	87.8	86.2	87.8	87.7	87.8
Pressure psia	0.742	49.7	24.7	0.15	0.15	17.4	0.15	30	0.175
Mass VFrac	0.029	0	0	1	1	0	1	0	1
Mass SFrac	0	0	0	0	0	0.985	0	0	0
*** ALL PHASES ***									
Mass Flow lb/hr	2283.137	2283.137	2283.137	3393.232	3.397	6716.833	3.397	14277.862	3393.232
Volume Flow cuft/hr	8003.521	40.499	36.677	7.31E+06	7312.788	19.354	7312.788	231.957	6.27E+06
Enthalpy Btu/hr	-2.73E+06	-2.80E+06	-3.08E+06	-1.94E+07	-19373.293	-3.00E+05	-19373.293	-4.68E+07	-1.94E+07
Density lb/cuft	0.285	56.375	62.25	0	0	347.052	0	61.554	0.001
Mass Flow lb/hr									
1:4-B-01	112.376	112.376	112.376	13.558	0.014	38.278	0.014	8649.229	13.558
WATER	0	0	0	3338.933	3.342	15.089	3.342	3409.572	3338.933
DEXTR-01	447.09	447.09	447.09	0	0	1.979	0	447.123	0
CARBO-01	0	0	0	10.887	0.011	0	0.011	11.03	10.887
OLEIC-01	929.299	929.299	929.299	0	0	0	0	929.442	0
LYSIN-01	40.912	40.912	40.912	0	0	0.181	0	40.915	0
GLYCI-01	40.912	40.912	40.912	0	0	0.181	0	40.915	0
ISOLE-01	40.912	40.912	40.912	0	0	0.181	0	40.915	0
LEUCI-01	40.912	40.912	40.912	0	0	0.181	0	40.915	0
METHI-01	40.912	40.912	40.912	0	0	0.181	0	40.915	0
L-PHE-01	40.912	40.912	40.912	0	0	0.181	0	40.915	0
THREO-01	40.912	40.912	40.912	0	0	0.181	0	40.915	0
TRYPT-01	40.912	40.912	40.912	0	0	0.181	0	40.915	0
TYROS-01	40.912	40.912	40.912	0	0	0.181	0	40.915	0
VALIN-01	40.912	40.912	40.912	0	0	0.181	0	40.915	0
INOSI-01	28.413	28.413	28.413	0	0	0.126	0	28.415	0
NIACI-01	0.019	0.019	0.019	0.125	0	0.032	0	7.316	0.125
POTAS-01	29.735	29.735	29.735	0	0	0.132	0	29.737	0
MAGNE-01	29.735	29.735	29.735	0	0	0.132	0	29.737	0
CALCI-01	29.735	29.735	29.735	0	0	0.132	0	29.737	0
SULFU-01	29.735	29.735	29.735	0	0	0.132	0	29.737	0
SODIU-01	29.735	29.735	29.735	0	0	0.132	0	29.737	0
IRON	29.735	29.735	29.735	0	0	0.132	0	29.737	0
ZINC	29.735	29.735	29.735	0	0	0.132	0	29.737	0
MANGA-01	29.735	29.735	29.735	0	0	0.132	0	29.737	0
COPPE-01	29.735	29.735	29.735	0	0	0.132	0	29.737	0
CHROM-01	29.735	29.735	29.735	0	0	0.132	0	29.737	0
MOLYB-01	29.735	29.735	29.735	0	0	0.132	0	29.737	0
COBAL-01	29.735	29.735	29.735	0	0	0.132	0	29.737	0
HYDRO-01	0	0	0	29.729	0.03	0.132	0.03	29.737	29.729
ETHYL-01	0	0	0	0	0	43.943	0	0	0
CELLU-01	0	0	0	0	0	6613.865	0	0	0


CO2TOATM
BDOOUT
OLEICOUT
OLPUROUT
OLPVALVE
VINASSE
TOBOTVAL
LLEBOTV
LLETOPPU
OLESPLIT
WRMBDOVL
CO2EXIT
WARMBDO
TOOLEMIX
OLPUMPVA
LLEMIN
TOMIXER
VINMIXER
LLE
2OLPVALV
OLEPUMP
BDOPUMP
OLEICPUR
WTRP30
WTR2LLE
DSLVCO2O
CO2VENT2
OLEICREC
PUMPWATE
WTRVALVE
CO2COMP
CO2INRXN
OLETOLLE
MEDIAOUT
FLASHVAP
VAPOROUT
WATERNOV
CO2VENT
BDO
SOLIDOUT
CONDENSE

	2OLPVALV	BDO	BDOOUT	CO2INRXN	CO2TOAT	CO2VENT	CO2VENT2	CONDENSE	DSLVCO2O	LLEMIN	MEDIAOUT	OLEICOUT	OLEICPUR	OLEICREC
*** VAPOR PHASE ***														
Density lb/cuft				0.132	0.13			0.001	0.061					
Viscosity cP				0.015	0.015			0.01	0.015					
*** LIQUID PHASE ***														
Density lb/cuft	55.425	64.461	64.449		64.578			63.264	57.82	59.412	55.572	55.418	55.431	55.421
Viscosity cP	36.839	129.02	127.598		0.632			1.487	0.297	2.226	0.405	36.608	37.026	36.723
Surface Ten dyne/cm	32.761	46.912	46.89		64.185			76.808	59.166	81.22	22.608	32.749	32.771	32.755
Temperature F	65.8	49.9	50.2	86	83			41	200	65.6	86.8	66.1	65.6	66
Pressure psia	74.7	0.074	40	17.405	17.405			0.15	17.405	24.7	14.692	24.7	24.7	49.7
Mass VFrac	0	0	0	1	0.988			0.037	0.535	0	0	0	0	0
Mass SFrac	0	0	0	0	0			0	0	0	0	0	0	0
*** ALL PHASES ***														
Mass Flow lb/hr	929.303	8601.312	8601.312	4231.381	4358.415	0	0	3393.232	127.034	4620.035	9929.282	185.861	929.303	743.442
Volume Flow cuft/hr	16.767	133.435	133.459	32167.077	33144.36			215430.498	1112.172	77.763	178.675	3.354	16.765	13.414
Enthalpy Btu/hr	-1.17E+06	-2.11E+07	-2.10E+07	-1.63E+07	-1.69E+07	0	0	-2.29E+07	-6.35E+05	-2.42E+07	-2.32E+07	-2.33E+05	-1.17E+06	-9.32E+05
Density lb/cuft	55.425	64.461	64.449	0.132	0.131			0.016	0.114	59.412	55.572	55.418	55.431	55.421
Mass Flow lb/hr														
1:4-B-01	0	8523.251	8523.251	0	0.001			13.558	0.001	125.933	0	0	0	0
WATER	0	70.671	70.671	0	91.177			3338.933	91.177	3247.757	0	0	0	0
DEXTR-01	0	0	0	0	0			0	0	447.09	0	0	0	0
CARBO-01	0	0.144	0.144	4231.381	4237.913			10.887	6.533	4.354	0	0	0	0
OLEIC-01	929.303	0.067	0.067	0	0			0	0	0	0	185.861	929.303	743.442
LYSIN-01	0	0	0	0	0			0	0	40.912	0	0	0	0
GLYCI-01	0	0	0	0	0			0	0	40.912	0	0	0	0
ISOLE-01	0	0	0	0	0			0	0	40.912	0	0	0	0
LEUCI-01	0	0	0	0	0			0	0	40.912	0	0	0	0
METHI-01	0	0	0	0	0			0	0	40.912	0	0	0	0
L-PHE-01	0	0	0	0	0			0	0	40.912	0	0	0	0
THREO-01	0	0	0	0	0			0	0	40.912	0	0	0	0
TRYPT-01	0	0	0	0	0			0	0	40.912	0	0	0	0
TYROS-01	0	0	0	0	0			0	0	40.912	0	0	0	0
VALIN-01	0	0	0	0	0			0	0	40.912	0	0	0	0
INOSI-01	0	0	0	0	0			0	0	28.413	0	0	0	0
NIACI-01	0	7.171	7.171	0	0			0.125	0	0.144	0	0	0	0
POTAS-01	0	0	0	0	0			0	0	29.735	0	0	0	0
MAGNE-01	0	0	0	0	0			0	0	29.735	0	0	0	0
CALCI-01	0	0	0	0	0			0	0	29.735	0	0	0	0
SULFU-01	0	0	0	0	0			0	0	29.735	0	0	0	0
SODIU-01	0	0	0	0	0			0	0	29.735	0	0	0	0
IRON	0	0	0	0	0			0	0	29.735	0	0	0	0
ZINC	0	0	0	0	0			0	0	29.735	0	0	0	0
MANGA-01	0	0	0	0	0			0	0	29.735	0	0	0	0
COPPE-01	0	0	0	0	0			0	0	29.735	0	0	0	0
CHROM-01	0	0	0	0	0			0	0	29.735	0	0	0	0
MOLYB-01	0	0	0	0	0			0	0	29.735	0	0	0	0
COBAL-01	0	0	0	0	0			0	0	29.735	0	0	0	0
HYDRO-01	0	0.009	0.009	0	29.324			29.729	29.324	0.405	0	0	0	0
ETHYL-01	0	0	0	0	0			0	0	0	9929.282	0	0	0
CELLU-01	0	0	0	0	0			0	0	0	0	0	0	0

	OLPUROUT	SOLIDOUT	TOBOTVAL	TOMIXER	TOOLEMIX	VAPOROUT	VINASSE	WARMBDO	WATERNOV	WTR2LLE	WTRP30
*** VAPOR PHASE ***											
Density lb/cuft						0.001					
Viscosity cP						0.01					
*** LIQUID PHASE ***											
Density lb/cuft	55.421	60.486	59.411	59.408	55.421		59.194	64.453	63.264	63.259	63.261
Viscosity cP	36.723	2.407	2.224	2.22	36.723		1.235	128.103	1.487	1.484	1.486
Surface Ten dyne/cm	32.755	55.253	81.216	81.206	32.755		60.157	46.898	76.808	76.79	76.799
Temperature F	66	86.2	65.6	65.7	66	41	67.1	50.1	41	41.2	41.1
Pressure psia	49.7	17.4	42.4	17.4	49.7	0.15	39.7	65	0.15	24.7	49.7
Mass VFrac	0	0	0	0	0	1	0	0	0	0	0
Mass SFrac	0	0.985	0	0	0	0	0.311	0	0	0	0
*** ALL PHASES ***											
Mass Flow lb/hr	185.861	6716.833	4620.035	4620.035	929.303	127.034	21266.151	8601.312	3266.198	3266.198	3266.198
Volume Flow cuft/hr	3.354	19.354	77.764	77.768	16.768	215378.87	265.181	133.45	51.628	51.632	51.63
Enthalpy Btu/hr	-2.33E+05	-3.00E+05	-2.42E+07	-2.42E+07	-1.17E+06	-5.85E+05	-4.77E+07	-2.10E+07	-2.23E+07	-2.23E+07	-2.23E+07
Density lb/cuft	55.421	347.052	59.411	59.408	55.421	0.001	80.195	64.453	63.264	63.259	63.261
Mass Flow lb/hr											
1:4-B-01	0	38.278	125.933	125.933	0	0.001	164.211	8523.251	13.557	13.557	13.557
WATER	0	15.089	3247.757	3247.757	0	91.177	3262.846	70.671	3247.757	3247.757	3247.757
DEXTR-01	0	1.979	447.09	447.09	0	0	449.068	0	0	0	0
CARBO-01	0	0	4.354	4.354	0	6.533	4.354	0.144	4.354	4.354	4.354
OLEIC-01	185.861	0	0	0	929.303	0	0	0.067	0	0	0
LYSIN-01	0	0.181	40.912	40.912	0	0	41.093	0	0	0	0
GLYCI-01	0	0.181	40.912	40.912	0	0	41.093	0	0	0	0
ISOLE-01	0	0.181	40.912	40.912	0	0	41.093	0	0	0	0
LEUCI-01	0	0.181	40.912	40.912	0	0	41.093	0	0	0	0
METHI-01	0	0.181	40.912	40.912	0	0	41.093	0	0	0	0
L-PHE-01	0	0.181	40.912	40.912	0	0	41.093	0	0	0	0
THREO-01	0	0.181	40.912	40.912	0	0	41.093	0	0	0	0
TRYPT-01	0	0.181	40.912	40.912	0	0	41.093	0	0	0	0
TYROS-01	0	0.181	40.912	40.912	0	0	41.093	0	0	0	0
VALIN-01	0	0.181	40.912	40.912	0	0	41.093	0	0	0	0
INOSI-01	0	0.126	28.413	28.413	0	0	28.539	0	0	0	0
NIACI-01	0	0.032	0.144	0.144	0	0	0.176	7.171	0.125	0.125	0.125
POTAS-01	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
MAGNE-01	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
CALCI-01	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
SULFU-01	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
SODIU-01	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
IRON	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
ZINC	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
MANGA-01	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
COPPE-01	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
CHROM-01	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
MOLYB-01	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
COBAL-01	0	0.132	29.735	29.735	0	0	29.867	0	0	0	0
HYDRO-01	0	0.132	0.405	0.405	0	29.324	0.537	0.009	0.405	0.405	0.405
ETHYL-01	0	43.943	0	0	0	0	9973.225	0	0	0	0
CELLU-01	0	6613.865	0	0	0	0	6613.865	0	0	0	0

Process Description

Physical Properties

The utilization of the Aspen Plus programming software limited our availability of components to those in the program's data banks. Molasses and Corn Steep Liquor (CSL) were not available components, so to model them, we separated each material into its constituents. To model the molasses feed, we obtained the theoretical amount of sugar needed by the *E. coli* cells from the Genomatica Patent Application, then divided by 0.54 since molasses is 54% sugars. Molasses is also 20% water, 12% ash, and 16% other compounds (Paturau). To model the CSL feed, we obtained the theoretical amount of media needed by the cells from the Genomatica Patent Application and operated at 25% excess. CSL is about 50% solids, such as ash, 24% amino acids, 9% minerals, 10% vitamins, and 7% other compounds. We inputted the individual vitamins, minerals, amino acids, and solids into Aspen to model the remaining constituents of the molasses and CSL. The Aspen software also required us to assign a property method to model the non-ideal interactions among our components. We narrowed down our options to the two most widely accepted property methods, NRTL-RK or UNIQUAC. Though both yield non-ideal estimates of properties, we were advised by Mr. Bruce M. Vrana, one of our consultants from DuPont, that NRTL-RK is more readily used in industry, so we imitated industry standards to ensure consistent results.

Unit Operations

Water Sterilization Skid

The use of organic compounds and sensitive *E. coli* cells requires our feed water to be sterile. Purchasing sterile water would be very expensive and subject to contamination during transport and storage. As advised by Mr. Tieri, we decided to purchase a water sterilization skid. This apparatus will allow us to sterilize our feed water immediately before it is sent to the fermenter, which will prevent any contaminants from entering and potentially spoiling our batch of cells. We will purchase process water, which comes in varying forms of purity depending on the source, but will not be sterile. This process water will be stored in our water storage tank and sent to the sterilization skid when necessary. Since we require about 10,000 gallons per day of water, we decided to purchase two units of the commercial TV-Reverse Osmosis-10,000 from RO Consumables for about $9,900 each (RO Consumables, 2001). We decided to purchase a second unit as a replacement in case the first unit breaks down. The sterilization skid also contains a pump that will operate with a requirement of 2 - 3 horsepower.

Molasses Sterilizer and Holding Tanks

Our sister facility produces blackstrap molasses (a concentrated form of traditional molasses with higher nutrient content) as a byproduct of their system and will sell it to us at a discounted price for our feedstock. However, since the facility produces molasses as a byproduct and it does not come from a bio-based system, the sterility of the feed is not guaranteed. Moreover, we will have to store the molasses in one of three 800,000-gallon storage tanks for one month during which production in the sister sugar and ethanol factory is halted; so, contamination may occur then as well. The holding tank will also provide a steady flow of molasses to the fermenter irrespective of upstream disturbances. The feed from this holding tank must be sterilized to ensure that the feed to the fermenter is contaminant-free. The molasses pumped from the sister facility to our molasses storage tanks will be supplied at 1.11 times the required rate for our production process and will be continuously sterilized immediately before entering the fermenter to 266°F with hot steam to kill most bacteria and microorganisms (Kristiansen, Mattey, & Linden, 2002). The 1.11 ratio of feed to output of the storage tanks is to account for the nine months of our sister plant's continuous operation and an additional one month of storage. The excess molasses flow rate will accumulate in the molasses storage tank over nine months, resulting in a sufficient amount of molasses to be sent through the sterilizer of molasses for the additional one month of production in which the sugar refinery is out of operation.

Fermenters

Seed Fermenter

The seed fermenter is an 18,500-liter batch fermenter that allows the *E. coli* to replicate under aerobic conditions and a pH of 7. This batch process lasts 24 hours and provides 10% more cells than needed. These extra cells are used to start the next batch when needed, so new cells are not required to be delivered. The cells can be reused as there is little to no mutations in the *E. coli* as stated in Patent Application 20090075351. The seed fermenter is only in use once a month when the cells in the main fermenters need to be changed. It is sterilized after use, which takes around 20 hours.

Primary Fermenters

In the initial design phase, we decided to replicate the bench-scale laboratory work done by our Research and Development team. Based on the information in Patent Application 20090075351 we began modeling our feeds and fermenter design. The bench-scale work was done with both a 10-liter batch and a 10-liter continuous fermenter. We modeled our simulation as a continuous process, since we decided our final process would be more economical operating continuously as the batch time required to produce a sufficient amount of BDO was 24 hours. Since the continuous process yielded as much BDO in a day as the batch process did in one batch, the continuous process would be more economical due to the ability to run 24/7 and not need the extra time to clean and sterilize between each batch.

Based on the requirement of 50 million pounds per year of BDO produced, we scaled up the amount of sugar the *E. coli* cells needed based on the Reaction 1 given below and in Patent

Application 20090075351 assuming a 90% conversion of sugar to BDO and the CSL in 20% excess.

$$\text{CSL} + \text{Dextrose} \dashrightarrow 2\,\text{BDO} + \text{CO}_2 \qquad \text{(Reaction 1)}$$

Since Reaction 1 given above is an anaerobic process, the fermenters are kept at 1.2 bar by sparging in carbon dioxide. The fermenters are also kept at a pH below 6 by a concentrated hydrochloric acid control stream. The low pH of the environment severely inhibits the replication of the *E. coli* and allows the complete conversion of feed to BDO (Burgard, Van Dien, Burk, & Niu). An agitator continuously rotates inside each of the fermenters to provide a well-mixed solution so the *E. coli* have greater access to the minerals. The agitators will be run at a moderate 7 RPM in order to ensure low shear stress on the cells, while also ensuring proper mixing in the reactor.

Use of Two Fermenters

We utilize two equally-sized 43,000-liter fermenters that handle 50% of the input rather than a single large one, though it is less cost efficient, to allow for possible glitches in our system. A sensitivity analysis determined two smaller reactors operating at 50% capacity would cost us about $1.1 million total, whereas one large fermenter operating at 70% capacity would cost us $890,000. We believe the extra $200,000 cost for the two smaller reactors will be a beneficial initial investment. If a set of cells is bad, if the reactor is not pressurized correctly, or if the pH deviates too far from the suggested range, the single spoiled fermenter will need to be shut down, sterilized and the problem fixed. This will result in significant downtime and loss of revenue. By using two smaller reactors running at 50% capacity, if one fermenter goes down, the whole production process will not be stopped and when both fermenters come back on line, the capacity can be increased to allow extra BDO production to make up for the losses.

Cleaning - Sterilization/CIP

The *E. coli* in the fermenters need to be replaced once a month but the fermenters only need to be sterilized every other month, which is five to six times per year. The clean-in-place (CIP) process will take about 30 hours for each fermenter. Cleaning of the remaining equipment will take place once a year during the two months of downtime in the Brazilian rainy season. Appendix G shows a sample operating schedule.

Aspen Simulation of Fermenters

In Aspen, the fermenter was modeled as an RSTOIC reactor, which allowed us to input reactions as well as the degree of conversion. However, due to the lack of CSL in the Aspen databanks, the reaction would not balance due to the improper amounts of elements being fed in. Thus, just using an atomic species balance, ethyl acetate was used to model the CSL in the RSTOIC and was removed immediately after the fermenter to avoid any interactions it may have with the other components in the process.

Centrifuge

The solid-liquid separation system we pursued was a centrifuge. The benefit of a centrifuge is that some of the models can be run continuously without constant need to be cleaned or have their filters replaced. The specific device we implemented was a continuous reciprocating pusher. Despite its extra capital cost, we chose this device over the other style, a continuous solid scroll bowl, since the solid scroll bowl is not suitable for handling fragile materials, such as our *E. coli* (Sinnott, 2005). The continuous reciprocating pusher will efficiently separate the ash in the molasses (12%) (Paturau), ash in the CSL (17%) (Corn Refiners Association, 2006) and the *E. coli* cell solids from the BDO, water, and dissolved minerals liquid stream.

The solid output will contain some *E. coli*, but 80% of the solids stream will be recycled to the fermenter to prevent the concentration of cells in the fermenter from varying too much. The recycle stream will also be frequently analyzed and tested to ensure the recycled *E. coli* cells are robust enough to be sent back to the fermenter. The purged stream will be sold back to our sister plant as vinasse, a common type of fertilizer. *E. coli* will be present in the vinasse, but it is assumed that they are not harmful to the environment and can be released with no ill side effects. The liquid overflow will be captured and held in an eight-hour holding tank before being fed into the distillation column. This allows the feed into the column to remain constant in case of upstream fluctuations.

Distillation Tower

We are using one vacuum distillation tower with five dual-flow trays and a partial condenser to separate out the minerals, solutes, and other impurities in the feed stream using oleic acid. The distillate will contain both water and oleic acid and enter a partial condenser. The partial condenser will condense the BDO, but not the water. A portion of the BDO will be sent back to the column as reflux, while the other portion will be sent to day tanks as the final product. The water, on the other hand, will be sent to another flash column, where the carbon dioxide that came out of solution when the pressure changed, will be drawn off. The remaining water will be sent to the decanter.

Dual flow trays were chosen to allow the solid particles to easily travel through the trays without restricting the flow due to sediment build-up. We calculated seven theoretical stages to allow for two trays above and two trays below the feed stream.

The distillation column will have to operate at high vacuum in order to attain optimal separation of BDO from the water, while also decreasing the reboiler temperature to 450°F, at which high-pressure steam can still be used as a heat source. Moreover, any reboiler temperatures higher than 450°F are hard to manage. The high temperature of the reboiler, in addition to the corrosive acids and minerals in the bottoms stream, require the use of Monel-400, over stainless steel or Nickel-200, as the design material of the distillation tower reboiler. The original design with the distillation tower operating at atmospheric pressure resulted in a reboiler temperature of 733°F. A blower above the column condenser will reduce the tower pressure to 0.076 psia.

Decanter

In order to recover the minerals contained in our oleic acid stream from the distillation tower, we will feed this stream to a decanter along with water. The water-soluble minerals will dissolve in the water and be mixed in with the solid purge from the centrifuge to be sold as the fertilizer, vinasse, to our sister sugar and ethanol facility. The pure oleic acid will be recycled to the distillation tower to perform its original duty as a mineral sink. A purge stream will be used to remove excess oleic acid and prevent buildup in the system. Fresh oleic acid will also be fed into the distillation tower to reduce the buildup of minerals, in the event that the decanting does not completely transfer the minerals to the water. A static mixer will be placed before the decanter to allow thorough mixing of the oleic acid, minerals and water. The residence time of the decanter is estimated to be about 30 minutes to allow for complete transfer. This was calculated based on the settling time of a droplet of oleic acid in water using the following equation:

$$t = 3*\text{viscosity}/\text{sp.gr difference} \qquad \text{(Equation 4)}$$

in which t = decanter residence time in minutes, viscosity = viscosity of predominant continuous liquid phase in centipoise (water for us since water flow rate = 3255 pounds per hour and oleic acid flow rate = 930 pounds per hour), and specific gravity difference = the g/cm^3 difference in densities of the liquid phases (USBCD, 2008).

Aspen could not accurately account for the binary interactions between oleic acid and water, and calculated the two to be highly miscible. Oleic acid, as mentioned previously, is highly hydrophobic, so the two phases will remain distinct. Mr. Vrana advised us to not always trust Aspen's interactions and to model the decanter as a SEP2 to provide a more realistic separation.

Multistage Compressor vs. Blowers

From the distillation column's reflux accumulator we have a single step blower that increases the pressure from 0.071 psi to 0.15 psi of the water vapor. We cool the vapor to 41°F to allow the water condense and subsequently flash out the carbon dioxide which is compressed with a multistage compressor to 1.2 bar. This compressor requires only 28 horsepower. Our industry consultant suggested we use a multistage compressor in place of our first blower to raise our pressure from 0.071 psi to above atmospheric, and then condenser that vapor stream, again using a flash, with a blower this time, to remove the carbon dioxide. This method lead to an 1100 horsepower requirement and it raised our steam temperature to 1700°F. Though the cooler could use regular water rather than cooling water, it is a waste of energy as we need to cool the stream enough to have it enter the decanter at around 120°F.

Day Tanks

After separation from the steam in the reflux condenser, the product stream will be held into one of two 11,500-gallon, eight-hour day tanks before being shipped to our customers. The day tank's eight hour holdup time is used to test the purity and composition of our product to ensure its quality before it is sent to customers. While one tank is being tested, the other tank will continue to be filled by the product stream from the reflux condenser. When testing is done and the tank empties, the role of each of the tanks will switch, allowing for continuous operation, while maintaining the accuracy of the tests.

Material and Product Delivery: Trains vs. Trucks

For incoming and outgoing deliveries, we are going to be using trucks, as opposed to a rail system. On the materials side, we will be receiving two 5,500-gallon trucks of process water per day, one truck of oleic acid every four days, and seven trucks of CSL every four days. On the product side, we will be exporting five trucks of our product every day.

We chose trucking due to the resulting inefficiency from filling rail cars. Tanker trains, which hold 30,000 gallons (Guide to Railcars), would require us to import1/3 of a tanker a day of process water, 1/10 of a tanker of oleic acid once every four days and two tankers of corn steep liquor every four days. Though fewer trains are needed, it is a waste of money to bring in tankers that are not close to filled. We also considered slowing down the frequency of the train deliveries, but this would require more significant costs in holding tanks and other storage.

Energy Balances and Utility Requirements

Table 2: Overall Material and Energy Balances

		In	Out	Diff
Components				
	(LB/HR)			
	1:4-B-01	0.0	8687.3	-100.00%
	WATER	3421.8	3421.8	0.00%
	DEXTR-01	9132.5	449.1	1933.64%
	CARBO-01	0.0	4241.5	-100.00%
	OLEIC-01	186.0	185.9	0.04%
	LYSIN-01	41.1	41.1	0.01%
	GLYCI-01	41.1	41.1	0.01%
	ISOLE-01	41.1	41.1	0.01%
	LEUCI-01	41.1	41.1	0.01%
	METHI-01	41.1	41.1	0.01%
	L-PHE-01	41.1	41.1	0.01%
	THREO-01	41.1	41.1	0.01%
	TRYPT-01	41.1	41.1	0.01%
	TYROS-01	41.1	41.1	0.01%
	VALIN-01	41.1	41.1	0.01%
	INOSI-01	28.5	28.5	0.01%
	NIACI-01	7.3	7.3	0.00%
	POTAS-01	29.9	29.9	0.01%
	MAGNE-01	29.9	29.9	0.01%
	CALCI-01	29.9	29.9	0.01%
	SULFU-01	29.9	29.9	0.01%
	SODIU-01	29.9	29.9	0.01%
	IRON	29.8	29.8	0.01%
	ZINC	29.8	29.8	0.01%
	MANGA-01	29.8	29.8	0.01%
	COPPE-01	29.8	29.8	0.01%
	CHROM-01	29.9	29.9	0.01%
	MOLYB-01	29.9	29.9	0.01%
	COBAL-01	29.9	29.8	0.01%
	HYDRO-01	44.6	44.6	0.00%
	ETHYL-01	14202.7	9961.2	42.58%
	CELLU-01	6612.1	6612.1	0.00%
Total Balance				
	MOLE(LBMOL/HR)	456.139	552.536	-0.174463
	MASS(LB/HR)	34411.9	34411.7	5.26E-06
	ENTHALPY(BTU/HR)	-8.46E+07	-8.59E+07	1.54E-02

Table 3: Utilities Consumption and Costing

	Utility	Unit	Required Ratio	Unit	Utility Cost	Unit
1	High Pressure Steam (633 psig)	lb	3,702	lb per ton of 1,4 Butanediol	$5.489E-03	per lb
2	Low Pressure Steam (150 psig)	lb	365	lb per ton of 1,4 Butanediol	$3.504E-03	per lb
3	Refrigeration, 100°F	ton	1.98	ton per ton of 1,4 Butanediol	$6.93	per ton
4	Brine Cooling Water	1000 gal	0.95	1000 gal per ton of 1,4 Butanediol	$0.075	per 1000 gal
5	Electricity	kWh	26	kWh per ton of 1,4 Butanediol	$0.044	per kWh
6	Treatment of Waste Oleic	ton	0.093	ton per ton of 1,4 Butanediol	$60.00	per ton
7	Sterilization of Water	ton	0.47	ton per ton of 1,4 Butanediol	$60.00	per ton
	Total Weighted Average:				$70	per ton of 1,4 Butanediol

The enthalpy entering the system was calculated to be -84.6 million BTU per hour, while the enthalpy leaving the system was calculated to be -85.9 million BTU per hour. This is a difference of 1.3 million BTU per hour, which is comparable to the utilities provided to the system.

Specification Sheets, Equipment List, and Descriptions

The following pages detail the sizes and costs of the various equipment in our plant.

Compiled Costing for 1,4 BDO

Equipment

Qty	Item	Description	Eqpt BM Cost	Price	Notes
1	B-100	Seed Fermenter Blower	$119,120	$119,120	Scaled down Main Fermenter volumetric flow rate
2	B-101	Main Fermenter Blower	$216,797	$433,595	Used volumetric flow rate from ASPEN
1	B-102	Distillation Tower Blower	$4,005	$4,005	
1	B-103	Compressor	$55,302	$55,302	Assumed centrifugal, cast iron/carbon-steel, with electric motor drive
1	C-100	Centrifuge	$710,113	$710,113	Type = Continuous Reciprocating Pusher
1	D-100	Decanter	$83,544	$83,544	Assumed 50% capacity, AR=5, residence time = 30 mins
1	E-100	Molasses Pasteurizer	$46,666	$46,666	Assumed Fixed head, carbon steel/stainless steel
1	E-101	Distillation Feed HX	$46,151	$46,151	Assumed Fixed head, carbon steel/stainless steel
1	E-102	Distillation Tower Condenser	$86,744	$86,744	Assumed Fixed head, carbon steel/stainless steel
1	E-103	Distillation Tower Reboiler	$257,968	$257,968	Assumed Fixed head, carbon steel/stainless steel
1	E-104	Distillation Vapor Condenser	$63,330	$63,330	Assumed Fixed head, carbon steel/stainless steel
1	F-100	Seed Fermenter Vessel	$352,551	$352,551	Assumed 10% Extra, Stainless Steel
1	F-100b	Seed Fermenter Agitator	$3,713	$3,713	
2	F-101	Fermenter Vessel	$534,026	$1,068,052	Assumed 50% capacity, AR=3, batch time = 24 hours
2	F-101b	Fermenter Vessel Agitator	$4,594	$9,188	
28	P-10X	Generic Pump (est.)	$18,771	$525,576	Used Stainless Steel
34	P-10Xb	Generic Pump Motor (est.)	$1,181	$40,161	Based on Molasses Feed Pump; Flow rate out of range
2	P-100	Oleic Feed Pump	$8,387	$16,775	Used Stainless Steel; Flow rate out of range
8	P-101, 103	Molasses Feed Pump	$17,026	$136,211	Need 1 before and after storage; Head assumed at 20 ft.
2	P-105	Media Feed Pump	$24,278	$48,556	Used Stainless Steel
2	P-106	Water Feed Pump	$12,623	$25,245	Used Cast Iron - as corrosion-resistance not needed
1	T-100	Oleic Acid Distillation Tower	$4,234,361	$4,234,361	Used Monel-400 for heat & corrosion resistance
3	V-100	Molasses Storage tank	$270,345	$811,034	Based on Feed Pumps, Assume 20% Extra
1	V-101	Pre-Distillation Storage Tank	$41,547	$41,547	Assumed 8 hour residence time (as advised)
1	V-102	Reflux Accumulator Drum	$68,601	$68,601	Aspect Ratio = 2, Residence Time = 5 mins, 50% capacity
1	V-103	Water Vapor Flash Drum	$62,483	$62,483	
2	V-104,105	BDO Product Storage Tank	$31,115	$62,230	RT = 8 hours
1	VZ-10X	Media Storage Tank	$227,665	$227,665	Assumed 1 week residence time
1	VZ-10X	Sterile Water Storage Tank	$34,777	$34,777	Assumed 1 day residence time
1	VZ-10X	Oleic Feed Storage Tank	$22,508	$22,508	Assumed 1 week residence time
1		Packaged Boiler	$4,195	$4,195	http://ingramswaterandair.com/burnham-electronic-ignition-model-173000-p-14755.html?osCsid=c43ae024ef6a220ec6f0eb6f9bcd1c05
2		Water Sterilization Skid	$9,898	$19,796	http://www.roconsumables.com/reverseosmosis.html
		Total		$9,721,761.92	

EQUIPMENT COSTING

Equipment Category	**Fans, Blowers, Compressors**	
Subtype 1	**Blowers**	
Subtype 2	**Centrifugal (turbo) blower**	
Construction Material	**Stainless Steel**	
Blade Type	**Cast Aluminum Blades**	
Inlet Volumetric Flow, Qi, ft^3/min	**16082.1335**	Since Seed is half Main, used half of Main total Flow Rate
Inlet Pressure, psi	**14.7**	
Outlet Pressure, psi	**17.40932642**	
Specific Heat Ratio, k	**1.4**	heat capacity ratio of CO2 at 20 degrees C http://en.wikipedia.org/wiki/Heat_capacity_ratio
Equipment Base f.o.b. Cost	**$ 79,413.33**	
Material Factor	2.50	
Blade Type Factor	0.60	
Bare-Module Factor	1	
CE Index	500	
Equipment Bare-Module Cost	**$ 119,119.99**	
Notes/Base Case	See Page 565: Includes electric motor drive, Assuming Nb=0.75	

EQUIPMENT COSTING		
Equipment Category	**Fans, Blowers, Compressors**	
Subtype 1	**Blowers**	
Subtype 2	**Centrifugal (turbo) blower**	
Construction Material	**Cast Iron**	
Blade Type	**Cast Aluminum Blades**	
Inlet Volumetric Flow, Qi, ft^3/min	**16082.1335**	
Inlet Pressure, psi	**17.40452856**	
Outlet Pressure, psi	**42.40452856**	
Specific Heat Ratio, k	**1.4**	heat capacity ratio of CO2 at 20 degrees C http://en.wikipedia.org/wiki/Heat_capacity_ratio
Equipment Base f.o.b. Cost	**$ 361,329.12**	
Material Factor	1.00	
Blade Type Factor	0.60	
Bare-Module Factor	1	
CE Index	500	
Equipment Bare-Module Cost	**$ 216,797.47**	
Notes/Base Case	See Page 565: Includes electric motor drive, Assuming Nb=0.75	

EQUIPMENT COSTING		
Equipment Category	**Fans, Blowers, Compressors**	
Subtype 1	**Blowers**	
Subtype 2	**Centrifugal (turbo) blower**	
Construction Material	**Cast Iron**	
Blade Type	**Cast Aluminum Blades**	
Inlet Volumetric Flow, Qi, ft^3/min	**235153.3333**	
Inlet Pressure, psi	**0.073758**	
Outlet Pressure, psi	**0.081134**	
Specific Heat Ratio, k	**1.33**	heat capacity ratio of H2O at 20 degrees C http://en.wikipedia.org/wiki/Heat_capacity_ratio
Equipment Base f.o.b. Cost	**$ 6,675.26**	
Material Factor	1.00	
Blade Type Factor	0.60	
Bare-Module Factor	1	
CE Index	500	
Equipment Bare-Module Cost	**$ 4,005.16**	
Notes/Base Case	See Page 565: Includes electric motor drive, Assuming Nb=0.75	

EQUIPMENT COSTING		
Equipment Category	**Fans, Blowers, Compressors**	
Subtype 1	**Compressors**	
Subtype 2	**Centrifugal Compressor**	
Construction Material	**Cast Iron/Carbon-Steel**	
Drive	**Electric Motor Drive**	
Bare-Module Type	**Use Bare-Module Factor**	
Consumed Power, Hp	**25**	
Applicable Range	200 - 30,000 Hp	
Equipment Base f.o.b. Cost	**$**	**25,721.97**
Material Factor		1.00
Drive Factor		1.00
Bare-Module Factor		2.15
CE Index		500
Equipment Bare-Module Cost	**$**	**55,302.23**
Notes/Base Case	See Page 565: Includes drive	

EQUIPMENT COSTING		
Equipment Category	**Solid-Liquid Separators**	
Subtype 1	**Centrifuges**	
Subtype 2	**Continuous Reciprocating Pusher**	
Bare-Module Type	**Use Bare-Module Factor**	
Tons solids/hr, S	**16.819**	
Applicable Range	1 - 20 tons solids/hr	
Equipment Base f.o.b. Cost	**$**	**349,809.27**
Bare-Module Factor		2.03
CE Index		500
Equipment Bare-Module Cost	**$**	**710,112.81**
Notes/Base Case	See Page 585: Stainless steel	

VESSEL SIZING				
Vessel Type	**Horizontal**	**Vessel Material (Cost)**		**Stainless Steel 304**
		Material Factor		1.7
Height/Length (ft)	**11.8**			
Diameter (ft)	**2.4**	**Vessel Cost - Eq. (22.53)**	**$**	**24,996.69**
		Platforms & Ladders Cost - Eq. (22.55)	**$**	**2,394.83**
Operating Pressure (psig)	**0**			
Design Pressure (psig)	0	**Tray/Packing?**		**Neither**
Material (Stress)	**Carbon Steel (SA-285 Grade C)**			
Design Temperature (F)	**-20 to 650**			
Maximum Allowable Stress (psig)	13750			
Minimum Wall Thickness				
Inside Diameter Range	Up to 4 ft			
Minimum Thickness (in.)	0.25			
Weld Efficiency				
Wall Thickness Range	Up to 1.25 in.	**Total f.o.b. Purchase Cost**	**$**	**27,391.52**
Efficiency	0.85			
		Bare-Module Factor		3.05
Estimated Wall Thickness (in.)	0.0000			
Corrosion Allowance (in.)	0.125	**Total Bare-Module Cost**	**$**	**83,544.14**
Vessel Wall Thickness (in) - Eq. (22.60)	**0.375**			
Enter Round Thickness (in.)				
Vessel Weight (lbs) - Eq. (22.59)	**1605**			

Flow in (from ASPEN)	51.523	ft^3/hr	0.858717	ft^3/min
water viscosity (predominant continuous liquid phase)	1	cp		
density of oleic acid	0.895	g/cm^3		
density of water	1	g/cm^3		
specific gravity difference in densities	0.105	g/cm^3		
decanter residence time calculated using following equation: 3*viscosity/sp.gr difference	28.57143	min	0.47619	hrs
rounded residence time	30	mins		
Volume (decanter)	25.7615	ft^3	192.7086	gallons
Container liquid percent full at operation	50%			
Volume (decanter) if liquid volume at container % full	51.523	ft^3	385.4173	gallons

Assuming Aspect Ratio = L/D = 5

Vdecanter = pi*r^2*L = pi*r^2*10*r

r (radius)	**1.17928**	**ft**
D (diameter)	**2.358561**	**ft**
L (length)	**11.7928**	**ft**

E-100-Molasses Pasteurizer HX-Sizing

$Q_{aspen} = m_{molasses}C_{p,molasses}\Delta T$ = 1,201,620 BTU/hr

Hot - Tube (A) – steam

Cold - Shell (B) – molasses

Cold (B) in T = 86°F

m = 15,746 lb/hr (or 7,142 kg/hr)

Cold (B) out T = 266°F

ΔT_B = 180°F

$C_{P,molasses}$ = 0.424 BTU/(lb*R)

$C_{p,steam}$ = 1 BTU/(lb*R)

Driving Force = 45°F

Temperature to match = 311°F

Steam choice = 150 psig

Sat temp (Hot in T) = 366°F

Latent Heat of Vap = 857.5 BTU/lb

Steam Flow Rate = 1,401.3 lb/hr

Hot out T = 366.0°F

LMTD = ((366 – 86) – (366 – 266))/ln((366 – 86)/(366 – 266)) = 174.8°F

U = 45 BTU/(sqft*°F*hr) [40 – 50 for tar, as on page 488 of Seider textbook]

Therefore, A = 152.7 sqft

EQUIPMENT COSTING

Equipment Category	**Heat Exchangers**
Subtype 1	**Shell & Tube**
Subtype 2	**Fixed Head**
Shell/Tube Material	**Carbon Stell/Stainless Steel**
Tube Length	**20 ft**
Pressure	**Select to use Pressure Factor**
Bare-Module Type	**Use Bare-Module Factor**
Surface Area, A, ft^2	**153**
Applicable Range	150 - 12,000 ft^2
Shell-Side Pressure, psig	**37.8**
Applicable Range	0 - 2,000 psig
Equipment Base f.o.b. Cost	**$ 9,376.56**
Material Factor	2.81
Tube Length Factor	1.00
Pressure Factor	1.00
Bare-Module Factor	3.17
CE Index	500
Equipment Bare-Module Cost	**$ 46,665.65**
Notes/Base Case	See Page 475, 570: 1 in. OD, 16 BWG carbon-steel tubes, 20 ft long, square or triangular pitch

E-101-Distillation Feed HX-Sizing

Q_{aspen} = 272,789 BTU/hr

Hot - Tube (A) – oleic + minerals

Cold - Shell (B) – distillation feed

Cold (B) in T = 87.5°F

m_{cold} = 14,284 lb/hr (or 6,479 kg/hr)

Cold (B) out T = 119.2°F

ΔT_B = 31.7°F

Hot (A) in T = 390.2°F

Hot (A) out T = 120°F

ΔT_A = 270.2°F

$C_{P,oleic}$ = 0.5129 BTU/(lb*R)

$C_{p,distillation\ feedr}$ = 0.6128415 BTU/(lb*R)

Q = U*A*LMTD = 272,789.4 BTU/hr

LMTD = ((390.2 – 87.5) – (120 – 119.2))/ln((390.2 – 87.5)/(120 – 119.2)) = 50.86°F

U = 40 BTU/(sqft*°F*hr) [20 – 60 for organic-organic, as on page 488 of Seider textbook]

Therefore, A = 134.09 sqft

EQUIPMENT COSTING

Equipment Category	**Heat Exchangers**
Subtype 1	**Shell & Tube**
Subtype 2	**Fixed Head**
Shell/Tube Material	**Carbon Stell/Stainless Steel**
Tube Length	**20 ft**
Pressure	**Select to use Pressure Factor**
Bare-Module Type	**Use Bare-Module Factor**
Surface Area, A, ft^2	**134**
Applicable Range	150 - 12,000 ft^2
Shell-Side Pressure, psig	**15.3**
Applicable Range	0 - 2,000 psig
Equipment Base f.o.b. Cost	**$ 9,306.91**
Material Factor	2.79
Tube Length Factor	1.00
Pressure Factor	1.00
Bare-Module Factor	3.17
CE Index	500
Equipment Bare-Module Cost	**$ 46,150.92**
Notes/Base Case	See Page 475, 570: 1 in. OD, 16 BWG carbon-steel tubes, 20 ft long, square or triangular pitch

E-102-Distillation Tower Condenser-Sizing

$Q_{condenser}$ = 8,006,613.7 BTU/hr

Hot - Tube (A) – bdo + water,

Cold - Shell (B) – cooling water

Hot (A) in T = 227.13°F

m = 12,010.678 lb/hr

Hot (A) out T = 71.695°F

ΔT_A = 270.2°F

$C_{P,bdo+water}$ = .5845897 BTU/(lb*R)

$C_{p,cooling\ water}$ = 0.7643 BTU/(lb*R)

Driving Force = 10°F

Temperature to match = Hot out T – Driving Force = 61.7°F

Cold Flow Rate = 27,626.0 lb/hr

Cold out T = Cold in + $m_{hot}*C_{p,bdo+water}*\Delta T_A/(m_{cold}*C_{p,cooling\ water})$ = 61.7°F

Q = U*A*LMTD = 8,006,613.7 BTU/hr

LMTD = ((227.13 – 10) – (71.69 – 61.7))/ln((227.13 – 10)/(71.69 – 61.7)) = 67.3°F

U = 100 BTU/(sqft*°F*hr) [50 – 150 for organic-water, as on page 488 of Seider textbook]

Therefore, A = 1189.8 sqft

EQUIPMENT COSTING

Equipment Category	**Heat Exchangers**	
Subtype 1	**Shell & Tube**	
Subtype 2	**Fixed Head**	
Shell/Tube Material	**Carbon Stell/Stainless Steel**	
Tube Length	**20 ft**	
Pressure	**Select to use Pressure Factor**	
Bare-Module Type	**Use Bare-Module Factor**	
Surface Area, A, ft^2	**1190**	
Applicable Range	150 - 12,000 ft^2	
Shell-Side Pressure, psig	**-14.59**	
Applicable Range	0 - 2,000 psig	
Equipment Base f.o.b. Cost	**$**	**16,367.45**
Material Factor		3.13
Tube Length Factor		1.00
Pressure Factor		1.00
Bare-Module Factor		3.17
CE Index		500
Equipment Bare-Module Cost	**$**	**86,744.45**
Notes/Base Case	See Page 475, 570: 1 in. OD, 16 BWG carbon-steel tubes, 20 ft long, square or triangular pitch	

E-103-Distillation Tower Reboiler-Sizing

Q_{aspen} = 11,492,172.2 BTU/hr

Hot - Tube (A) – steam

Cold - Shell (B) – Oleic + minerals

Cold (B) in T = 321.17°F = 160.65°C

m = 2,273.486 (lb/hr = 1,031.25 kg/hr)

Cold (B) out T = 449.25°F = 231.81°C

ΔT_B = 71.16°C or 128.09°F

$C_{P,oleic\&minerals}$ = .5128926 Btu/(lb*R)

$C_{p,water}$ = 1 Btu/(lb*R)

Reboiler Heat Duty = 11,492,172.2 BTU/hr

Driving force = 45°F

Temperature to match = Cold out T + Driving Force = 494.3°F

Steam Choice: 633 psig

Hot (A) in T = Sat temp = 494.25°F

Latent Heat of Vap = 721.9 BTU/lb

Steam Flow Rate = Heat Duty/Latent Heat of Vap = 15,919.3 lb/hr

Hot out T = 494.3°F since steam will condense but remain at Hot in T

Q = U*A*LMTD = 11,492,172.2 BTU/hr

LMTD = ((494.25 – 321.2)-(494.3 – 449.3))/ln((494.25 – 321.2)/(494.3 – 449.3)) = 95.1°F

U = 20.0 BTU/(sqft*°F*hr) [15 – 25 for No. 6 fuel oil, as on page 488 of Seider textbook]

Therefore, A = 6,043.5 sqft

EQUIPMENT COSTING		
Equipment Category	**Heat Exchangers**	
Subtype 1	**Shell & Tube**	
Subtype 2	**Fixed Head**	
Shell/Tube Material	**Carbon Stell/Stainless Steel**	
Tube Length	**20 ft**	
Pressure	**Select to use Pressure Factor**	
Bare-Module Type	**Use Bare-Module Factor**	
Surface Area, A, ft^2	**6,043**	
Applicable Range	150 - 12,000 ft^2	
Shell-Side Pressure, psig	**-14.03**	
Applicable Range	0 - 2,000 psig	
Equipment Base f.o.b. Cost	**$**	**45,865.94**
Material Factor		3.45
Tube Length Factor		1.00
Pressure Factor		1.00
Bare-Module Factor		3.17
CE Index		500
Equipment Bare-Module Cost	**$**	**257,967.64**
Notes/Base Case	See Page 475, 570: 1 in. OD, 16 BWG carbon-steel tubes, 20 ft long, square or triangular pitch	

E-104-Distillation Vapor Condenser-Sizing

Q_{aspen} = 3,502,311.4 BTU/hr

Cold - Shell (A) – Brine

Hot - Tube (B) – Water

Hot (B) in T = 71.7°F = 39.83°C

m = 3,384.06 lb/hr (m = 1,535.01 kg/hr)

Hot (B) out T = 41°F = 22.78°C

ΔT_B = 17.05°C or 30.7°F = about 31°F

$C_{p,water}$ = 1 Btu/(lb*R)

$C_{P,brine}$ = .7643 Btu/(lb*R)

Cold (A) in T (Sat temp) = 10°F

Driving Force = 10°F

Temperature to match = ΔT_B = 31°F

m_{cold} = Cold Mass Flow = 6473.08 lb/hr

Cold (A) out = Cold (A) in + $m_{hot}*C_{p,water}*\Delta T_B/(m_{cold}*C_{p,brine})$ = 31°F

Q = U*A*LMTD = 3,502,311.4 BTU/hr

LMTD = ((71.7 – 10)-(41 – 31))/ln((71.7 – 10)/(41 – 31))= 28.4°F

U = 325 BTU/(sqft*°F*hr) [250 – 4000 for water/water, as on page 489 of Seider textbook]

Therefore, A = 379.4 sqft

EQUIPMENT COSTING		
Equipment Category	**Heat Exchangers**	
Subtype 1	**Shell & Tube**	
Subtype 2	**Fixed Head**	
Shell/Tube Material	**Carbon Stell/Stainless Steel**	
Tube Length	**8 ft**	
Pressure	**Select to use Pressure Factor**	
Bare-Module Type	**Use Bare-Module Factor**	
Surface Area, A, ft^2	**380**	
Applicable Range	150 - 12,000 ft^2	
Shell-Side Pressure, psig	**-14.55**	
Applicable Range	0 - 2,000 psig	
Equipment Base f.o.b. Cost	**$**	**10,835.97**
Material Factor		2.94
Tube Length Factor		1.25
Pressure Factor		1.00
Bare-Module Factor		3.17
CE Index		500
Equipment Bare-Module Cost	**$**	**63,329.74**
Notes/Base Case	See Page 475, 570: 1 in. OD, 16 BWG carbon-steel tubes, 20 ft long, square or triangular pitch	

F-100-Seed Fermenter-Sizing

Continuous Bench-Scale cell concentration = 4 g/L

Continuous Bench-Scale Volume = 10 L

Continuous Bench-Scale amount of cells = 4 g/L * 10 L = 40 g

Calculated Continuous Scale up Factor = 3700

Scaled up Bench-Scale cell amount = 40 g * 3700 = 148,000 g cells

Batch Bench-Scale cell concentration = 8 g/L

Batch Bench-Scale Volume = 10 L

Batch Bench-Scale amount of cells = 8 g/L * 10 L = 80 g cells

Batch scale up factor = 148,000 g cells / 80 g cells = 1850

Volume Batch Scale up factor = 1850

Batch Bench-Scale Volume = 10 L

Scaled up Volume = Batch Bench-Scale Volume * Batch Scale up factor = 18,500 L

VESSEL SIZING

Vessel Type	**Vertical**	**Vessel Material (Cost)**	**Stainless Steel 304**
		Material Factor	1.7
Height/Length (ft)	**19.53**		
Diameter (ft)	**6.51**	**Vessel Cost - Eq. (22.54)**	**$ 72,930.69**
		Platforms & Ladders Cost - Eq. (22.56)	**$ 11,817.16**
Operating Pressure (psig)	**2.7045**		
Design Pressure (psig)	10	**Tray/Packing?**	**Neither**
Material (Stress)	**Carbon Steel (SA-285 Grade C)**		
Design Temperature (F)	**-20 to 650**		
Maximum Allowable Stress (psig)	13750		
Minimum Wall Thickness			
Inside Diameter Range	6 - 8 ft		
Minimum Thickness (in.)	0.375		
Weld Efficiency			
Wall Thickness Range	Up to 1.25 in.	**Total f.o.b. Purchase Cost**	**$ 84,747.85**
Efficiency	0.85		
		Bare-Module Factor	4.16
Estimated Wall Thickness (in.)	0.0404		
Corrosion Allowance (in.)	0.125	**Total Bare-Module Cost**	**$ 352,551.05**
Vessel Wall Thickness (in) - Eq. (22.60/22.62)	**0.500**		
Enter Round Thickness (in.)			
Vessel Weight (lbs) - Eq. (22.59)	**10396**		

EQUIPMENT COSTING	
Equipment Category	**Agitators**
Subtype 1	**Propeller, closed vessel**
Subtype 2	
Motor Hp	**2**
Applicable Range	1 - 8 Hp
Equipment Base f.o.b. Cost	**$ 3,712.69**
Bare-Module Factor	1
CE Index	500
Equipment Bare-Module Cost	**$ 3,712.69**
Notes/Base Case	See Page 580: Includes motor shaft, direct coupling to motor, pressures up to 150 psig

F-101-Fermenter-Sizing

Sugar in → 9,132.429 lb/hr

Sugar out → 457.368 lb/hr

$C_{out} = C_{in}$ * exp(-t/tau) where t = batch time & tau = residence time

t = 24 hours

Therefore tau = 8.0158 hours

tau * $U_{total} = V_{reactor}$ where U_{total} = total volumetric flow rate to reactor (258.433 ft^3/hr) & $V_{reactor}$ = volume of reactor

$V_{reactor}$ = 2071.55 ft^3 = 15,496.3 gallons

Assuming **2 reactors** operate at **70% capacity**, $V_{reactor}$ needs to be 2071.55/2/0.7 = 1479.69 ft^3

Assuming Aspect Ratio = H/D = 3, $V_{reactor}$ = pi*r^2*h = pi*r^2*6r

Therefore, reactor dimensions are: r = 4.2818 ft; D = 8.5635 ft; H = 25.6905 ft

VESSEL SIZING				
Vessel Type	**Vertical**	**Vessel Material (Cost)**		**Stainless Steel 304**
		Material Factor		1.7
Height/Length (ft)	**25.7**			
Diameter (ft)	**8.6**	**Vessel Cost - Eq. (22.54)**	**$**	**110,742.92**
		Platforms & Ladders Cost - Eq. (22.56)	**$**	**17,628.72**
Operating Pressure (psig)	**2.709**			
Design Pressure (psig)	10	**Tray/Packing?**		**Neither**
Material (Stress)	**Carbon Steel (SA-285 Grade C)**			
Design Temperature (F)	**-20 to 650**			
Maximum Allowable Stress (psig)	13750			
Minimum Wall Thickness				
Inside Diameter Range	8 - 10 ft			
Minimum Thickness (in.)	0.4375			
Weld Efficiency				
Wall Thickness Range	Up to 1.25 in.	**Total f.o.b. Purchase Cost**	**$**	**128,371.64**
Efficiency	0.85			
		Bare-Module Factor		4.16
Estimated Wall Thickness (in.)	0.0528			
Corrosion Allowance (in.)	0.125	**Total Bare-Module Cost**	**$**	**534,026.02**
Vessel Wall Thickness (in) - Eq. (22.60/22.62)	**0.563**			
Enter Round Thickness (in.)				
Vessel Weight (lbs) - Eq. (22.59)	**20328**			

EQUIPMENT COSTING	
Equipment Category	**Agitators**
Subtype 1	**Propeller, closed vessel**
Subtype 2	
Motor Hp	**7**
Applicable Range	1 - 8 Hp
Equipment Base f.o.b. Cost	**$ 4,593.89**
Bare-Module Factor	1
CE Index	500
Equipment Bare-Module Cost	**$ 4,593.89**
Notes/Base Case	See Page 580: Includes motor shaft, direct coupling to motor, pressures up to 150 psig

EQUIPMENT COSTING	
Equipment Category	**Pumps**
Subtype 1	**External Gear Pumps**
Subtype 2	
Construction Material	**Cast Iron**
Bare-Module Type	**Use Bare-Module Factor**
Flow Rate, gpm	**8.36**
Applicable Range	10 - 900 gpm
Equipment Base f.o.b. Cost	**$ 3,825.07**
Material Factor	1.00
Bare-Module Factor	3.3
CE Index	500
Equipment Bare-Module Cost	**$ 12,622.74**
Notes/Base Case	See Page 559: Includes base plate and driver coupling, but not electric motor

water flow rate -->	1,553,400.00	gram/hr
water density -->	1	gram/ml
volume conversion -->	0.000264172	gallons/ml

(CSL = 1.3 gm/ml + 0.3 for gm/ml MgSO4, K3PO4, NH4Cl estimate)

Assuming Water flow operation for 2 off-season months in addition to 9 in-season months

EQUIPMENT COSTING	
Equipment Category	**Pumps**
Subtype 1	**External Gear Pumps**
Subtype 2	
Construction Material	**Stainless Steel**
Bare-Module Type	**Use Bare-Module Factor**
Flow Rate, gpm	**9.3**
Applicable Range	10 - 900 gpm
Equipment Base f.o.b. Cost	**$ 3,959.61**
Material Factor	2.00
Bare-Module Factor	3.3
CE Index	500
Equipment Bare-Module Cost	**$ 17,026.34**
Notes/Base Case	See Page 559: Includes base plate and driver coupling, but not electric motor

molasses flow rate per tank -->	2,557,037.04	gram/hr
molasses density -->	1.48	gram/ml
volume conversion -->	0.000264172	gallons/ml

Assuming Molasses flow at 7.67 mio g/hr, operation for 2 off-season months in addition to 9 in-season months

EQUIPMENT COSTING		
Equipment Category	**Pumps**	
Subtype 1	**Electric Motors**	
Subtype 2		
Enclosure Type	**Totally Enclosed, fan-cooled, 1 to 250 Hp, 1800 RPM**	
Bare-Module Type	**Use Bare-Module Factor**	
Flow Rate, Q, gpm	**27.9**	
Applicable Range	50 - 5,000 gpm	
Head, H, ft	**20**	
Density, lb/gal	**12.351129**	
Equipment Base f.o.b. Cost	**$**	**328.12**
Enclosure Type Factor		1.30
Bare-Module Factor		3.3
CE Index		500
Equipment Bare-Module Cost	**$**	**1,181.22**
Notes/Base Case	See Page 262, 559:	

EQUIPMENT COSTING	
Equipment Category	**Pumps**
Subtype 1	**External Gear Pumps**
Subtype 2	
Construction Material	**Stainless Steel**
Bare-Module Type	**Use Bare-Module Factor**
Flow Rate, gpm	**25.1**
Applicable Range	10 - 900 gpm
Equipment Base f.o.b. Cost	**$ 5,646.10**
Material Factor	2.00
Bare-Module Factor	3.3
CE Index	500
Equipment Bare-Module Cost	**$ 24,278.21**
Notes/Base Case	See Page 559: Includes base plate and driver coupling, but not electric motor

media flow rate -->	7,463,987.58	gram/hr
media density -->	1.6	gram/ml
volume conversion -->	0.000264172	gallons/ml

(CSL = 1.3 gm/ml + 0.3 for gm/ml MgSO4, K3PO4, NH4Cl estimate)

Assuming Media flow operation for 2 off-season months in addition to 9 in-season months

EQUIPMENT COSTING	
Equipment Category	**Pumps**
Subtype 1	**External Gear Pumps**
Subtype 2	
Construction Material	**Cast Iron**
Bare-Module Type	**Use Bare-Module Factor**
Flow Rate, gpm	**8.36**
Applicable Range	10 - 900 gpm
Equipment Base f.o.b. Cost	**$ 3,825.07**
Material Factor	1.00
Bare-Module Factor	3.3
CE Index	500
Equipment Bare-Module Cost	**$ 12,622.74**
Notes/Base Case	See Page 559: Includes base plate and driver coupling, but not electric motor

water flow rate -->	1,553,400.00	gram/hr
water density -->	1	gram/ml
volume conversion -->	0.000264172	gallons/ml

(CSL = 1.3 gm/ml + 0.3 for gm/ml MgSO4, K3PO4, NH4Cl estimate)

Assuming Water flow operation for 2 off-season months in addition to 9 in-season months

EQUIPMENT COSTING	
Equipment Category	**Pumps**
Subtype 1	**External Gear Pumps**
Subtype 2	
Construction Material	**Stainless Steel**
Bare-Module Type	**Use Bare-Module Factor**
Flow Rate, gpm	**12.42**
Applicable Range	10 - 900 gpm
Equipment Base f.o.b. Cost	**$ 4,365.25**
Material Factor	2.00
Bare-Module Factor	3.3
CE Index	500
Equipment Bare-Module Cost	**$ 18,770.56**
Notes/Base Case	See Page 559: Includes base plate and driver coupling, but not electric motor

average flow rate -->	3,000,000.00	gram/hr
average density -->	1.3	gram/ml
volume conversion -->	0.000264172	gallons/ml

Assuming Water flow operation for 2 off-season months in addition to 9 in-season months

VESSEL SIZING

Vessel Type	**Tower**	**Vessel Material (Cost)**		**Monel-400**
		Material Factor		3.6
Height/Length (ft)	**27**			
Diameter (ft)	**15.45**	**Vessel Cost - Eq. (22.57)**	$	**739,194.47**
		Platforms & Ladders Cost - Eq. (22.58)	$	**23,911.11**
Operating Pressure (psig)	**-14.626**			
Design Pressure (psig)	-15	**Tray/Packing?**		**Tray**
	Low-Alloy Steel (SA-387B)	**Tray Type**		**Sieve**
	-20 to 650	Tray Type Factor		1
	15000			
		Tray Material		**Monel**
Material (Elasticity)	**Low-Alloy Steel - 650 F**	Material Factor		4.0364
Modulus of Elasticty (psi)	27000000			
		Number of Trays		**5**
Minimum Wall Thickness		Tray Number Factor		9.2
Inside Diameter Range	10 - 12 ft			
Minimum Thickness (in.)	0.5			
		Tray Cost - Eq. (22.67)	$	**254,769.58**
Weld Efficiency				
Wall Thickness Range		**Total f.o.b. Purchase Cost**	$	**1,017,875.16**
Efficiency				
		Bare-Module Factor		4.16
Estimated Wall Thickness (in.)	0.8497			
Corrosion Allowance (in.)	0.125	**Total Bare-Module Cost**	$	**4,234,360.65**
Vessel Wall Thickness (in) - Eq. (22.63/22.64)	**0.975**			
Enter Round Thickness (in.)				
Vessel Weight (lbs) - Eq. (22.59)	**76436**			

A	B	D	C
2	**Column Sizing Data**		
3	Out of Top Condenser (lb/hr)	12019	12019.157
4	Out of Bottom Reboiler (lb/hr)	2265	2264.608
5	Dist. Reflux Flow - L (lb/hr)	6906	=8632.684*D20
6	Top Vapor Flow - V (lb/hr)	3386	=3386.473
7	L_density (lb/cuft)	55.6	55.63126
8	V_density (lb/cuft)	0.00024	=2.4*10^(-4)
9	L_surf tension (dyne/cm)	130.9	130.8542
10	**Column Sizing Factors**		
11	F{ST} (surf. tension)	1.46	=(D9/20)^(0.2)
12	F{LG}	0.0042	=D5/D6*(D8/D7)^(1/2)
13	C{SB}	0.39	0.39
14	F{F} (no fouling)	1	1
15	F{HA} (relative area of sieve tray)	0.1	0.1
16	**Column Sizing Parameters**		
17	U{flooding} (ft/s)	27.34	=D13*D11*D14*D15*((D7-D8)/D8)^(1/2)
18	U (ft/s)	23.24	=0.85*D17
19	Volum. Flow Rate of Top Vapor (cuft/s)	3920	=(D6/D8)/3600
20	Reflux Ratio	0.8	0.8
21	Column Diameter (ft)	15.45	=((4*D19)/(0.9*PI()*D18))^(0.5)
22	Number of Stages	7	7
23	Spacing between Trays (ft)	2	2
24	Height of Column (ft)	27	=(D22-2)*D23+6+4+1+6

EQUIPMENT COSTING		
Equipment Category	**Storage Tanks**	
Subtype 1	**Cone Roof**	
Subtype 2		
Volume, V, gal	**793152**	Difference in rate of pumps, for 9 months
Applicable Range	10,000 - 1,000,000 gal	Additional leeway of 20%
		Require 3 such tanks to stay under 1 mio gal
Equipment Base f.o.b. Cost	**$ 270,344.57**	
Bare-Module Factor	1	
CE Index	500	
Equipment Bare-Module Cost	**$ 270,344.57**	
Notes/Base Case	See Page 588: Carbon steel, pressure to 3 psig	

EQUIPMENT COSTING		
Equipment Category	**Storage Tanks**	
Subtype 1	**Cone Roof**	
Subtype 2		
Volume, V, gal	**20,160.30**	
Applicable Range	10,000 - 1,000,000 gal	
Equipment Base f.o.b. Cost	**$**	**41,546.97**
Bare-Module Factor		1
CE Index		500
Equipment Bare-Module Cost	**$**	**41,546.97**
Notes/Base Case	See Page 588: Carbon steel, pressure to 3 psig	

volumetric flow -->	235.816	cuft/hr
volumetric flow -->	1764.026304	gal/hr
residence time -->	8	hr
70% capacity -->	20160.30062	

VESSEL SIZING

Vessel Type	**Vertical**	**Vessel Material (Cost)**		**Stainless Steel 304**
		Material Factor		1.7
Height/Length (ft)	**4**			
Diameter (ft)	**2**	**Vessel Cost - Eq. (22.54)**	**$**	**14,881.09**
		Platforms & Ladders Cost - Eq.	**$**	**1,609.42**
Operating Pressure (psig)	**-14.58857**			
Design Pressure (psig)	-15	**Tray/Packing?**		**Neither**
Material (Elasticity)	**Carbon Steel - 200 F**			
Modulus of Elasticty (psi)	29500000			
Minimum Wall Thickness				
Inside Diameter Range	Up to 4 ft			
Minimum Thickness (in.)	0.25			
Weld Efficiency				
Wall Thickness Range		**Total f.o.b. Purchase Cost**	**$**	**16,490.52**
Efficiency				
		Bare-Module Factor		4.16
Estimated Wall Thickness (in.)	-0.0653			
Corrosion Allowance (in.)	0.125	**Total Bare-Module Cost**	**$**	**68,600.55**
Vessel Wall Thickness (in) - Eq. (22.63/22.64)	**0.375**			
Enter Round Thickness (in.)				
Vessel Weight (lbs) - Eq. (22.59)	**547**			

Aspect Ratio (H/D)	2	
Residence Time	5	mins
50% capacity		
Reflux Rate	9608.54269	lb/hr
Reflux Density	63.771	lb/cuft
Reflux Vol	12.55605041	cuft
Diameter	1.999452346	ft

V-103-Flash Drum-Sizing

LBS/HOUR=	3384.055	**INPUT ONLY ONE OF THESE**				
KG/HOUR=						
Top Flow Out=**INPUT THIS VALUE**	127.248					
Bottom Flow Out	3256.807					
VAPOR FRACTION	0.037602	VAPDENSITY=	0.00058965	1	1 FOR LBS/FT3	OR 2 FOR KG/CUM
LIQUID FRACTION=	0.962398	LIQDENSITY=	63.2627			
		VAPDENSITY=	0.00058965	LBS/FT3		
L/D (Aspect Ratio) =	3	LIQDENSITY=	63.2627	LBS/FT3		
HOLD-UP TIME,MIN.=	5					
FRACTION OF DRUM FULL FOR HORIZ	0.5					
KFACTOR=	1		**1=DEFAULT=0.27**	0.27		
			2=USER INPUT			
				KFACTOR	FT/SEC	METERS/SEC
VELOCITYALLOWED,FT.SEC=	327.5467			0.27	88.43761	26.95578228
VFLOW RATE ,CUF/SEC	59.94476					
LFLOW RATE ,CUF/SEC	0.0143					
FLOW, LBS/HOUR=	3384.055	LBS/HOUR TOTAL				
AREA REQ'D FOR VAPOR FT2	0.67782	ACTUAL=	0.929338748	FT2ACTUAL	GREATER THAN C18?OK! IF NOT THEN THIS AREA MUST BE ADDED TO THE LIQUID AREA REQ'D	
VOLUME OF LIQUID HELD,FT3 FOR GIVEN HOLD UP TIME	4.29		0.121481113	meter3		
HEIGHT OF LIQUID IF VERTICAL,FT FOR GIVEN HOLDUP TIME&C18 AREA	6.3292		1.929140142	meters		
DIAMETER FOR DRUM AT GIVEN HOLD UP, FEET FOR GIVEN %FULL	1.538747		0.469010056	meters		
LENGTH OF LIQUID IF HORIZONTAL,FT FOR GIVEN HOLD UP TIME, %FULL	4.616241		1.407030169	meters		
AREA REQ'D FOR LIQUID FT2 AT C9 FULL DRUM, HORIZONTAL	0.929339		0.28326245	meter2		
Height of Drum (ft)	12.6584		3.858280285	meters		

VESSEL SIZING

Vessel Type	**Vertical**	**Vessel Material (Cost)**		**Carbon Steel**
		Material Factor		1
Height/Length (ft)	**12.66**			
Diameter (ft)	**1.54**	**Vessel Cost - Eq. (22.54)**	**$**	**12,024.91**
		Platforms & Ladders Cost - Eq. (22.56)	**$**	**2,995.04**
Operating Pressure (psig)	**-14.55**			
Design Pressure (psig)	-15	**Tray/Packing?**		**Neither**
Material (Elasticity)	**Carbon Steel - -20 F**			
Modulus of Elasticty (psi)	30200000			
Minimum Wall Thickness				
Inside Diameter Range	Up to 4 ft			
Minimum Thickness (in.)	0.25			
Weld Efficiency				
Wall Thickness Range		**Total f.o.b. Purchase Cost**	**$**	**15,019.96**
Efficiency				
		Bare-Module Factor		4.16
Estimated Wall Thickness (in.)	-0.0224			
Corrosion Allowance (in.)	0.125	**Total Bare-Module Cost**	**$**	**62,483.03**
Vessel Wall Thickness (in) - Eq. (22.63/22.64)	**0.375**			
Enter Round Thickness (in.)				
Vessel Weight (lbs) - Eq. (22.59)	**1050**			

V-104-BDOProductStorageTank

EQUIPMENT COSTING		
Equipment Category	**Storage Tanks**	
Subtype 1	**Cone Roof**	
Subtype 2		
Volume, V, gal	**11,436.13**	
Applicable Range	10,000 - 1,000,000 gal	
Equipment Base f.o.b. Cost	**$**	**31,114.89**
Bare-Module Factor		1
CE Index		500
Equipment Bare-Module Cost	**$**	**31,114.89**
Notes/Base Case	See Page 588: Carbon steel, pressure to 3 psig	

volumetric flow -->	133.769	cuft/hr
volumetric flow -->	1000.66168	gal/hr
residence time -->	8	hr
70% capacity -->	11436.13348	

EQUIPMENT COSTING		
Equipment Category	**Storage Tanks**	
Subtype 1	**Cone Roof**	
Subtype 2		
Volume, V, gal	**566,292.12**	
Applicable Range	10,000 - 1,000,000 gal	
Equipment Base f.o.b. Cost	**$**	**227,665.14**
Bare-Module Factor		1
CE Index		500
Equipment Bare-Module Cost	**$**	**227,665.14**
Notes/Base Case	See Page 588: Carbon steel, pressure to 3 psig	

volumetric flow -->	315.426	cuft/hr
volumetric flow -->	2359.550502	gal/hr
residence time -->	168	hr
70% capacity -->	566292.1204	

EQUIPMENT COSTING

Equipment Category	**Storage Tanks**
Subtype 1	**Cone Roof**
Subtype 2	
Volume, V, gal	**6,061.02**
Applicable Range	10,000 - 1,000,000 gal
Equipment Base f.o.b. Cost	**$ 22,508.37**
Bare-Module Factor	1
CE Index	500
Equipment Bare-Module Cost	**$ 22,508.37**
Notes/Base Case	See Page 588: Carbon steel, pressure to 3 psig

volumetric flow -->	3.376	cuft/hr
volumetric flow -->	25.25423552	gal/hr
residence time -->	168	hr
70% capacity -->	6061.016525	

EQUIPMENT COSTING	
Equipment Category	**Storage Tanks**
Subtype 1	**Cone Roof**
Subtype 2	
Volume, V, gal	**14,224.36**
Applicable Range	10,000 - 1,000,000 gal
Equipment Base f.o.b. Cost	**$ 34,777.03**
Bare-Module Factor	1
CE Index	500
Equipment Bare-Module Cost	**$ 34,777.03**
Notes/Base Case	See Page 588: Carbon steel, pressure to 3 psig

volumetric flow -->	55.461	cuft/hr
volumetric flow -->	414.8771197	gal/hr
residence time -->	24	hr
70% capacity -->	14224.35839	

Fixed-Capital Investment Summary

Table 4Table 4. Equipment Cost Summary

Equipment Description	Type	Bare Module Cost
Name		
1x B-100 Seed Fermenter Blower	Process Machinery	$ 119,000
2x B-101 Main Fermenter Blower	Process Machinery	$ 434,000
1x B-102 Distillation Tower Blower	Process Machinery	$ 4,000
1x B-103 Compressor	Process Machinery	$ 55,000
1x C-100 Centrifuge	Process Machinery	$ 710,000
1x D-100 Decanter	Fabricated Equipment	$ 84,000
1x E-100 Molasses Pasteurizer	Fabricated Equipment	$ 47,000
1x E-101 Distillation Feed HX	Fabricated Equipment	$ 46,000
1x E-102 Distillation Tower Condenser	Fabricated Equipment	$ 87,000
1x E-103 Distillation Tower Reboiler	Fabricated Equipment	$ 258,000
1x E-104 Distillation Vapor Condenser	Fabricated Equipment	$ 63,000
1x F-100 Seed Fermenter Vessel	Fabricated Equipment	$ 353,000
1x F-100b Seed Fermenter Agitator	Process Machinery	$ 4,000
2x F-101 Fermenter Vessel	Fabricated Equipment	$ 1,068,000
2x F-101b Fermenter Vessel Agitator	Process Machinery	$ 9,000
28x P-10X Generic Pump (est.)	Process Machinery	$ 526,000
34x P-10Xb Generic Pump Motor (est.)	Process Machinery	$ 40,000
8x P-101,103 Molasses Feed Pump	Process Machinery	$ 136,000
2x P-100 Oleic Feed Pump	Process Machinery	$ 17,000
2x P-105 Media Feed Pump	Process Machinery	$ 49,000
2x P-106 Water Feed Pump	Process Machinery	$ 25,000
1x T-100 Oleic Acid Distillation Tower	Fabricated Equipment	$ 4,234,000
3x V-100 Molasses Storage tank	Fabricated Equipment	$ 811,000
1x V-101 Pre-Distillation Storage Tank	Fabricated Equipment	$ 42,000
1x V-102 Reflux Accumulator Drum	Fabricated Equipment	$ 69,000
1x V-103 Water Vapor Flash Drum	Fabricated Equipment	$ 62,000
2x V-104,105 BDO Product Storage Tank	Fabricated Equipment	$ 62,000
1x VZ-10X Media Storage Tank	Fabricated Equipment	$ 228,000
1x VZ-10X Sterile Water Storage Tank	Fabricated Equipment	$ 35,000
1x VZ-10X Oleic Feed Storage Tank	Fabricated Equipment	$ 23,000
1x Packaged Boiler	Fabricated Equipment	$ 4,000
2x Water Sterilization Skid	Process Machinery	$ 20,000
Total		***$9,724,000***
Adjusted to CE = 560.4 (Dec. 2010)		***$10,899,000***

summarizes the fixed capital investments required for this project. Cost estimates from Seider, et al. were used to calculate these values. The total equipment cost is estimated to be $9.7 million. The cost correlations used the CE Index from 2006, where CE = 500. When updated to account for the current CE Index from December 2010, where CE = 560.4 (Chemical Engineering, 2010), the total equipment cost becomes $10.9 million.

The most expensive equipment is our vacuum distillation tower at $4.23 million. The vacuum distillation tower is an integral part of our process, separating the impurities from the final product. Moreover, the impurities contain solids and other components which have the potential to foul up normal material. Furthermore, the column operates at extremely high temperatures and at low pressures, so the tower requires special materials.

Table 4. Equipment Cost Summary

Equipment Description Name	Type	Bare Module Cost
1x B-100 Seed Fermenter Blower	Process Machinery	$ 119,000
2x B-101 Main Fermenter Blower	Process Machinery	$ 434,000
1x B-102 Distillation Tower Blower	Process Machinery	$ 4,000
1x B-103 Compressor	Process Machinery	$ 55,000
1x C-100 Centrifuge	Process Machinery	$ 710,000
1x D-100 Decanter	Fabricated Equipment	$ 84,000
1x E-100 Molasses Pasteurizer	Fabricated Equipment	$ 47,000
1x E-101 Distillation Feed HX	Fabricated Equipment	$ 46,000
1x E-102 Distillation Tower Condenser	Fabricated Equipment	$ 87,000
1x E-103 Distillation Tower Reboiler	Fabricated Equipment	$ 258,000
1x E-104 Distillation Vapor Condenser	Fabricated Equipment	$ 63,000
1x F-100 Seed Fermenter Vessel	Fabricated Equipment	$ 353,000
1x F-100b Seed Fermenter Agitator	Process Machinery	$ 4,000
2x F-101 Fermenter Vessel	Fabricated Equipment	$ 1,068,000
2x F-101b Fermenter Vessel Agitator	Process Machinery	$ 9,000
28x P-10X Generic Pump (est.)	Process Machinery	$ 526,000
34x P-10Xb Generic Pump Motor (est.)	Process Machinery	$ 40,000
8x P-101,103 Molasses Feed Pump	Process Machinery	$ 136,000
2x P-100 Oleic Feed Pump	Process Machinery	$ 17,000
2x P-105 Media Feed Pump	Process Machinery	$ 49,000
2x P-106 Water Feed Pump	Process Machinery	$ 25,000
1x T-100 Oleic Acid Distillation Tower	Fabricated Equipment	$ 4,234,000
3x V-100 Molasses Storage tank	Fabricated Equipment	$ 811,000
1x V-101 Pre-Distillation Storage Tank	Fabricated Equipment	$ 42,000
1x V-102 Reflux Accumulator Drum	Fabricated Equipment	$ 69,000
1x V-103 Water Vapor Flash Drum	Fabricated Equipment	$ 62,000

Equipment Description	**Type**	**Bare Module Cost**
Name		
2x V-104,105 BDO Product Storage Tank	Fabricated Equipment	$ 62,000
1x VZ-10X Media Storage Tank	Fabricated Equipment	$ 228,000
1x VZ-10X Sterile Water Storage Tank	Fabricated Equipment	$ 35,000
1x VZ-10X Oleic Feed Storage Tank	Fabricated Equipment	$ 23,000
1x Packaged Boiler	Fabricated Equipment	$ 4,000
2x Water Sterilization Skid	Process Machinery	$ 20,000
Total		***$9,724,000***
Adjusted to CE = 560.4 (Dec. 2010)		***$10,899,000***

Operating Costs and Economic Analysis

Profitability Analysis

Results

The NPV of the project (at a discount rate of 11.88%) is $283 million, indicating an extremely profitable project. The IRR was calculated to be 157%, and ROI in the third year of production (when 100% production capacity has been reached) is 254%. All three of these profitability measurements indicate that the project is definitely profitable. Future research may need to be conducted to find out if additional equipment is needed in the actual plant, or if we were too optimistic on our pricing for the raw materials and utilities.

Calculating the Discount Rate

We chose a discount rate of 10.7%, which reflects the average Weighted Average Cost of Capital (WACC) of various firms in the chemicals industry. WACC takes into account the various sources of capital available to a firm, and weights it accordingly. For most firms, this is just debt and equity, resulting in the following formula:

$$\text{WACC} = \frac{MV_e}{MV_d + MV_e} \cdot R_e + \frac{MV_d}{MV_d + MV_e} \cdot R_d \cdot (1 - t) \quad \text{(Equation 5)}$$

where MV_e is the market value of equity (usually, market capitalization), MV_d is the market value of debt (usually, straight from the company's balance sheet), R_e is the return on equity, R_d is the return on debt and t is the corporate tax rate. R_e can be calculated based on historical averages, the Capital Asset Pricing Model (CAPM), the Fama-French model, or any other pricing model. R_d can be determined from the company's balance sheet, or by using credit ratings.

To determine WACC for our company, we used DuPont, LyondellBassell, and Dow Chemical as comparables, and calculated an industry WACC of 11.9%. We also considered using Mitsubishi; however, since it is a Japanese company, the capital structure of the firm differs, and this may result in inappropriate weightings. We also included preferred equity in our WACC calculations. Market values of equity were calculated from market capitalization of the corresponding firm, and returns on equity were calculated based on Jack Treynor's and William Sharpe's Capital Asset Pricing Model ("CAPM"). The cost of debt was subdivided into short term and long term debt, and the credit ratings of BASF (A), DuPont (A), and DOW (BBB-) reflect each firm's cost of debt. Finally, the cost of each source of capital was calculated using a weighted average of all firms, and then the final industry WACC was calculated based on this weighted average. See Appendix B for details on the calculations.

Revenue

The primary source of revenue from this project is the sales of BDO. In 2010 Q3, the market price for BDO in the American market ranged from $2,420 per ton to $2,480 per ton (ICIS, 2010). Conservative revenue estimates were calculated from the low end of this range. At 8600 pounds per hour, we estimate average annual revenues of $65.2 million (in real, or inflation-adjusted, terms). However, since our process is specialized and produces BDO in an environmentally friendly manner, we may even be able to charge a premium.

We forecast that this market price will remain similar for the lifetime of the project, barring inflation. Due to the profitability of this bio-based process for BDO production, it may be possible that other firms will try to enter the market, ultimately pushing market prices of BDO further down.

The secondary source of revenue from this project is from the sales of the co-product, vinasse. The vinasse will be sold back to our sister plant at a 70% discount of the current fertilizer price, or $142.50 per ton. At a rate of 1.31 tons of vinasse per ton of BDO, we expect additional revenue of $5 million per year.

Licensing

We found that the profit margins for chemical intermediates produced by major chemical companies are about 25% (BASF, 2011). In contrast, our profit margins are an astounding 96%. Since our company does not have the resources to convert all existing BDO processes to this highly profitable method, nor does it have the resources to make lots of these plants, we will license this technology out to other companies interested in manufacturing BDO. Based on the profitability of this bio-based process and the profitability of the current process, prospective companies should be willing to pay as much as $1700 per ton of BDO produced.

Operating Costs

Raw materials amount to $205 per ton of BDO. This is an order of magnitude less than the selling price of BDO ($2,420 per ton). Of these, the largest cost per ton of BDO is the primary raw product, molasses, which costs $138 per ton of BDO produced. Utilities amount to $70 per ton of BDO. Most of the processing is performed by the genetically modified *E. coli*. However, since it is our company that developed this species, we assume that R&D expenditures are sunk costs. Moreover, since we own the patent for the bacteria, we assumed that we can acquire the bacteria for free.

Working Capital

Our calculations include 30 days' worth of accounts receivable, cash reserves, and accounts payable. Four days' worth of BDO inventory and two days' worth of raw materials inventory are also factored in.

Taxes and Depreciation

The Brazilian corporate income tax is 34%, which is made up of a 15% basic tax, a surtax of 10% on income over BRL 240,000, and an additional 9% for social contribution. For tax purposes, depreciation is normally straight-line, but for companies working three shifts, such as our plant, 200% of the standard rates, also called double declining balance (WorldWide-Tax, 2010).

Sensitivity Analysis

The most IRR-sensitive variable is the product price since the BDO product is our primary revenue source. High fluctuations in this price, although unlikely, will result in high variations in sales due to our large production rate.

Additional sensitivity analysis data can be found within the profitability analysis tables.

Profitability Measures

The Internal Rate of Return (IRR) for this project is 156.52%

The Net Present Value (NPV) of this project in 2012 is $ 252,968,100

ROI Analysis (Third Production Year)

Annual Sales	**73,082,590**
Annual Costs	**(15,190,016)**
Depreciation	**(1,166,121)**
Pretax Income	**56,726,453**
Income Tax Expense	**(8,098,509)**
Net Income	**48,627,944**
Total Capital Investment	**20,496,100**
ROI	**237.25%**

Sensitivity Analyses

	Vary Initial Value by +/-
x-axis	50%
y-axis	50%

Total Permanent Investment						**Product Price**					
	$1,200	$1,500	$1,700	$1,900	$2,200	***$2,420***	$2,700	$3,200	$4,200	$5,900	$8,900
$7,288,000	181.16%	224.38%	263.36%	298.75%	331.07%	**360.71%**	387.99%	413.21%	436.58%	458.30%	478.54%
$8,746,000	145.56%	181.11%	213.68%	243.74%	271.62%	**297.59%**	321.84%	344.56%	365.90%	385.98%	404.91%
$10,204,000	119.54%	149.15%	176.53%	202.07%	226.01%	**248.55%**	269.83%	289.96%	309.05%	327.19%	344.44%
$11,661,000	100.07%	125.09%	148.36%	170.20%	190.83%	**210.39%**	228.99%	246.72%	263.66%	279.86%	295.39%
$13,119,000	85.13%	106.58%	126.59%	145.45%	163.34%	**180.39%**	196.69%	212.32%	227.32%	241.75%	255.64%
$14,576,512	**73.41%**	**92.05%**	**109.46%**	**125.90%**	**141.55%**	**156.52%**	**170.88%**	**184.69%**	**198.00%**	**210.86%**	**223.28%**
$16,034,000	64.02%	80.42%	95.73%	110.21%	124.02%	**137.25%**	149.97%	162.25%	174.12%	185.60%	196.75%
$17,492,000	56.37%	70.94%	84.54%	97.41%	109.70%	**121.48%**	132.84%	143.82%	154.44%	164.76%	174.78%
$18,949,000	50.02%	63.11%	75.30%	86.84%	97.85%	**108.42%**	118.63%	128.50%	138.07%	147.37%	156.43%
$20,407,000	44.68%	56.54%	67.56%	77.98%	87.92%	**97.48%**	106.70%	115.63%	124.30%	132.73%	140.95%
$21,865,000	40.12%	50.97%	61.01%	70.48%	79.52%	**88.20%**	96.59%	104.71%	112.60%	120.29%	127.78%

	Product Price										
Vinasse Price	$1,200	$1,500	$1,700	$1,900	$2,200	***$2,420***	$2,700	$3,200	$4,200	$5,900	$8,900
$0.00	57.00%	76.70%	94.83%	111.82%	127.91%	**143.25%**	157.93%	172.03%	185.61%	198.70%	211.35%
$85.50	66.95%	85.96%	103.64%	120.29%	136.11%	**151.22%**	165.71%	179.63%	193.05%	206.00%	218.52%
$99.75	68.57%	87.49%	105.10%	121.70%	137.48%	**152.55%**	167.00%	180.90%	194.29%	207.21%	219.71%
$114.00	70.19%	89.01%	106.55%	123.10%	138.84%	**153.87%**	168.29%	182.16%	195.53%	208.43%	220.90%
$128.25	71.81%	90.53%	108.01%	124.50%	140.20%	**155.20%**	169.58%	183.42%	196.76%	209.64%	222.09%
$142.50	**73.41%**	**92.05%**	**109.46%**	**125.90%**	**141.55%**	**156.52%**	**170.88%**	**184.69%**	**198.00%**	**210.86%**	**223.28%**
$156.75	75.01%	93.56%	110.90%	127.30%	142.91%	**157.84%**	172.17%	185.95%	199.24%	212.07%	224.48%
$171.00	76.60%	95.07%	112.35%	128.70%	144.26%	**159.16%**	173.45%	187.21%	200.47%	213.28%	225.67%
$185.25	78.19%	96.57%	113.79%	130.09%	145.62%	**160.48%**	174.74%	188.47%	201.71%	214.49%	226.86%
$199.50	79.77%	98.07%	115.23%	131.48%	146.97%	**161.80%**	176.03%	189.73%	202.94%	215.70%	228.05%
$213.75	81.35%	99.57%	116.67%	132.87%	148.32%	**163.11%**	177.32%	190.99%	204.18%	216.92%	229.24%

	Total Permanent Investment										
Variable Costs	$7,288,000	$8,746,000	$10,204,000	$11,661,000	$13,119,000	***$14,576,512***	$16,034,000	$19,241,000	$25,013,000	$35,018,000	$52,527,000
$5,497,480	393.19%	323.86%	270.07%	228.27%	195.48%	**169.42%**	148.43%	131.28%	117.09%	105.21%	95.16%
$6,596,976	386.69%	318.61%	265.77%	224.70%	192.47%	**166.85%**	146.20%	129.33%	115.36%	103.67%	93.78%
$7,696,472	380.20%	313.35%	261.47%	221.13%	189.46%	**164.27%**	143.97%	127.37%	113.64%	102.13%	92.39%
$8,795,968	373.70%	308.10%	257.17%	217.55%	186.44%	**161.69%**	141.73%	125.41%	111.90%	100.58%	91.00%
$9,895,464	367.21%	302.84%	252.86%	213.97%	183.42%	**159.11%**	139.49%	123.45%	110.17%	99.03%	89.60%
$10,994,960	**360.71%**	**297.59%**	**248.55%**	**210.39%**	**180.39%**	**156.52%**	**137.25%**	**121.48%**	**108.42%**	**97.48%**	**88.20%**
$12,094,456	354.21%	292.33%	244.24%	206.80%	177.36%	**153.92%**	135.00%	119.51%	106.68%	95.92%	86.80%
$13,193,951	347.71%	287.06%	239.92%	203.21%	174.33%	**151.33%**	132.75%	117.54%	104.93%	94.35%	85.39%
$14,293,447	341.20%	281.80%	235.61%	199.61%	171.30%	**148.73%**	130.49%	115.56%	103.18%	92.79%	83.98%
$15,392,943	334.70%	276.53%	231.28%	196.01%	168.25%	**146.12%**	128.23%	113.58%	101.42%	91.21%	82.56%
$16,492,439	328.20%	271.26%	226.96%	192.41%	165.21%	**143.51%**	125.96%	111.59%	99.65%	89.64%	81.14%

	Fixed Costs										
Inflation	$1,827,000	$2,193,000	$2,558,000	$2,923,000	$3,289,000	***$3,654,258***	$4,020,000	$4,824,000	$6,271,000	$8,779,000	$13,169,000
2.90%	164.78%	163.31%	161.85%	160.40%	158.94%	**157.50%**	156.05%	154.61%	153.18%	151.75%	150.32%
3.50%	164.62%	163.15%	161.68%	160.22%	158.76%	**157.30%**	155.85%	154.41%	152.96%	151.53%	150.09%
4.10%	164.46%	162.98%	161.51%	160.04%	158.57%	**157.11%**	155.65%	154.20%	152.75%	151.30%	149.86%
4.70%	164.30%	162.81%	161.33%	159.86%	158.38%	**156.91%**	155.45%	153.99%	152.53%	151.08%	149.63%
5.30%	164.13%	162.64%	161.16%	159.67%	158.19%	**156.72%**	155.24%	153.78%	152.31%	150.85%	149.39%
5.88%	**163.97%**	**162.47%**	**160.98%**	**159.49%**	**158.00%**	**156.52%**	**155.04%**	**153.56%**	**152.09%**	**150.62%**	**149.16%**
6.50%	163.80%	162.30%	160.80%	159.30%	157.81%	**156.32%**	154.83%	153.34%	151.86%	150.39%	148.92%
7.10%	163.64%	162.12%	160.62%	159.11%	157.61%	**156.11%**	154.62%	153.13%	151.64%	150.15%	148.67%
7.60%	163.47%	161.95%	160.43%	158.92%	157.41%	**155.90%**	154.40%	152.90%	151.41%	149.92%	148.43%
8.20%	163.30%	161.77%	160.25%	158.73%	157.21%	**155.70%**	154.19%	152.68%	151.18%	149.67%	148.18%
8.80%	163.12%	161.59%	160.06%	158.53%	157.01%	**155.49%**	153.97%	152.45%	150.94%	149.43%	147.93%

		Product Price										
		$1,200	$1,500	$1,700	$1,900	$2,200	***$2,420***	$2,700	$3,200	$4,200	$5,900	$8,900
Capital Cost	$4,862,000	120.52%	149.76%	176.81%	202.05%	225.72%	**248.01%**	269.06%	288.99%	307.90%	325.87%	342.97%
	$5,834,000	106.36%	132.43%	156.65%	179.35%	200.76%	**221.01%**	240.25%	258.55%	276.01%	292.67%	308.62%
	$6,807,000	95.41%	119.01%	140.99%	161.66%	181.22%	**199.80%**	217.51%	234.42%	250.61%	266.14%	281.04%
	$7,779,000	86.64%	108.26%	128.43%	147.44%	165.47%	**182.65%**	199.06%	214.79%	229.89%	244.41%	258.38%
	$8,752,000	79.45%	99.44%	118.11%	135.73%	152.48%	**168.46%**	183.77%	198.48%	212.62%	226.26%	239.42%
	$9,724,000	**73.41%**	**92.05%**	**109.46%**	**125.90%**	**141.55%**	**156.52%**	**170.88%**	**184.69%**	**198.00%**	**210.86%**	**223.28%**
	$10,696,000	68.26%	85.75%	102.08%	117.52%	132.23%	**146.31%**	159.83%	172.86%	185.44%	197.61%	209.39%
	$11,669,000	63.81%	80.30%	95.70%	110.27%	124.16%	**137.46%**	150.26%	162.60%	174.53%	186.08%	197.27%
	$12,641,000	59.91%	75.54%	90.13%	103.93%	117.10%	**129.72%**	141.87%	153.60%	164.94%	175.94%	186.62%
	$13,614,000	56.46%	71.34%	85.21%	98.34%	110.86%	**122.88%**	134.45%	145.63%	156.46%	166.96%	177.16%
	$14,586,000	53.38%	67.59%	80.83%	93.36%	105.31%	**116.78%**	127.84%	138.53%	148.88%	158.93%	168.71%

		Variable Costs										
		$5,497,000	$6,597,000	$7,696,000	$8,796,000	$9,895,000	***$10,994,960***	$12,094,000	$14,513,000	$18,867,000	$26,414,000	$39,621,000
Capital Cost	$4,862,000	269.28%	265.03%	260.78%	256.53%	252.27%	**248.01%**	243.75%	239.48%	235.21%	230.94%	226.66%
	$5,834,000	239.76%	236.02%	232.27%	228.52%	224.77%	**221.01%**	217.25%	213.49%	209.73%	205.95%	202.18%
	$6,807,000	216.59%	213.24%	209.89%	206.53%	203.16%	**199.80%**	196.43%	193.06%	189.68%	186.30%	182.91%
	$7,779,000	197.88%	194.84%	191.80%	188.75%	185.70%	**182.65%**	179.59%	176.52%	173.46%	170.39%	167.31%
	$8,752,000	182.43%	179.64%	176.85%	174.06%	171.26%	**168.46%**	165.66%	162.85%	160.04%	157.22%	154.40%
	$9,724,000	**169.42%**	**166.85%**	**164.27%**	**161.69%**	**159.11%**	**156.52%**	**153.92%**	**151.33%**	**148.73%**	**146.12%**	**143.51%**
	$10,696,000	158.32%	155.92%	153.52%	151.12%	148.71%	**146.31%**	143.89%	141.47%	139.05%	136.62%	134.19%
	$11,669,000	148.70%	146.46%	144.22%	141.97%	139.72%	**137.46%**	135.20%	132.94%	130.67%	128.39%	126.11%
	$12,641,000	140.30%	138.19%	136.08%	133.96%	131.84%	**129.72%**	127.59%	125.46%	123.32%	121.18%	119.04%
	$13,614,000	132.87%	130.88%	128.89%	126.89%	124.89%	**122.88%**	120.87%	118.85%	116.83%	114.81%	112.78%
	$14,586,000	126.27%	124.38%	122.48%	120.59%	118.69%	**116.78%**	114.87%	112.96%	111.04%	109.12%	107.19%

Cash Flow Summary

Year	Percentage of Design Capacity	Product Unit Price	Sales	Capital Costs	Working Capital	Var Costs	Total Costs	Fixed Costs	Depreciation	Taxable Income	Taxes	Net Earnings	Cash Flow	Cumulative Net Present Value	Cumulative Net Present Value at 11.88%
2012	0%		-	-	-	-	-	-	-	-	-	-	-	-	-
2013	0%		-	-	-	-	-	-	-	-	-	-	-	-	-
2014	0%		-	-	-	-	-	-	-	-	-	-	-	-	-
2015	0%		-	(14,576,500)	(2,959,800)	-	-	-	-	-	-	-	(17,536,300)	(12,522,203)	(12,522,200)
2016	45%	$2,420.00	32,595,400	-	(1,479,900)	(4,947,700)	(8,601,990)	(3,654,300)	(1,682,900)	22,310,500	(3,280,300)	19,030,200	19,233,200	(246,619)	(246,600)
2017	68%	$2,562.30	51,768,000	-	(1,479,900)	(7,858,000)	(11,727,116)	(3,869,100)	(1,458,500)	38,582,400	(5,558,300)	33,024,000	33,002,600	18,580,607	18,580,600
2018	90%	$2,712.96	73,082,600	-	-	(11,093,400)	(15,190,016)	(4,096,600)	(1,264,000)	56,628,500	(8,084,800)	48,543,700	49,807,800	43,977,606	43,977,600
2019	90%	$2,872.48	77,379,800	-	-	(11,745,700)	(16,083,189)	(4,337,500)	(1,095,500)	60,201,200	(8,585,000)	51,616,200	52,711,700	68,001,297	68,001,300
2020	90%	$3,041.38	81,929,800	-	-	(12,436,300)	(17,028,881)	(4,592,600)	(949,400)	63,951,500	(9,110,000)	54,841,500	55,790,900	90,728,376	90,728,400
2021	90%	$3,220.22	86,747,300	-	-	(13,167,600)	(18,030,179)	(4,862,600)	(822,800)	67,894,200	(9,662,000)	58,232,200	59,055,100	112,230,686	112,230,700
2022	90%	$3,409.56	91,848,000	-	-	(13,941,800)	(19,090,353)	(5,148,500)	(713,100)	72,044,500	(10,243,000)	61,801,500	62,514,600	132,575,648	132,575,600
2023	90%	$3,610.05	97,248,700	-	-	(14,761,600)	(20,212,866)	(5,451,300)	(618,000)	76,417,700	(10,855,300)	65,562,400	66,180,500	151,826,635	151,826,600
2024	90%	$3,822.32	102,966,900	-	-	(15,629,600)	(21,401,383)	(5,771,800)	(535,600)	81,029,800	(11,501,000)	69,528,900	70,064,500	170,043,288	170,043,300
2025	90%	$4,047.07	109,021,300	-	-	(16,548,600)	(22,659,784)	(6,111,200)	(464,200)	85,897,300	(12,182,400)	73,714,900	74,179,100	187,281,796	187,281,800
2026	90%	$4,285.04	115,431,800	-	-	(17,521,700)	(23,992,179)	(6,470,500)	(402,300)	91,037,300	(21,848,900)	69,188,300	69,590,700	201,736,743	201,736,700
2027	90%	$4,537.00	122,219,200	-	-	(18,551,900)	(25,402,919)	(6,851,000)	(348,700)	96,467,600	(23,152,200)	73,315,400	73,664,000	215,413,044	215,413,000
2028	90%	$4,803.77	129,405,700	-	-	(19,642,800)	(26,896,611)	(7,253,800)	(302,200)	102,206,900	(24,529,600)	77,677,200	77,979,400	228,353,231	228,353,200
2029	90%	$5,086.24	137,014,700	-	-	(20,797,800)	(28,478,132)	(7,680,300)	(261,900)	108,274,700	(25,985,900)	82,288,800	82,550,700	240,597,384	240,597,400
2030	90%	$5,385.31	145,071,200	-	5,919,600	(22,020,700)	(30,152,646)	(8,131,900)	(227,000)	114,691,500	(27,526,000)	87,165,600	93,312,100	252,968,074	252,968,100

General Information

Process Title: **Renewable 1,4 Butanediol from Molasses**
Product: **1,4 Butanediol**
Plant Site Location: **São Paulo, Brazil**
Site Factor: **0.92**
CE Index: **560.40**
Operating Hours per Year: **6960**
Operating Days Per Year: **290**
Operating Factor: **0.7945**

Product Information

This Process will Yield

4	ton of 1,4 Butanediol per hour
103	ton of 1,4 Butanediol per day
29,931	ton of 1,4 Butanediol per year

Price **$2,420.00** **per ton**

Chronology

Year	Action	Distribution of Permanent Investment	Production Capacity	Depreciation 15 yr Double-Declining	Product Price
2012	Design		0.0%		
2013	Design		0.0%		
2014	Design		0.0%		
2015	Construction	100%	0.0%		
2016	Production	0%	45.0%		$2,420
2017	Production	0%	67.5%		$2,562
2018	Production	0%	90.0%		$2,713
2019	Production		90.0%		$2,872
2020	Production		90.0%		$3,041
2021	Production		90.0%		$3,220
2022	Production		90.0%		$3,410
2023	Production		90.0%		$3,610
2024	Production		90.0%		$3,822
2025	Production		90.0%		$4,047
2026	Production		90.0%		$4,285
2027	Production		90.0%		$4,537
2028	Production		90.0%		$4,804
2029	Production		90.0%		$5,086
2030	Production		90.0%		$5,385

Equipment Costs

Equipment Description		Bare Module Cost
1x B-100 Seed Fermenter Blower	Process Machinery	$119,120
2x B-101 Main Fermenter Blower	Process Machinery	$433,595
1x B-102 Distillation Tower Blower	Process Machinery	$4,005
1x B-103 Compressor	Process Machinery	$55,302
1x C-100 Centrifuge	Process Machinery	$710,113
1x D-100 Decanter	Fabricated Equipment	$83,544
1x E-100 Molasses Pasteurizer	Fabricated Equipment	$46,666
1x E-101 Distillation Feed HX	Fabricated Equipment	$46,151
1x E-102 Distillation Tower Condenser	Fabricated Equipment	$86,744
1x E-103 Distillation Tower Reboiler	Fabricated Equipment	$257,968
1x E-104 Distillation Vapor Condenser	Fabricated Equipment	$63,330
1x F-100 Seed Fermenter Vessel	Fabricated Equipment	$352,551
1x F-100b Seed Fermenter Agitator	Process Machinery	$3,713
2x F-101 Fermenter Vessel	Fabricated Equipment	$1,068,052
2x F-101b Fermenter Vessel Agitator	Process Machinery	$9,188
28x P-10X Generic Pump (est.)	Process Machinery	$525,576
34x P-10Xb Generic Pump Motor (est.)	Process Machinery	$40,161
8x P-101, P-103 Molasses Feed Pump	Process Machinery	$136,211
2x P-100 Oleic Feed Pump	Process Machinery	$16,775
2x P-105 Media Feed Pump	Process Machinery	$48,556
2x P-106 Water Feed Pump	Process Machinery	$25,245
1x T-100 Oleic Acid Distillation Tower	Fabricated Equipment	$4,234,361
3x V-100 Molasses Storage tank	Fabricated Equipment	$811,034
1x V-101 Pre-Distillation Storage Tank	Fabricated Equipment	$41,547
1x V-102 Reflux Accumulator Drum	Fabricated Equipment	$68,601
1x V-103 Water Vapor Flash Drum	Fabricated Equipment	$62,483
2x V-104,105 BDO Product Storage Tank	Fabricated Equipment	$62,230
1x VZ-10X Media Storage Tank	Fabricated Equipment	$227,665
1x VZ-10X Sterile Water Storage Tank	Fabricated Equipment	$34,777
1x VZ-10X Oleic Feed Storage Tank	Fabricated Equipment	$22,508
1x Packaged Boiler	Fabricated Equipment	$4,000
2x Water Sterilization Skid	Process Machinery	$20,000
Total		***$9,721,771***

Raw Materials

	Raw Material:	Unit:	Required Ratio:		Cost of Raw Material:	
1	Molasses	ton	1.966283	ton per ton of 1,4 Butanediol	$70.000	per ton
2	Oleic Acid	ton	0.0216254	ton per ton of 1,4 Butanediol	$1,270.00	per ton
3	Cornsteep Liquor	ton	0.7946053	ton per ton of 1,4 Butanediol	$50.00	per ton
4	Water	ton	0.0707592	ton per ton of 1,4 Butanediol	$0.00	per ton

Total Weighted Average: $204.834 per ton of 1,4 Butanediol

Byproducts

	Byproduct:	Unit:	Ratio to Product		Byproduct Selling Price	
1	Vinasse	ton	1.3149634	ton per ton of 1,4 Butanediol	$142.500	per ton

Total Weighted Average: $187.382 per ton of 1,4 Butanediol

Utilities

	Utility:	Unit:	Required Ratio		Utility Cost	
1	High Pressure Steam (633	lb	3701.7324	lb per ton of 1,4 Butanediol	$5.489E-03	per lb
2	Low Pressure Steam (150	lb	365.47137	lb per ton of 1,4 Butanediol	$3.504E-03	per lb
3	Refrigeration, 10degF	ton	1.9822753	ton per ton of 1,4 Butanediol	$6.926	per ton
#REF!	#REF!	#REF!	#REF!	#REF!	#REF!	#REF!
5	Electricity	kWh	26.130217	kWh per ton of 1,4 Butanediol	$0.044	per kWh
6	Treatment of Waste Oleic	ton	0.093	ton per ton of 1,4 Butanediol	$60.000	per ton
7	Sterilization of Water	ton	0.4708755	ton per ton of 1,4 Butanediol	$60.00	per ton

Total Weighted Average: $70.375 per ton of 1,4 Butanediol

Variable Costs

General Expenses:

Selling / Transfer Expenses:	**3.00%**	**of Sales**
Direct Research:	**4.80%**	**of Sales**
Allocated Research:	**0.50%**	**of Sales**
Administrative Expense:	**2.00%**	**of Sales**
Management Incentive Compensation:	**1.25%**	**of Sales**

Working Capital

Accounts Receivable	⇨	**30**	**Days**
Cash Reserves (excluding Raw Materials)	⇨	**30**	**Days**
Accounts Payable	⇨	**30**	**Days**
1,4 Butanediol Inventory	⇨	**4**	**Days**
Raw Materials	⇨	**2**	**Days**

Total Permanent Investment

Cost of Site Preparations:	**5.00%**	**of Total Bare Module Costs**
Cost of Service Facilities:	**5.00%**	**of Total Bare Module Costs**
Allocated Costs for utility plants and related facilities:	**$0**	
Cost of Contingencies and Contractor Fees:	**18.00%**	**of Direct Permanent Investment**
Cost of Land:	**2.00%**	**of Total Depreciable Capital**
Cost of Royalties:	**$0**	
Cost of Plant Start-Up:	**10.00%**	**of Total Depreciable Capital**

Fixed Costs

Operations

Operators per Shift:	**5**	**(assuming 5 shifts)**
Direct Wages and Benefits:	**$25**	**/operator hour**
Direct Salaries and Benefits:	**15%**	**of Direct Wages and Benefits**
Operating Supplies and Services:	**6%**	**of Direct Wages and Benefits**
Technical Assistance to Manufacturing:	**$0.00**	**per year, for each Operator per Shift**
Control Laboratory:	**$0.00**	**per year, for each Operator per Shift**

Maintenance

Wages and Benefits:	**4.50%**	**of Total Depreciable Capital**
Salaries and Benefits:	**25%**	**of Maintenance Wages and Benefits**
Materials and Services:	**100%**	**of Maintenance Wages and Benefits**
Maintenance Overhead:	**5%**	**of Maintenance Wages and Benefits**

Operating Overhead

General Plant Overhead:	**7.10%**	**of Maintenance and Operations Wages and Benefits**
Mechanical Department Services:	**2.40%**	**of Maintenance and Operations Wages and Benefits**
Employee Relations Department:	**5.90%**	**of Maintenance and Operations Wages and Benefits**
Business Services:	**7.40%**	**of Maintenance and Operations Wages and Benefits**

Property Taxes and Insurance

Property Taxes and Insurance:	**2%**	**of Total Depreciable Capital**

Straight Line Depreciation

Direct Plant:	8.00%	**of Total Depreciable Capital, less 1.18 times the Allocated Costs for Utility Plants and Related Facilities**
Allocated Plant:	6.00%	**of 1.18 times the Allocated Costs for Utility Plants and Related Facilities**

Other Annual Expenses

Rental Fees (Office and Laboratory Space):	**$0**
Licensing Fees:	**$0**
Miscellaneous:	**$0**

Depletion Allowance

Annual Depletion Allowance:	**$0**

Variable Cost Summary

Variable Costs at 100% Capacity:

General Expenses

Selling / Transfer Expenses:	$	2,173,025
Direct Research:	$	3,476,841
Allocated Research:	$	362,171
Administrative Expense:	$	1,448,684
Management Incentive Compensation:	$	905,427
Total General Expenses	$	8,366,148

Raw Materials	$204.83	per ton of 1,4 Butanediol		$6,130,998
Byproducts	$187.38	per ton of 1,4 Butanediol		($5,608,629)
Utilities	$70.38	per ton of 1,4 Butanediol		$2,106,442
Total Variable Costs			**$**	**10,994,960**

Fixed Cost Summary

Operations

Direct Wages and Benefits	$	1,313,407
Direct Salaries and Benefits	$	197,011
Operating Supplies and Services	$	78,804
Technical Assistance to Manufacturing	$	-
Control Laboratory	$	-
Total Operations	**$**	**1,589,222**

Maintenance

Wages and Benefits	$	567,979
Salaries and Benefits	$	141,995
Materials and Services	$	567,979
Maintenance Overhead	$	28,399
Total Maintenance	**$**	**1,306,351**

Operating Overhead

General Plant Overhead:	$	157,648
Mechanical Department Services:	$	53,289
Employee Relations Department:	$	131,003
Business Services:	$	164,309
Total Operating Overhead	**$**	**506,249**

Property Taxes and Insurance

Property Taxes and Insurance:	$	252,435

Other Annual Expenses

Rental Fees (Office and Laboratory Space):	$	-
Licensing Fees:	$	-
Miscellaneous:	$	-
Total Other Annual Expenses	**$**	**-**
Total Fixed Costs	**$**	**3,654,258**

Investment Summary

Bare Module Costs		
Fabricated Equipment	$ 7,576,000	
Process Machinery	$ 2,148,000	
Spares	$ -	
Storage	$ -	
Other Equipment	$ -	
Catalysts	$ -	
Computers, Software, Etc.	$ -	
Total Bare Module Costs:		**$ 9,724,000**
Direct Permanent Investment		
Cost of Site Preparations:	$ 486,200	
Cost of Service Facilities:	$ 486,200	
Allocated Costs for utility plants and related facilities:	$ -	
Direct Permanent Investment		**$ 10,696,400**
Total Depreciable Capital		
Cost of Contingencies & Contractor Fees	$ 1,925,352	
Total Depreciable Capital		**$ 12,621,752**
Total Permanent Investment		
Cost of Land:	$ 252,435	
Cost of Royalties:	$ -	
Cost of Plant Start-Up:	$ 1,262,175	
Total Permanent Investment - Unadjusted		$ 14,136,362
Site Factor		0.92
CE Index Adjustment (over CE [2006] = 500)		1.12
Total Permanent Investment		**$ 14,576,512**

Working Capital

	2015	2016	2017
Accounts Receivable	$ 2,679,072	$ 1,339,536	$ 1,339,536
Cash Reserves	$ 213,067	$ 106,534	$ 106,534
Accounts Payable	$ (304,672)	$ (152,336)	$ (152,336)
1,4 Butanediol Inventory	$ 357,210	$ 178,605	$ 178,605
Raw Materials	$ 15,118	$ 7,559	$ 7,559
Total	**$ 2,959,794**	**$ 1,479,897**	**$ 1,479,897**
Present Value at 11.88%	*$ 2,113,509*	*$ 944,543*	*$ 844,246*

Total Capital Investment	**$ 18,478,811**

Process Title: ***Renewable 1,4 Butanediol from Molasses***
Product: ***1,4 Butanediol***
Plant Site Location: ***São Paulo, Brazil***

Timeline:

Number of Years for Design	3	(must be whole number)
Number of Years for Construction	1	(must be whole number)
Number of Years for Production	15	
Total Number of Years for Project	19	
Start Year	2012	
Site Factor	0.92	for Brazil
CE Index	560.40	Dec. 2010 Prelim

Continuous Operation:

Days per Year	290	
OR		
Hours per Year	0	
OR		
Operating Factor	0.0000	(if multiple entries, "Operating Factor" is used)
Production Capacity	90%	of Design Capacity
Start production at	50%	of Production Capacity
Years to achieve full capacity	2	
Number of Shifts	5	
Asset Life	15	years
Depreciation Schedule	Double-Declining	
Brazilian Real / US Dollar	1.6581	
Income Tax Rate Lower Tier	24%	Brazil Corporate Income Tax
Income Tax Rate Upper Tier	34%	
Income Tax Rate Tier Threshhold	$2,600,000	BRL
Income Tax Rate Tier Threshhold	$1,568,060	USD
Cost of Capital (for the NPV Calculation)	11.9%	(discount rate)
General Inflation Rate	5.9%	
Product Inflation Rate		

Product Information:

Enter Product Units (i.e. lb, gram, gal, etc)	*ton*	
Price Per Unit	$2,420.00	per ton
Number of units per:	(Specify ONE of the three. If multiple entries, "Year" is used.)	
Year	-	ton per Year
OR		
Day	-	ton per Day
OR		
Hour	4.30	ton per Hour

Raw Materials

	Raw Material:	Unit:	Required Ratio:		Cost of Raw Material:	
1	Molasses	ton	1.97	ton per ton of 1,4 Butanediol	$70.000	per ton
2	Oleic Acid	ton	0.02	ton per ton of 1,4 Butanediol	$1270.000	per ton
3	Cornsteep Liquor	ton	0.79	ton per ton of 1,4 Butanediol	$50.000	per ton
4	Water	ton	0.07	ton per ton of 1,4 Butanediol	$1.800E-03	per ton
5						
6						
7						
8						
9						
10						
	Total Weighted Average:				$204.834	per ton of 1,4 Butanediol

Byproducts

	Byproduct:	Unit:	Ratio to Product		Byproduct Selling Price	
1	Vinasse	ton	1.31	ton per ton of 1,4 Butanediol	$142.500	per ton
2						
3						
4						
5						
6						
7						
8						
9						
10						
	Total Weighted Average:				$187.382	per ton of 1,4 Butanediol

Utilities

	Utility:	Unit:	Required Ratio		Utility Cost		
1	High Pressure Steam (6	lb	3,701.73	lb per ton of 1,4 Butanediol	$5.489E-03	per lb	90 Off/10 On
2	Low Pressure Steam (1	lb	365.47	lb per ton of 1,4 Butanediol	$3.504E-03	per lb	90 Off/10 On
3	Refrigeration, 10degF	ton	1.98	ton per ton of 1,4 Butanediol	$6.926	per ton	90 Off/10 On
4	Brine Cooling Water	1000 gal	0.95	1000 gal per ton of 1,4 Butanediol	$0.075	per 1000 gal	On
5	Electricity	kWh	2.61E+01	kWh per ton of 1,4 Butanediol	$0.044	per kWh	90 Off/10 On
6	Treatment of Waste Olei	ton	0.093	ton per ton of 1,4 Butanediol	$60.000	per ton	On
7	Sterilization of Water	ton	0.47	ton per ton of 1,4 Butanediol	$60.000	per ton	On
8							
9							
10							
	Total Weighted Average:				$70.375	per ton of 1,4 Butanediol	

Selling Price Worksheet

This worksheet is optional. It may be used to adjust the product selling prices each year. Your inputs for the product prices, adjusted using the inflation rates, are entered as default values. To change, enter a price into the "Manual Input Price" column.

Year	Calculated Unit Price	Manual Input Price	Price to Be Used
2016	$2,420.00		$2,420.00
2017	$2,562.30		$2,562.30
2018	$2,712.96		$2,712.96
2019	$2,872.48		$2,872.48
2020	$3,041.38		$3,041.38
2021	$3,220.22		$3,220.22
2022	$3,409.56		$3,409.56
2023	$3,610.05		$3,610.05
2024	$3,822.32		$3,822.32
2025	$4,047.07		$4,047.07
2026	$4,285.04		$4,285.04
2027	$4,537.00		$4,537.00
2028	$4,803.77		$4,803.77
2029	$5,086.24		$5,086.24
2030	$5,385.31		$5,385.31

Other Variable Costs

General Expenses

Selling / Transfer Expenses:	3.00% of Sales
Direct Research:	4.80% of Sales
Allocated Research:	0.50% of Sales
Administrative Expense:	2.00% of Sales
Management Incentive Compensation:	1.25% of Sales

Working Capital

Accounts Receivable	➪	**30 Days**
Cash Reserves (excluding Raw Materials)	➪	**30 Days**
Accounts Payable	➪	**30 Days**
1,4 Butanediol Inventory	➪	**4 Days**
Raw Materials	➪	**2 Days**

Total Permanent Investment

		% of Total Permanent Investment	
Year:	**2015**	100%	(default is first year of Construction,
	2016	0%	otherwise over-ride this year)
	2017	0%	
	2018	0%	

Cost of Site Preparations:	5.00%	of Total Bare Module Costs
Cost of Service Facilities:	5.00%	of Total Bare Module Costs
Allocated Costs for utility plants and related facilities:	$0	
Cost of Contingencies and Contractor Fees:	18.00%	of Direct Permanent Investment
Cost of Land:	2.00%	of Total Depreciable Capital
Cost of Royalties:	$0	
Cost of Plant Start-Up:	10.00%	of Total Depreciable Capital

Equipment Costs

Equipment Description	Type	Bare Module Cost
Name		
1x B-100 Seed Fermenter Blower	Process Machinery	$ 119,000
2x B-101 Main Fermenter Blower	Process Machinery	$ 434,000
1x B-102 Distillation Tower Blower	Process Machinery	$ 4,000
1x B-103 Compressor	Process Machinery	$ 55,000
1x C-100 Centrifuge	Process Machinery	$ 710,000
1x D-100 Decanter	Fabricated Equipment	$ 84,000
1x E-100 Molasses Pasteurizer	Fabricated Equipment	$ 47,000
1x E-101 Distillation Feed HX	Fabricated Equipment	$ 46,000
1x E-102 Distillation Tower Condenser	Fabricated Equipment	$ 87,000
1x E-103 Distillation Tower Reboiler	Fabricated Equipment	$ 258,000
1x E-104 Distillation Vapor Condenser	Fabricated Equipment	$ 63,000
1x F-100 Seed Fermenter Vessel	Fabricated Equipment	$ 353,000
1x F-100b Seed Fermenter Agitator	Process Machinery	$ 4,000
2x F-101 Fermenter Vessel	Fabricated Equipment	$ 1,068,000
2x F-101b Fermenter Vessel Agitator	Process Machinery	$ 9,000
28x P-10X Generic Pump (est.)	Process Machinery	$ 526,000
34x P-10Xb Generic Pump Motor (est.)	Process Machinery	$ 40,000
8x P-101, P-103 Molasses Feed Pump	Process Machinery	$ 136,000
2x P-100 Oleic Feed Pump	Process Machinery	$ 17,000
2x P-105 Media Feed Pump	Process Machinery	$ 49,000
2x P-106 Water Feed Pump	Process Machinery	$ 25,000
1x T-100 Oleic Acid Distillation Tower	Fabricated Equipment	$ 4,234,000
3x V-100 Molasses Storage tank	Fabricated Equipment	$ 811,000
1x V-101 Pre-Distillation Storage Tank	Fabricated Equipment	$ 42,000
1x V-102 Reflux Accumulator Drum	Fabricated Equipment	$ 69,000
1x V-103 Water Vapor Flash Drum	Fabricated Equipment	$ 62,000
2x V-104,105 BDO Product Storage Tank	Fabricated Equipment	$ 62,000
1x VZ-10X Media Storage Tank	Fabricated Equipment	$ 228,000
1x VZ-10X Sterile Water Storage Tank	Fabricated Equipment	$ 35,000
1x VZ-10X Oleic Feed Storage Tank	Fabricated Equipment	$ 23,000
1x Packaged Boiler	Fabricated Equipment	$ 4,000
2x Water Sterilization Skid	Process Machinery	$ 20,000
Total		***$ 9,724,000***
Adjusted to CE = 560.4 (Dec. 2010)		***$ 10,899,000***

Fixed Costs

Operations

Operators per Shift:	5	(assuming 5 shifts)
Direct Wages and Benefits:	$25	/operator hour
Direct Salaries and Benefits:	15%	of Direct Wages and Benefits
Operating Supplies and Services:	6%	of Direct Wages and Benefits
Technical Assistance to Manufacturing:	$0.00	per year, for each Operator per Shift
Control Laboratory:	$0.00	per year, for each Operator per Shift

Maintenance

Wages and Benefits:	4.50%	of Total Depreciable Capital
Salaries and Benefits:	25.00%	of Maintenance Wages and Benefits
Materials and Services:	100.00%	of Maintenance Wages and Benefits
Maintenance Overhead:	5.00%	of Maintenance Wages and Benefits

Operating Overhead

General Plant Overhead:	7.10%	of Maintenance and Operations Wages and Benefits
Mechanical Department Services:	2.40%	of Maintenance and Operations Wages and Benefits
Employee Relations Department	5.90%	of Maintenance and Operations Wages and Benefits
Business Services	7.40%	of Maintenance and Operations Wages and Benefits

Property Taxes and Insurance

Property Taxes and Insurance:	2.00%	of Total Depreciable Capital

Straight Line Depreciation

Direct Plant:	8.00%	of Total Depreciable Capital, less	1.18	times the Allocated Costs for Utility Plants and Related Facilities
Allocated Plant:	6.00%	of	1.18	times the Allocated Costs for Utility Plants and Related Facilities

Other Annual Expenses

Rental Fees (Office and Laboratory Space):	$0
Licensing Fees:	$0
Miscellaneous:	$0

Depletion Allowance

Annual Depletion Allowance:	$0

Other Important Considerations

Startup

The plant will be shut down once a year, and therefore, will incur startup costs at the beginning of the year. The plant startup procedure is estimated to take 35.5 hours. Startup costs amount to approximately $51,000 per year. Further details on startup costs can be found in Appendix B.

Packaged Boiler

During our nine months of continuous operation, we will be provided steam from our sister sugar and ethanol production facility as a byproduct of their process at a discounted rate. In order to supply steam during the one month in the Brazil rainy season when our sister sugar and ethanol facility ceases operation and our facility will continue to run, we will need to purchase a packaged boiler, as advised by Mr. Tieri. The specific device we chose is a Burnham Electronic Ignition Model from Ingram's Water & Air Equipment for $4,195.00. This model is a traditional insulated cast iron heat exchanger that runs at 173,000 BTU. It will provide steam at an I=B=R Net Steam Rating of 542 square feet.

Conclusions and Recommendations

The innovative, environmentally-friendly technology we developed will convert renewable feedstocks into BDO in fewer steps than traditional petrochemical routes, with no toxic byproducts and low greenhouse gas emissions. Upon completing the design of our BDO production facility, a profitability analysis determined our process to be highly profitable. Despite being limited to 290 days of operation in our São Paulo, Brazil location, our plant is able to produce 50 million pounds of BDO per year with a low total permanent investment of $13.5 million. An economic analysis shows that the NPV of our facility 15 years after construction will be $283 million with an IRR of 157% at a BDO selling price of $2,420 per ton. Future research may need to be conducted to find out if additional equipment is needed in the actual plant, or if we were too optimistic on our pricing for the raw materials and utilities. The selling price of our BDO is at the low range of the U.S. market pricing of $2,420 to $2,840 per ton, obtained from the third quarter ICIS market report of 2010. We also manufacture vinasse as a co-product alongside our BDO, which will be sold back to our sister sugar and ethanol facility for their use as fertilizer. The additional revenues from selling this product at a 70% discounted price will offset the majority of our raw material expenses. The raw materials required, which are blackstrap molasses, oleic acid, CSL, and water, will cost us about $200 per ton of BDO produced, whereas our vinasse will return about $190 per ton of BDO produced. The utilities required in our facility, such as steam, refrigeration, cooling water, electricity, and waste treatment, will result in an additional cost of $70 per ton of BDO produced.

Although our design is economically feasible, it is highly contingent on the passing of the patent application of Genomatica, Inc., who genetically engineered the *E. coli* cells. Further research may need to be performed on the specific *E. coli* cells in order to verify the BDO yields measured by Genomatica, Inc., as reported in their patent application. Any variations in these results will require alterations in our design process and profitability analysis. However, due to our current profit margins, we are still confident that this design will remain economically attractive. In addition, we recommend building a pilot plant in order to determine whether additional equipment or utilities will be required. Finally, additional research on vendors may be performed to optimize the cost of raw materials, while still maintaining sufficient quality.

Acknowledgements

We would like to thank Professor Leonard A. Fabiano, Professor Daniel A. Hammer, Mr. Stephen M. Tieri, Mr. Bruce M. Vrana, Mr. Adam A. Brostow, and Mr. David M. Kolesar for their guidance, ideas and patience over the course of this project. Their assistance in choosing a location, optimizing process designs, and helping us understand the finer details of our plant design were invaluable. We would especially like to thank Mr. Tieri for his assistance with the background of the project. We would also like to thank Mr. Kolesar for his assistance with proper assignments of control valves. We would also like to thank our classmates for their continuous (not batch) support throughout this semester.

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Appendix A: Aspen Data

```
BLOCK:  2CNTPUMP MODEL: PUMP
----------------------------
  INLET STREAM:          WITHCELL
  OUTLET STREAM:         HPWCELL
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN             OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           625.951         625.951         0.00000
      MASS(LB/HR   )           56850.8         56850.8       -0.255967E-15
      ENTHALPY(BTU/HR  )      -0.701543E+08   -0.701520E+08   -0.330328E-04

                        ***  INPUT DATA  ***
   OUTLET PRESSURE  PSIA                                   39.7000
   PUMP EFFICIENCY                                          0.70000
   DRIVER EFFICIENCY                                        1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                             30
   TOLERANCE                                                0.000100000

                         ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  CUFT/HR                          393.135
   PRESSURE CHANGE  PSI                                    22.2955
   NPSH AVAILABLE   FT-LBF/LB                              35.0246
   FLUID POWER  HP                                          0.63746
   BRAKE POWER  HP                                          0.91066
   ELECTRICITY  KW                                          0.67908
   PUMP EFFICIENCY USED                                     0.70000
   NET WORK REQUIRED  HP                                    0.91066
   HEAD DEVELOPED FT-LBF/LB                                53.0739

BLOCK:  ADDINCO2 MODEL: MIXER
-----------------------------
  INLET STREAMS:         NOSOLID     CO2DISSO
  OUTLET STREAM:         WITHC02
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN             OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           413.113         413.113         0.00000
      MASS(LB/HR   )           23277.7         23277.7       -0.156286E-15
      ENTHALPY(BTU/HR  )      -0.686957E+08   -0.686957E+08    0.640749E-10

                        ***  INPUT DATA  ***
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                                    30
  CONVERGENCE TOLERANCE                                      0.000100000
  OUTLET PRESSURE:  MINIMUM OF INLET STREAM PRESSURES

BLOCK:  BDOPUMP  MODEL: PUMP
----------------------------
  INLET STREAM:          BDO
  OUTLET STREAM:         WARMBDO
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
```

```
                                  IN             OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           98.5592         98.5592         0.00000
      MASS(LB/HR   )           8601.31         8601.31         0.00000
      ENTHALPY(BTU/HR  )      -0.210506E+08   -0.210483E+08   -0.108796E-03

                        ***  INPUT DATA  ***
   OUTLET PRESSURE  PSIA                                   65.0000
   PUMP EFFICIENCY                                          0.70000
   DRIVER EFFICIENCY                                        1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                             30
   TOLERANCE                                                0.000100000

                         ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  CUFT/HR                          133.435
   PRESSURE CHANGE  PSI                                    64.9260
   NPSH AVAILABLE   FT-LBF/LB                               0.0
   FLUID POWER  HP                                          0.63006
   BRAKE POWER  HP                                          0.90009
   ELECTRICITY  KW                                          0.67120
   PUMP EFFICIENCY USED                                     0.70000
   NET WORK REQUIRED  HP                                    0.90009
   HEAD DEVELOPED FT-LBF/LB                               145.039

BLOCK:  BLOWER   MODEL: MIXER
-----------------------------
  INLET STREAMS:         TOBLWR      WTRVAPOR
  OUTLET STREAM:         BLOWN
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN             OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           186.740         186.740        0.185301E-07
      MASS(LB/HR   )           3396.63         3396.63       -0.873187E-08
      ENTHALPY(BTU/HR  )      -0.193733E+08   -0.193733E+08   -0.645543E-07

                        ***  INPUT DATA  ***
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                                    30
  CONVERGENCE TOLERANCE                                      0.000100000
  OUTLET PRESSURE   PSIA                                    0.15000

BLOCK:  CFGVALVE MODEL: VALVE
-----------------------------
  INLET STREAM:          NOSOLHPR
  OUTLET STREAM:         TOMIX
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN             OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           300.417         300.417         0.00000
      MASS(LB/HR   )           13348.4         13348.4         0.00000
      ENTHALPY(BTU/HR  )      -0.456749E+08   -0.456749E+08    0.132118E-07

                        ***  INPUT DATA  ***
```

```
  VALVE PRESSURE DROP       PSI                         25.0000
  VALVE FLOW COEF CALC.                                NO

                      FLASH SPECIFICATIONS:
  NPHASE                                                2
  MAX NUMBER OF ITERATIONS                             30
  CONVERGENCE TOLERANCE                                 0.000100000

                      ***  RESULTS  ***

  VALVE OUTLET PRESSURE     PSIA                        74.7000

BLOCK:  CNTVALVE MODEL: VALVE
-----------------------------
  INLET STREAM:          HPWCELL
  OUTLET STREAM:         2CFUGE
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
  TOTAL BALANCE
     MOLE(LBMOL/HR)            625.951         625.951         0.00000
     MASS(LB/HR   )            56850.8         56850.8         0.00000
     ENTHALPY(BTU/HR  )      -0.701520E+08   -0.701520E+08    0.198478E-11

                      ***  INPUT DATA  ***

  VALVE PRESSURE DROP       PSI                         25.0000
  VALVE FLOW COEF CALC.                                NO

                      FLASH SPECIFICATIONS:
  NPHASE                                                2
  MAX NUMBER OF ITERATIONS                             30
  CONVERGENCE TOLERANCE                                 0.000100000

                      ***  RESULTS  ***

  VALVE OUTLET PRESSURE     PSIA                        14.7000

BLOCK:  CO2COMP  MODEL: MCOMPR
------------------------------
  INLET STREAMS:    VAPOROUT     TO STAGE   1
  OUTLET STREAMS:   DSLVCO2O   FROM STAGE   2
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
  TOTAL BALANCE
     MOLE(LBMOL/HR)            6.01377         6.01377         0.00000
     MASS(LB/HR   )            127.034         127.034         0.00000
     ENTHALPY(BTU/HR  )       -584897.        -634863.        0.787030E-01

                      ***  INPUT DATA  ***

  ISENTROPIC CENTRIFUGAL COMPRESSOR
  NUMBER OF STAGES                                        2
  FINAL PRESSURE, PSIA                                   17.4045

                COMPRESSOR SPECIFICATIONS PER STAGE

  STAGE                     MECHANICAL      ISENTROPIC
  NUMBER                    EFFICIENCY      EFFICIENCY


    1                        1.000          0.7200
    2                        1.000          0.7200
```

```
                COOLER SPECIFICATIONS PER STAGE

  STAGE     PRESSURE DROP     TEMPERATURE
  NUMBER    PSI               F

    1       0.000             200.0
    2       0.000             200.0

                      ***  RESULTS  ***

  FINAL PRESSURE, PSIA                                      17.4045
  TOTAL WORK REQUIRED, HP                                   24.3989
  TOTAL COOLING DUTY , BTU/HR                         -112,047.

                      ***  PROFILE  ***

                      COMPRESSOR PROFILE

  STAGE      OUTLET       PRESSURE     OUTLET
  NUMBER     PRESSURE     RATIO        TEMPERATURE
             PSIA                      F

    1        1.616        10.77        590.8
    2        17.40        10.77        893.8

  STAGE      INDICATED      BRAKE
  NUMBER     HORSEPOWER     HORSEPOWER
             HP             HP
    1        10.58          10.58
    2        13.82          13.82

  STAGE      HEAD           VOLUMETRIC
  NUMBER     DEVELOPED      FLOW
             FT-LBF/LB      CUFT/HR
    1       0.1187E+06     0.2154E+06
    2       0.1551E+06     0.2633E+05

                      COOLER PROFILE

  STAGE      OUTLET          OUTLET       COOLING     VAPOR
  NUMBER     TEMPERATURE     PRESSURE     LOAD        FRACTION
             F               PSIA         BTU/HR

    1         200.0           1.616       -.1935E+05   1.000
    2         200.0           17.40       -.9270E+05  0.4574

BLOCK:  CO2EXIT  MODEL: MIXER
-----------------------------
  INLET STREAMS:         CO2INRXN    CO2VENT2    CO2VENT     DSLVCO2O
  OUTLET STREAM:         CO2TOATM
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            102.160         102.160         0.00000
      MASS(LB/HR   )            4358.41         4358.41       -0.208676E-15
      ENTHALPY(BTU/HR  )      -0.168948E+08   -0.168948E+08   -0.254397E-08

                      ***  INPUT DATA  ***
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                                   30
  CONVERGENCE TOLERANCE                                     0.000100000
  OUTLET PRESSURE:  MINIMUM OF INLET STREAM PRESSURES

BLOCK:  CO2SPLIT MODEL: FSPLIT
------------------------------
  INLET STREAM:          CO2
```

```
  OUTLET STREAMS:        CO2DISSO    CO2INRXN
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                     ***  MASS AND ENERGY BALANCE  ***
                                   IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            96.3970         96.3970        0.147420E-15
      MASS(LB/HR   )            4242.41         4242.41        0.214382E-15
      ENTHALPY(BTU/HR  )      -0.163023E+08   -0.163023E+08   -0.228514E-15

                         ***  INPUT DATA  ***

 FRACTION OF FLOW                STRM=CO2DISSO FRAC=         0.0026000

                          ***  RESULTS  ***

 STREAM= CO2DISSO       SPLIT=          0.0026000   KEY=  0    STREAM-ORDER=   1
         CO2INRXN                       0.99740           0                    2

BLOCK:  CONDNSOR MODEL: HEATER
------------------------------
  INLET STREAM:          ROCOMP
  OUTLET STREAM:         CONDENSE
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                     ***  MASS AND ENERGY BALANCE  ***
                                   IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            186.553         186.553         0.00000
      MASS(LB/HR   )            3393.23         3393.23        0.134016E-15
      ENTHALPY(BTU/HR  )      -0.193539E+08   -0.228865E+08    0.154352

                         ***  INPUT DATA  ***
  TWO    PHASE  TP  FLASH
  SPECIFIED TEMPERATURE               F                        41.0000
  SPECIFIED PRESSURE                  PSIA                      0.15000
  MAXIMUM NO. ITERATIONS                                       30
  CONVERGENCE TOLERANCE                                         0.000100000


                          ***  RESULTS  ***
  OUTLET TEMPERATURE    F                                   41.000
  OUTLET PRESSURE       PSIA                               0.15000
  HEAT DUTY             BTU/HR                            -0.35326E+07
  OUTLET VAPOR FRACTION                                    0.32236E-01
  PRESSURE-DROP CORRELATION PARAMETER                       0.0000



  V-L PHASE EQUILIBRIUM :

     COMP             F(I)           X(I)           Y(I)           K(I)
     1:4-B-01         0.80643E-03    0.83323E-03    0.20183E-05    0.24223E-02
     WATER            0.99349        0.99855        0.84158        0.84280
     CARBO-01         0.13260E-02    0.54802E-03    0.24682E-01     45.040
     OLEIC-01         0.10312E-10    0.10656E-10    0.42237E-19    0.39640E-08
     L-PHE-01         0.75200E-14    0.77704E-14    0.12306E-17    0.15837E-03
     NIACI-01         0.54486E-05    0.56263E-05    0.11472E-06    0.20390E-01
     SULFU-01         0.42682E-13    0.44104E-13    0.76315E-19    0.17304E-05
     HYDRO-01         0.43707E-02    0.61515E-04    0.13374         2174.0

BLOCK:  DIST1    MODEL: RADFRAC
-------------------------------
   INLETS   - DIST1IN  STAGE   4
   OUTLETS  - WTRVAPOR STAGE   1
              BDODIST  STAGE   1
              OLESOLID STAGE   7
```

```
PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
 TOTAL BALANCE
    MOLE(LBMOL/HR)            303.707         303.707        0.321905E-07
    MASS(LB/HR   )            14277.9         14277.7        0.126836E-04
    ENTHALPY(BTU/HR  )      -0.465619E+08   -0.429852E+08   -0.768161E-01


                      **********************
                      ****  INPUT DATA  ****
                      **********************

****   INPUT PARAMETERS   ****

 NUMBER OF STAGES                                               7
 ALGORITHM OPTION                                            3-PHASE
 ABSORBER OPTION                                             NO
 INITIALIZATION OPTION                                       STANDARD
 HYDRAULIC PARAMETER CALCULATIONS                            NO
 INSIDE LOOP CONVERGENCE METHOD                              BROYDEN
 DESIGN SPECIFICATION METHOD                                 NESTED
 MAXIMUM NO. OF OUTSIDE LOOP ITERATIONS                        25
 MAXIMUM NO. OF INSIDE LOOP ITERATIONS                         10
 MAXIMUM NUMBER OF FLASH ITERATIONS                            30
 FLASH TOLERANCE                                                0.000100000
 OUTSIDE LOOP CONVERGENCE TOLERANCE                             0.000100000

****   COL-SPECS   ****

 MASS VAPOR DIST / TOTAL DIST                                   0.28290
 MASS REFLUX RATIO                                              0.80000
 MASS DISTILLATE RATE           LB/HR                       11,994.5

**** L2-STAGES SPECIFICATIONS ****

 TWO LIQUID PHASE CALCULATIONS ARE PERFORMED FOR    STAGE  TO  STAGE
                                                        2          4

**** L2-COMPS SPECIFICATIONS ****

 KEY COMPONENTS IN THE SECOND LIQUID PHASE        COMPONENT
                                                   OLEIC-01

****    PROFILES   ****

 P-SPEC          STAGE   1  PRES, PSIA                      0.17474

**** TRAY VAPORIZATION EFFICIENCY ****

                 STAGE   1  EFFICIENCY                      0.70000
                         2                                  0.70000
                         3                                  0.60000
                         4                                  0.60000
                         5                                  0.60000
                         6                                  0.50000
                         7                                  0.50000

                       *******************
                       ****  RESULTS  ****
                       *******************


***   COMPONENT SPLIT FRACTIONS   ***

                          OUTLET STREAMS
```

```
                        --------------
             WTRVAPOR     BDODIST      OLESOLID
COMPONENT:
1:4-B-01    .15676E-02   .98544       .12993E-01
WATER       .97927       .20727E-01   .12192E-11
DEXTR-01    0.0000       .82006E-13   1.0000
CARBO-01    .98698       .13025E-01   .17851E-07
OLEIC-01    .58469E-09   .71656E-04   .99993
LYSIN-01    0.0000       .24874E-08   1.0000
GLYCI-01    0.0000       .34583E-11   1.0000
ISOLE-01    0.0000       0.0000       1.0000
LEUCI-01    0.0000       0.0000       1.0000
METHI-01    0.0000       0.0000       1.0000
L-PHE-01    .56644E-11   .79364E-06   1.0000
THREO-01    0.0000       0.0000       1.0000
TRYPT-01    0.0000       0.0000       1.0000
TYROS-01    0.0000       0.0000       1.0000
VALIN-01    0.0000       0.0000       1.0000
INOSI-01    0.0000       0.0000       1.0000
NIACI-01    .17105E-01   .98030       .25921E-02
POTAS-01    0.0000       0.0000       1.0000
MAGNE-01    0.0000       0.0000       1.0000
CALCI-01    0.0000       0.0000       1.0000
SULFU-01    .85865E-11   .41863E-06   1.0000
SODIU-01    0.0000       0.0000       1.0000
IRON        0.0000       0.0000       1.0000
ZINC        0.0000       0.0000       1.0000
MANGA-01    0.0000       0.0000       1.0000
COPPE-01    0.0000       0.0000       1.0000
CHROM-01    0.0000       0.0000       1.0000
MOLYB-01    0.0000       0.0000       1.0000
COBAL-01    0.0000       0.0000       1.0000
HYDRO-01    .99970       .30351E-03   0.0000


***    SUMMARY OF KEY RESULTS    ***

 TOP STAGE TEMPERATURE          F                        87.7735
 BOTTOM STAGE TEMPERATURE       F                       449.694
 TOP STAGE LIQUID FLOW          LBMOL/HR                208.512
 BOTTOM STAGE LIQUID FLOW       LBMOL/HR                 18.5950
 TOP STAGE VAPOR FLOW           LBMOL/HR                186.553
 BOILUP VAPOR FLOW              LBMOL/HR                316.143
 MOLAR REFLUX RATIO                                       0.38565
 MOLAR BOILUP RATIO                                      17.0015
 CONDENSER DUTY (W/O SUBCOOL)   BTU/HR           -7,944,120.
 REBOILER DUTY                  BTU/HR                    0.115206+08

****   MAXIMUM FINAL RELATIVE ERRORS   ****

 BUBBLE POINT                    0.12001E-02  STAGE=  1 PHASE=L1
 COMPONENT MASS BALANCE          0.40744E-04  STAGE=  7 COMP=SODIU-01
 ENERGY BALANCE                  0.66360E-04  STAGE=  1


****    PROFILES   ****

**NOTE** REPORTED VALUES FOR STAGE LIQUID AND VAPOR RATES ARE THE FLOWS
         FROM THE STAGE INCLUDING ANY SIDE PRODUCT.

                                         ENTHALPY
STAGE TEMPERATURE   PRESSURE             BTU/LBMOL           HEAT DUTY
      F             PSIA          LIQUID       VAPOR          BTU/HR

  1   87.773       0.17474      -0.21205E+06 -0.10374E+06  -.79441+07
  2   241.11       0.19221      -0.20872E+06 -0.14080E+06
  3   263.07       0.30221      -0.20765E+06 -0.14253E+06
  4   277.59       0.41221      -0.20444E+06 -0.14474E+06
  5   307.85       0.52221      -0.20321E+06 -0.17563E+06
  6   325.81       0.63221      -0.20874E+06 -0.17525E+06
  7   449.69       0.74221      -0.14691E+06 -0.17593E+06   .11521+08

STAGE     FLOW RATE                  FEED RATE               PRODUCT RATE
           LBMOL/HR                  LBMOL/HR                 LBMOL/HR
      LIQUID     VAPOR       LIQUID    VAPOR    MIXED      LIQUID    VAPOR
  1  208.5      186.6                                      98.5591  186.5531
  2  125.8      395.1
  3  127.0      411.0                22.4405
  4  381.4      389.7       281.2668
  5  393.1      362.8
  6  334.7      374.5
  7  18.59      316.1                                      18.5949

STAGE     FLOW RATE               ENTHALPY
           LBMOL/HR               BTU/LBMO
      LIQUID1    LIQUID2       LIQUID1    LIQUID2
  1  208.5      0.000     -0.21205E+06 -0.21205E+06
  2  125.8      0.000     -0.20872E+06 -0.20872E+06
  3  127.0      0.000     -0.20765E+06 -0.20765E+06
  4  381.4      0.000     -0.20444E+06 -0.20444E+06
  5  393.1      0.000     -0.20321E+06 -0.20321E+06
  6  334.7      0.000     -0.20874E+06 -0.20874E+06
  7  18.59      0.000     -0.14691E+06 -0.14691E+06

   ****  MASS FLOW PROFILES  ****

STAGE     FLOW RATE                  FEED RATE               PRODUCT RATE
           LB/HR                      LB/HR                    LB/HR
      LIQUID     VAPOR       LIQUID    VAPOR    MIXED      LIQUID    VAPOR
  1 0.1820E+05  3393.                                    8601.3121 3393.2320
  2 0.1134E+05 0.2159E+05
  3 0.1146E+05 0.2333E+05            419.3241
  4 0.3500E+05 0.2304E+05  .13859+05
  5 0.3626E+05 0.3272E+05
  6 0.3452E+05 0.3398E+05
  7  2283.     0.3224E+05                                2283.1436

STAGE     FLOW RATE
           LB/HR
      LIQUID1    LIQUID2
  1 0.1820E+05  0.000
  2 0.1134E+05  0.000
  3 0.1146E+05  0.000
  4 0.3500E+05  0.000
  5 0.3626E+05  0.000
  6 0.3452E+05  0.000
  7  2283.      0.000


                        ****   MOLE-X-PROFILE     ****
  STAGE     1:4-B-01      WATER         DEXTR-01      CARBO-01      OLEIC-01
     1     0.95957       0.39802E-01   0.20649E-14   0.33122E-04   0.23921E-05
     2     0.99910       0.59335E-03   0.89534E-10   0.86873E-07   0.62605E-04
     3     0.99827       0.73468E-03   0.98753E-06   0.14075E-06   0.76538E-03
     4     0.95278       0.86976E-03   0.65091E-02   0.10656E-05   0.92708E-02
     5     0.95265       0.15518E-05   0.63201E-02   0.19188E-08   0.11354E-01
     6     0.87425       0.36205E-08   0.16429E-01   0.35425E-09   0.62555E-01
     7     0.67057E-01   0.12409E-10   0.13346       0.24061E-09   0.17693

                        ****   MOLE-X-PROFILE     ****
  STAGE     LYSIN-01      GLYCI-01      ISOLE-01      LEUCI-01      METHI-01
     1     0.70629E-11   0.19123E-13   0.15270E-61   0.15270E-61   0.13424E-61
     2     0.63080E-08   0.16037E-09   0.78290E-42   0.78290E-42   0.68825E-42
     3     0.24182E-05   0.53875E-06   0.24940E-22   0.24940E-22   0.21925E-22
     4     0.73567E-03   0.14296E-02   0.81788E-03   0.81788E-03   0.71900E-03
     5     0.72659E-03   0.13874E-02   0.79340E-03   0.79340E-03   0.69748E-03
     6     0.44543E-02   0.22842E-02   0.93177E-03   0.93177E-03   0.81913E-03
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   7     0.15050E-01   0.29309E-01   0.16773E-01   0.16773E-01   0.14745E-01

                       ****   MOLE-X-PROFILE     ****
STAGE     L-PHE-01      THREO-01      TRYPT-01      TYROS-01      VALIN-01
   1     0.19943E-08   0.16815E-61   0.98080E-62   0.11055E-61   0.17099E-61
   2     0.22450E-06   0.86213E-42   0.50285E-42   0.56679E-42   0.87664E-42
   3     0.12716E-04   0.27464E-22   0.16019E-22   0.18055E-22   0.27926E-22
   4     0.65777E-03   0.90064E-03   0.52532E-03   0.59211E-03   0.91581E-03
   5     0.68945E-03   0.87369E-03   0.50960E-03   0.57439E-03   0.88840E-03
   6     0.44345E-02   0.10261E-02   0.59847E-03   0.67456E-03   0.10433E-02
   7     0.13319E-01   0.18470E-01   0.10773E-01   0.12143E-01   0.18781E-01

                       ****   MOLE-X-PROFILE     ****
STAGE     INOSI-01      NIACI-01      POTAS-01      MAGNE-01      CALCI-01
   1     0.35833E-22   0.59104E-03   0.42607E-17   0.17852E-31   0.67014E-45
   2     0.26114E-15   0.23904E-03   0.56115E-12   0.22868E-21   0.29703E-30
   3     0.43609E-09   0.18016E-03   0.35121E-07   0.99932E-12   0.31388E-16
   4     0.41357E-03   0.17876E-03   0.19944E-02   0.32082E-02   0.19456E-02
   5     0.40120E-03   0.10544E-03   0.19347E-02   0.31122E-02   0.18874E-02
   6     0.48101E-03   0.65195E-04   0.22836E-02   0.36550E-02   0.22165E-02
   7     0.84814E-02   0.82834E-05   0.40899E-01   0.65793E-01   0.39900E-01

                       ****   MOLE-X-PROFILE     ****
STAGE     SULFU-01      SODIU-01      IRON          ZINC          MANGA-01
   1     0.39388E-08   0.63408E-23   0.26069E-61   0.50949E-28   0.26500E-61
   2     0.48977E-06   0.10085E-15   0.13365E-41   0.32925E-19   0.13586E-41
   3     0.34562E-04   0.64676E-09   0.42576E-22   0.72935E-11   0.43280E-22
   4     0.24500E-02   0.33918E-02   0.13962E-02   0.11925E-02   0.14193E-02
   5     0.24062E-02   0.32903E-02   0.13545E-02   0.11568E-02   0.13769E-02
   6     0.68934E-02   0.38647E-02   0.15907E-02   0.13586E-02   0.16170E-02
   7     0.49869E-01   0.69557E-01   0.28633E-01   0.24455E-01   0.29107E-01

                       ****   MOLE-X-PROFILE     ****
STAGE     COPPE-01      CHROM-01      MOLYB-01      COBAL-01      HYDRO-01
   1     0.22910E-61   0.27999E-61   0.15175E-61   0.24703E-61   0.25116E-05
   2     0.11746E-41   0.14355E-41   0.77800E-42   0.12665E-41   0.53813E-06
   3     0.37418E-22   0.45729E-22   0.24784E-22   0.40346E-22   0.88126E-06
   4     0.12271E-02   0.14996E-02   0.81276E-03   0.13231E-02   0.26536E-07
   5     0.11904E-02   0.14548E-02   0.78843E-03   0.12835E-02   0.19564E-10
   6     0.13980E-02   0.17085E-02   0.92594E-03   0.15074E-02   0.23646E-13
   7     0.25164E-01   0.30754E-01   0.16668E-01   0.27134E-01   0.22345E-16

                       ****   MOLE-X1-PROFILE    ****
STAGE     1:4-B-01      WATER         DEXTR-01      CARBO-01      OLEIC-01
   1     0.95957       0.39802E-01   0.20649E-14   0.33122E-04   0.23921E-05
   2     0.99910       0.59335E-03   0.89534E-10   0.86873E-07   0.62605E-04
   3     0.99827       0.73468E-03   0.98753E-06   0.14075E-06   0.76538E-03
   4     0.95278       0.86976E-03   0.65091E-02   0.10656E-05   0.92708E-02
   5     0.95265       0.15518E-05   0.63201E-02   0.19188E-08   0.11354E-01
   6     0.87425       0.36205E-08   0.16429E-01   0.35425E-09   0.62555E-01
   7     0.67057E-01   0.12409E-10   0.13346       0.24061E-09   0.17693

                       ****   MOLE-X1-PROFILE    ****
STAGE     LYSIN-01      GLYCI-01      ISOLE-01      LEUCI-01      METHI-01
   1     0.70629E-11   0.19123E-13   0.15270E-61   0.15270E-61   0.13424E-61
   2     0.63080E-08   0.16037E-09   0.78290E-42   0.78290E-42   0.68825E-42
   3     0.24182E-05   0.53875E-06   0.24940E-22   0.24940E-22   0.21925E-22
   4     0.73567E-03   0.14296E-02   0.81788E-03   0.81788E-03   0.71900E-03
   5     0.72659E-03   0.13874E-02   0.79340E-03   0.79340E-03   0.69748E-03
   6     0.44543E-02   0.22842E-02   0.93177E-03   0.93177E-03   0.81913E-03
   7     0.15050E-01   0.29309E-01   0.16773E-01   0.16773E-01   0.14745E-01

                       ****   MOLE-X1-PROFILE    ****
STAGE     L-PHE-01      THREO-01      TRYPT-01      TYROS-01      VALIN-01
   1     0.19943E-08   0.16815E-61   0.98080E-62   0.11055E-61   0.17099E-61
   2     0.22450E-06   0.86213E-42   0.50285E-42   0.56679E-42   0.87664E-42
   3     0.12716E-04   0.27464E-22   0.16019E-22   0.18055E-22   0.27926E-22
   4     0.65777E-03   0.90064E-03   0.52532E-03   0.59211E-03   0.91581E-03
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   5     0.68945E-03   0.87369E-03   0.50960E-03   0.57439E-03   0.88840E-03
   6     0.44345E-02   0.10261E-02   0.59847E-03   0.67456E-03   0.10433E-02
   7     0.13319E-01   0.18470E-01   0.10773E-01   0.12143E-01   0.18781E-01

                       ****   MOLE-X1-PROFILE    ****
STAGE     INOSI-01      NIACI-01      POTAS-01      MAGNE-01      CALCI-01
   1     0.35833E-22   0.59104E-03   0.42607E-17   0.17852E-31   0.67014E-45
   2     0.26114E-15   0.23904E-03   0.56115E-12   0.22868E-21   0.29703E-30
   3     0.43609E-09   0.18016E-03   0.35121E-07   0.99932E-12   0.31388E-16
   4     0.41357E-03   0.17876E-03   0.19944E-02   0.32082E-02   0.19456E-02
   5     0.40120E-03   0.10544E-03   0.19347E-02   0.31122E-02   0.18874E-02
   6     0.48101E-03   0.65195E-04   0.22836E-02   0.36550E-02   0.22165E-02
   7     0.84814E-02   0.82834E-05   0.40899E-01   0.65793E-01   0.39900E-01

                       ****   MOLE-X1-PROFILE    ****
STAGE     SULFU-01      SODIU-01      IRON          ZINC          MANGA-01
   1     0.39388E-08   0.63408E-23   0.26069E-61   0.50949E-28   0.26500E-61
   2     0.48977E-06   0.10085E-15   0.13365E-41   0.32925E-19   0.13586E-41
   3     0.34562E-04   0.64676E-09   0.42576E-22   0.72935E-11   0.43280E-22
   4     0.24500E-02   0.33918E-02   0.13962E-02   0.11925E-02   0.14193E-02
   5     0.24062E-02   0.32903E-02   0.13545E-02   0.11568E-02   0.13769E-02
   6     0.68934E-02   0.38647E-02   0.15907E-02   0.13586E-02   0.16170E-02
   7     0.49869E-01   0.69557E-01   0.28633E-01   0.24455E-01   0.29107E-01

                       ****   MOLE-X1-PROFILE    ****
STAGE     COPPE-01      CHROM-01      MOLYB-01      COBAL-01      HYDRO-01
   1     0.22910E-61   0.27999E-61   0.15175E-61   0.24703E-61   0.25116E-05
   2     0.11746E-41   0.14355E-41   0.77800E-42   0.12665E-41   0.53813E-06
   3     0.37418E-22   0.45729E-22   0.24784E-22   0.40346E-22   0.88126E-06
   4     0.12271E-02   0.14996E-02   0.81276E-03   0.13231E-02   0.26536E-07
   5     0.11904E-02   0.14548E-02   0.78843E-03   0.12835E-02   0.19564E-10
   6     0.13980E-02   0.17085E-02   0.92594E-03   0.15074E-02   0.23646E-13
   7     0.25164E-01   0.30754E-01   0.16668E-01   0.27134E-01   0.22345E-16

                       ****   MOLE-X2-PROFILE    ****
STAGE     1:4-B-01      WATER         DEXTR-01      CARBO-01      OLEIC-01
   1     0.95957       0.39802E-01   0.20649E-14   0.33122E-04   0.23921E-05
   2     0.99910       0.59335E-03   0.89534E-10   0.86873E-07   0.62605E-04
   3     0.99827       0.73468E-03   0.98753E-06   0.14075E-06   0.76538E-03
   4     0.95278       0.86976E-03   0.65091E-02   0.10656E-05   0.92708E-02
   5     0.95265       0.15518E-05   0.63201E-02   0.19188E-08   0.11354E-01
   6     0.87425       0.36205E-08   0.16429E-01   0.35425E-09   0.62555E-01
   7     0.67057E-01   0.12409E-10   0.13346       0.24061E-09   0.17693

                       ****   MOLE-X2-PROFILE    ****
STAGE     LYSIN-01      GLYCI-01      ISOLE-01      LEUCI-01      METHI-01
   1     0.70629E-11   0.19123E-13   0.15270E-61   0.15270E-61   0.13424E-61
   2     0.63080E-08   0.16037E-09   0.78290E-42   0.78290E-42   0.68825E-42
   3     0.24182E-05   0.53875E-06   0.24940E-22   0.24940E-22   0.21925E-22
   4     0.73567E-03   0.14296E-02   0.81788E-03   0.81788E-03   0.71900E-03
   5     0.72659E-03   0.13874E-02   0.79340E-03   0.79340E-03   0.69748E-03
   6     0.44543E-02   0.22842E-02   0.93177E-03   0.93177E-03   0.81913E-03
   7     0.15050E-01   0.29309E-01   0.16773E-01   0.16773E-01   0.14745E-01

                       ****   MOLE-X2-PROFILE    ****
STAGE     L-PHE-01      THREO-01      TRYPT-01      TYROS-01      VALIN-01
   1     0.19943E-08   0.16815E-61   0.98080E-62   0.11055E-61   0.17099E-61
   2     0.22450E-06   0.86213E-42   0.50285E-42   0.56679E-42   0.87664E-42
   3     0.12716E-04   0.27464E-22   0.16019E-22   0.18055E-22   0.27926E-22
   4     0.65777E-03   0.90064E-03   0.52532E-03   0.59211E-03   0.91581E-03
   5     0.68945E-03   0.87369E-03   0.50960E-03   0.57439E-03   0.88840E-03
   6     0.44345E-02   0.10261E-02   0.59847E-03   0.67456E-03   0.10433E-02
   7     0.13319E-01   0.18470E-01   0.10773E-01   0.12143E-01   0.18781E-01

                       ****   MOLE-X2-PROFILE    ****
STAGE     INOSI-01      NIACI-01      POTAS-01      MAGNE-01      CALCI-01
   1     0.35833E-22   0.59104E-03   0.42607E-17   0.17852E-31   0.67014E-45
   2     0.26114E-15   0.23904E-03   0.56115E-12   0.22868E-21   0.29703E-30
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   3    0.43609E-09   0.18016E-03   0.35121E-07   0.99932E-12   0.31388E-16
   4    0.41357E-03   0.17876E-03   0.19944E-02   0.32082E-02   0.19456E-02
   5    0.40120E-03   0.10544E-03   0.19347E-02   0.31122E-02   0.18874E-02
   6    0.48101E-03   0.65195E-04   0.22836E-02   0.36550E-02   0.22165E-02
   7    0.84814E-02   0.82834E-05   0.40899E-01   0.65793E-01   0.39900E-01

                      ****   MOLE-X2-PROFILE    ****
STAGE     SULFU-01      SODIU-01      IRON          ZINC          MANGA-01
   1    0.39388E-08   0.63408E-23   0.26069E-61   0.50949E-28   0.26500E-61
   2    0.48977E-06   0.10085E-15   0.13365E-41   0.32925E-19   0.13586E-41
   3    0.34562E-04   0.64676E-09   0.42576E-22   0.72935E-11   0.43280E-22
   4    0.24500E-02   0.33918E-02   0.13962E-02   0.11925E-02   0.14193E-02
   5    0.24062E-02   0.32903E-02   0.13545E-02   0.11568E-02   0.13769E-02
   6    0.68934E-02   0.38647E-02   0.15907E-02   0.13586E-02   0.16170E-02
   7    0.49869E-01   0.69557E-01   0.28633E-01   0.24455E-01   0.29107E-01

                      ****   MOLE-X2-PROFILE    ****
STAGE     COPPE-01      CHROM-01      MOLYB-01      COBAL-01      HYDRO-01
   1    0.22910E-61   0.27999E-61   0.15175E-61   0.24703E-61   0.25116E-05
   2    0.11746E-41   0.14355E-41   0.77800E-42   0.12665E-41   0.53813E-06
   3    0.37418E-22   0.45729E-22   0.24784E-22   0.40346E-22   0.88126E-06
   4    0.12271E-02   0.14996E-02   0.81276E-03   0.13231E-02   0.26536E-07
   5    0.11904E-02   0.14548E-02   0.78843E-03   0.12835E-02   0.19564E-10
   6    0.13980E-02   0.17085E-02   0.92594E-03   0.15074E-02   0.23646E-13
   7    0.25164E-01   0.30754E-01   0.16668E-01   0.27134E-01   0.22345E-16

                      ****   MOLE-Y-PROFILE     ****
STAGE     1:4-B-01      WATER         DEXTR-01      CARBO-01      OLEIC-01
   1    0.80643E-03   0.99349       0.38206E-25   0.13260E-02   0.10312E-10
   2    0.50684       0.49014       0.10898E-14   0.64363E-03   0.12625E-05
   3    0.53644       0.46072       0.27417E-10   0.60991E-03   0.19744E-04
   4    0.56848       0.43035       0.32193E-06   0.64316E-03   0.25012E-03
   5    0.99819       0.91434E-03   0.14555E-05   0.11202E-05   0.67628E-03
   6    0.99662       0.16288E-05   0.74743E-05   0.20021E-08   0.31326E-02
   7    0.92173       0.38327E-08   0.95447E-02   0.36093E-09   0.55828E-01

                      ****   MOLE-Y-PROFILE     ****
STAGE     LYSIN-01      GLYCI-01      ISOLE-01      LEUCI-01      METHI-01
   1    0.67440E-18   0.16159E-21   0.26925E-81   0.26925E-81   0.23670E-81
   2    0.37277E-11   0.10093E-13   0.80595E-62   0.80595E-62   0.70852E-62
   3    0.19333E-08   0.49113E-10   0.23974E-42   0.23974E-42   0.21075E-42
   4    0.78833E-06   0.17563E-06   0.81303E-23   0.81303E-23   0.71474E-23
   5    0.18830E-05   0.44894E-06   0.79973E-23   0.79973E-23   0.70305E-23
   6    0.15403E-04   0.10359E-05   0.94269E-23   0.94269E-23   0.82873E-23
   7    0.38311E-02   0.69452E-03   0.16772E-21   0.16772E-21   0.14745E-21

                      ****   MOLE-Y-PROFILE     ****
STAGE     L-PHE-01      THREO-01      TRYPT-01      TYROS-01      VALIN-01
   1    0.75199E-14   0.29650E-81   0.17294E-81   0.19493E-81   0.30149E-81
   2    0.10526E-08   0.88751E-62   0.51766E-62   0.58348E-62   0.90245E-62
   3    0.69224E-07   0.26400E-42   0.15398E-42   0.17356E-42   0.26844E-42
   4    0.41459E-05   0.89530E-23   0.52220E-23   0.58860E-23   0.91037E-23
   5    0.87452E-05   0.88066E-23   0.51366E-23   0.57897E-23   0.89549E-23
   6    0.62386E-04   0.10381E-22   0.60549E-23   0.68247E-23   0.10556E-22
   7    0.39119E-02   0.18470E-21   0.10773E-21   0.12142E-21   0.18781E-21

                      ****   MOLE-Y-PROFILE     ****
STAGE     INOSI-01      NIACI-01      POTAS-01      MAGNE-01      CALCI-01
   1    0.13462E-35   0.54486E-05   0.88345E-26   0.20038E-46   0.11816E-64
   2    0.18912E-22   0.31452E-03   0.22488E-17   0.94221E-32   0.35370E-45
   3    0.79964E-16   0.21742E-03   0.17183E-12   0.70024E-22   0.90956E-31
   4    0.14217E-09   0.21078E-03   0.11449E-07   0.32577E-12   0.10233E-16
   5    0.73531E-09   0.18750E-03   0.23748E-07   0.10254E-11   0.49904E-16
   6    0.18790E-08   0.11027E-03   0.28459E-07   0.15457E-11   0.95835E-16
   7    0.10416E-04   0.68543E-04   0.12139E-04   0.26824E-08   0.66063E-12

                      ****   MOLE-Y-PROFILE     ****
STAGE     SULFU-01      SODIU-01      IRON          ZINC          MANGA-01
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   1    0.42682E-13   0.24204E-34   0.45965E-81   0.10697E-41   0.46726E-81
   2    0.20789E-08   0.33466E-23   0.13759E-61   0.26891E-28   0.13986E-61
   3    0.15092E-06   0.30882E-16   0.40927E-42   0.10082E-19   0.41604E-42
   4    0.11268E-04   0.21084E-09   0.13880E-22   0.23777E-11   0.14109E-22
   5    0.19164E-04   0.53626E-09   0.13653E-22   0.73938E-11   0.13878E-22
   6    0.49589E-04   0.71667E-09   0.16093E-22   0.11053E-10   0.16359E-22
   7    0.43656E-02   0.59921E-06   0.28633E-21   0.17845E-07   0.33299E-19

                      ****   MOLE-Y-PROFILE     ****
STAGE     COPPE-01      CHROM-01      MOLYB-01      COBAL-01      HYDRO-01
   1    0.40396E-81   0.49370E-81   0.26757E-81   0.43558E-81   0.43707E-02
   2    0.12092E-61   0.14778E-61   0.80091E-62   0.13038E-61   0.20652E-02
   3    0.35968E-42   0.43958E-42   0.23824E-42   0.38784E-42   0.19848E-02
   4    0.12198E-22   0.14908E-22   0.80794E-23   0.13153E-22   0.44088E-04
   5    0.11999E-22   0.14664E-22   0.79472E-23   0.12938E-22   0.27897E-07
   6    0.14143E-22   0.17285E-22   0.93679E-23   0.15250E-22   0.20535E-10
   7    0.25164E-21   0.30754E-21   0.16667E-21   0.27133E-21   0.25036E-13

                      ****   K-VALUES: V-L1     ****
STAGE     1:4-B-01    WATER       DEXTR-01     CARBO-01    OLEIC-01    LYSIN-01
   1    1.1961-03     35.7011   2.6143-11     57.4299   6.1188-06   1.3568-07
   2       0.7245   1180.3318   1.7360-05   1.0589+04   2.8791-02   8.4360-04
   3       0.8958   1045.0377   4.6318-05   7218.9952   4.3011-02   1.3330-03
   4       0.9943    824.6937   8.2399-05   1005.6652   4.4956-02   1.7857-03
   5       1.7462    982.0663   3.8368-04    972.4429   9.9255-02   4.3186-03
   6       2.2797    899.8574   9.0929-04     11.2980      0.1001   6.9137-03
   7      27.4909    617.7070      0.1430      3.0002      0.6311      0.5091

                      ****   K-VALUES: V-L1     ****
STAGE     GLYCI-01    ISOLE-01    LEUCI-01    METHI-01    L-PHE-01    THREO-01
   1    1.1991-08      0.0         0.0         0.0      5.3676-06      0.0
   2    8.9831-05      0.0         0.0         0.0      6.6946-03      0.0
   3    1.5202-04      0.0         0.0         0.0      9.0756-03      0.0
   4    2.0471-04      0.0         0.0         0.0      1.0504-02      0.0
   5    5.3920-04      0.0         0.0         0.0      2.1138-02      0.0
   6    9.0666-04      0.0         0.0         0.0      2.8130-02      0.0
   7    4.7393-02      0.0         0.0         0.0         0.5874      0.0

                      ****   K-VALUES: V-L1     ****
STAGE     TRYPT-01    TYROS-01    VALIN-01    INOSI-01    NIACI-01    POTAS-01
   1       0.0         0.0         0.0      5.3025-14   1.3159-02   2.9497-09
   2       0.0         0.0         0.0      1.0328-07      1.8795   5.7217-06
   3       0.0         0.0         0.0      3.0594-07      2.0114   8.1571-06
   4       0.0         0.0         0.0      5.7267-07      1.9651   9.5666-06
   5       0.0         0.0         0.0      3.0534-06      2.9636   2.0455-05
   6       0.0         0.0         0.0      7.8064-06      3.3825   2.4918-05
   7       0.0         0.0         0.0      2.4562-03     16.5495   5.9359-04

                      ****   K-VALUES: V-L1     ****
STAGE     MAGNE-01    CALCI-01    SULFU-01    SODIU-01    IRON        ZINC
   1    1.5911-15   1.5248-21   1.5446-05   5.4202-12      0.0      2.9756-14
   2    5.8795-11   1.6982-15   6.0619-03   4.7365-08      0.0      1.1654-09
   3    1.1687-10   4.8346-15   7.2790-03   7.9622-08      0.0      2.3054-09
   4    1.6919-10   8.7618-15   7.6646-03   1.0358-07      0.0      3.3222-09
   5    5.4897-10   4.4049-14   1.3273-02   2.7158-07      0.0      1.0650-08
   6    8.4539-10   8.6406-14   1.4385-02   3.7074-07      0.0      1.6264-08
   7    8.1541-08   3.3115-11      0.1751   1.7229-05   3.5081-32   1.4595-06

                      ****   K-VALUES: V-L1     ****
STAGE     MANGA-01    COPPE-01    CHROM-01    MOLYB-01    COBAL-01    HYDRO-01
   1    4.9881-33      0.0         0.0         0.0         0.0      2495.6007
   2    1.2233-24      0.0         0.0         0.0         0.0      5486.0399
   3    6.3161-24      0.0         0.0         0.0         0.0      3752.3043
   4    1.6662-23      0.0         0.0         0.0         0.0      2769.4084
   5    1.7552-22      0.0         0.0         0.0         0.0      2376.8310
   6    5.2073-22      0.0         0.0         0.0         0.0      1737.4112
   7    2.2881-18      0.0         0.0         0.0         0.0      2240.8228
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                  ****   K-VALUES: V-L2     ****
STAGE     1:4-B-01    WATER       DEXTR-01    CARBO-01    OLEIC-01    LYSIN-01
   1      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   2      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   3      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   4      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   5      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   6      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   7      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING

                  ****   K-VALUES: V-L2     ****
STAGE     GLYCI-01    ISOLE-01    LEUCI-01    METHI-01    L-PHE-01    THREO-01
   1      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   2      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   3      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   4      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   5      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   6      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   7      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING

                  ****   K-VALUES: V-L2     ****
STAGE     TRYPT-01    TYROS-01    VALIN-01    INOSI-01    NIACI-01    POTAS-01
   1      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   2      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   3      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   4      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   5      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   6      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   7      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING

                  ****   K-VALUES: V-L2     ****
STAGE     MAGNE-01    CALCI-01    SULFU-01    SODIU-01    IRON        ZINC
   1      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   2      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   3      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   4      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   5      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   6      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   7      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING

                  ****   K-VALUES: V-L2     ****
STAGE     MANGA-01    COPPE-01    CHROM-01    MOLYB-01    COBAL-01    HYDRO-01
   1      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   2      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   3      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   4      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   5      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   6      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   7      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING

                  ****   K-VALUES: L2-L1    ****
STAGE     1:4-B-01    WATER       DEXTR-01    CARBO-01    OLEIC-01    LYSIN-01
   1      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   2      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   3      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   4      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   5      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   6      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   7      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING

                  ****   K-VALUES: L2-L1    ****
STAGE     GLYCI-01    ISOLE-01    LEUCI-01    METHI-01    L-PHE-01    THREO-01
   1      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   2      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   3      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   4      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   5      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   6      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   7      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING

                  ****   K-VALUES: L2-L1    ****
STAGE     TRYPT-01    TYROS-01    VALIN-01    INOSI-01    NIACI-01    POTAS-01
   1      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   2      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   3      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   4      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   5      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   6      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   7      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING

                  ****   K-VALUES: L2-L1    ****
STAGE     MAGNE-01    CALCI-01    SULFU-01    SODIU-01    IRON        ZINC
   1      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   2      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   3      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   4      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   5      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   6      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   7      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING

                  ****   K-VALUES: L2-L1    ****
STAGE     MANGA-01    COPPE-01    CHROM-01    MOLYB-01    COBAL-01    HYDRO-01
   1      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   2      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   3      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   4      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   5      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   6      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING
   7      MISSING     MISSING     MISSING     MISSING     MISSING     MISSING

                  ****   MASS-X-PROFILE     ****
STAGE      1:4-B-01      WATER         DEXTR-01      CARBO-01      OLEIC-01
   1     0.99092       0.82162E-02   0.42626E-14   0.16703E-04   0.77425E-05
   2     0.99936       0.11864E-03   0.17903E-09   0.42434E-07   0.19627E-03
   3     0.99717       0.14670E-03   0.19719E-05   0.68659E-07   0.23963E-02
   4     0.93556       0.17072E-03   0.12777E-01   0.51097E-06   0.28532E-01
   5     0.93070       0.30305E-06   0.12343E-01   0.91541E-09   0.34766E-01
   6     0.76403       0.63248E-09   0.28701E-01   0.15118E-09   0.17135
   7     0.49220E-01   0.18208E-11   0.19582       0.86243E-10   0.40703

                  ****   MASS-X-PROFILE     ****
STAGE      LYSIN-01      GLYCI-01      ISOLE-01      LEUCI-01      METHI-01
   1     0.11831E-10   0.16449E-13   0.22952E-61   0.22952E-61   0.22952E-61
   2     0.10235E-07   0.13362E-09   0.11398E-41   0.11398E-41   0.11398E-41
   3     0.39183E-05   0.44826E-06   0.36260E-22   0.36260E-22   0.36260E-22
   4     0.11718E-02   0.11693E-02   0.11689E-02   0.11689E-02   0.11689E-02
   5     0.11515E-02   0.11290E-02   0.11282E-02   0.11282E-02   0.11282E-02
   6     0.63145E-02   0.16627E-02   0.11852E-02   0.11852E-02   0.11852E-02
   7     0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01

                  ****   MASS-X-PROFILE     ****
STAGE      L-PHE-01      THREO-01      TRYPT-01      TYROS-01      VALIN-01
   1     0.37749E-08   0.22952E-61   0.22952E-61   0.22952E-61   0.22952E-61
   2     0.41161E-06   0.11398E-41   0.11398E-41   0.11398E-41   0.11398E-41
   3     0.23283E-04   0.36260E-22   0.36260E-22   0.36260E-22   0.36260E-22
   4     0.11839E-02   0.11689E-02   0.11689E-02   0.11689E-02   0.11689E-02
   5     0.12346E-02   0.11282E-02   0.11282E-02   0.11282E-02   0.11282E-02
   6     0.71036E-02   0.11852E-02   0.11852E-02   0.11852E-02   0.11852E-02
   7     0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01

                  ****   MASS-X-PROFILE     ****
STAGE      INOSI-01      NIACI-01      POTAS-01      MAGNE-01      CALCI-01
   1     0.73972E-22   0.83377E-03   0.19089E-17   0.49718E-32   0.30776E-45
   2     0.52215E-15   0.32662E-03   0.24351E-12   0.61687E-22   0.13213E-30
   3     0.87081E-09   0.24584E-03   0.15220E-07   0.26921E-12   0.13943E-16
   4     0.81181E-03   0.23978E-03   0.84959E-03   0.84958E-03   0.84958E-03
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   5    0.78353E-03   0.14072E-03   0.82000E-03   0.81999E-03   0.81999E-03
   6    0.84032E-03   0.77832E-04   0.86579E-03   0.86144E-03   0.86144E-03
   7    0.12445E-01   0.83055E-05   0.13024E-01   0.13024E-01   0.13024E-01

                      ****   MASS-X-PROFILE     ****
STAGE     SULFU-01      SODIU-01      IRON          ZINC          MANGA-01
   1    0.14472E-08   0.16704E-23   0.16682E-61   0.38175E-28   0.16682E-61
   2    0.17431E-06   0.25733E-16   0.82843E-42   0.23895E-19   0.82843E-42
   3    0.12284E-04   0.16480E-09   0.26354E-22   0.52862E-11   0.26354E-22
   4    0.85595E-03   0.84958E-03   0.84958E-03   0.84958E-03   0.84958E-03
   5    0.83641E-03   0.81999E-03   0.81999E-03   0.81999E-03   0.81999E-03
   6    0.21435E-02   0.86157E-03   0.86144E-03   0.86145E-03   0.86144E-03
   7    0.13024E-01   0.13024E-01   0.13024E-01   0.13024E-01   0.13024E-01

                      ****   MASS-X-PROFILE     ****
STAGE     COPPE-01      CHROM-01      MOLYB-01      COBAL-01      HYDRO-01
   1    0.16682E-61   0.16682E-61   0.16682E-61   0.16682E-61   0.10493E-05
   2    0.82843E-42   0.82843E-42   0.82843E-42   0.82843E-42   0.21777E-06
   3    0.26354E-22   0.26354E-22   0.26354E-22   0.26354E-22   0.35614E-06
   4    0.84958E-03   0.84958E-03   0.84958E-03   0.84958E-03   0.10542E-07
   5    0.81999E-03   0.81999E-03   0.81999E-03   0.81999E-03   0.77326E-11
   6    0.86144E-03   0.86144E-03   0.86144E-03   0.86144E-03   0.83604E-14
   7    0.13024E-01   0.13024E-01   0.13024E-01   0.13024E-01   0.66354E-17

                      ****   MASS-X1-PROFILE    ****
STAGE     1:4-B-01      WATER         DEXTR-01      CARBO-01      OLEIC-01
   1    0.99092       0.82162E-02   0.42626E-14   0.16703E-04   0.77425E-05
   2    0.99936       0.11864E-03   0.17903E-09   0.42434E-07   0.19627E-03
   3    0.99717       0.14670E-03   0.19719E-05   0.68659E-07   0.23963E-02
   4    0.93556       0.17072E-03   0.12777E-01   0.51097E-06   0.28532E-01
   5    0.93070       0.30305E-06   0.12343E-01   0.91541E-09   0.34766E-01
   6    0.76403       0.63248E-09   0.28701E-01   0.15118E-09   0.17135
   7    0.49220E-01   0.18208E-11   0.19582       0.86243E-10   0.40703

                      ****   MASS-X1-PROFILE    ****
STAGE     LYSIN-01      GLYCI-01      ISOLE-01      LEUCI-01      METHI-01
   1    0.11831E-10   0.16449E-13   0.22952E-61   0.22952E-61   0.22952E-61
   2    0.10235E-07   0.13362E-09   0.11398E-41   0.11398E-41   0.11398E-41
   3    0.39183E-05   0.44826E-06   0.36260E-22   0.36260E-22   0.36260E-22
   4    0.11718E-02   0.11693E-02   0.11689E-02   0.11689E-02   0.11689E-02
   5    0.11515E-02   0.11290E-02   0.11282E-02   0.11282E-02   0.11282E-02
   6    0.63145E-02   0.16627E-02   0.11852E-02   0.11852E-02   0.11852E-02
   7    0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01

                      ****   MASS-X1-PROFILE    ****
STAGE     L-PHE-01      THREO-01      TRYPT-01      TYROS-01      VALIN-01
   1    0.37749E-08   0.22952E-61   0.22952E-61   0.22952E-61   0.22952E-61
   2    0.41161E-06   0.11398E-41   0.11398E-41   0.11398E-41   0.11398E-41
   3    0.23283E-04   0.36260E-22   0.36260E-22   0.36260E-22   0.36260E-22
   4    0.11839E-02   0.11689E-02   0.11689E-02   0.11689E-02   0.11689E-02
   5    0.12346E-02   0.11282E-02   0.11282E-02   0.11282E-02   0.11282E-02
   6    0.71036E-02   0.11852E-02   0.11852E-02   0.11852E-02   0.11852E-02
   7    0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01

                      ****   MASS-X1-PROFILE    ****
STAGE     INOSI-01      NIACI-01      POTAS-01      MAGNE-01      CALCI-01
   1    0.73972E-22   0.83377E-03   0.19089E-17   0.49718E-32   0.30776E-45
   2    0.52215E-15   0.32662E-03   0.24351E-12   0.61687E-22   0.13213E-30
   3    0.87081E-09   0.24584E-03   0.15220E-07   0.26921E-12   0.13943E-16
   4    0.81181E-03   0.23978E-03   0.84959E-03   0.84958E-03   0.84958E-03
   5    0.78353E-03   0.14072E-03   0.82000E-03   0.81999E-03   0.81999E-03
   6    0.84032E-03   0.77832E-04   0.86579E-03   0.86144E-03   0.86144E-03
   7    0.12445E-01   0.83055E-05   0.13024E-01   0.13024E-01   0.13024E-01

                      ****   MASS-X1-PROFILE    ****
STAGE     SULFU-01      SODIU-01      IRON          ZINC          MANGA-01
   1    0.14472E-08   0.16704E-23   0.16682E-61   0.38175E-28   0.16682E-61
   2    0.17431E-06   0.25733E-16   0.82843E-42   0.23895E-19   0.82843E-42
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   3    0.12284E-04   0.16480E-09   0.26354E-22   0.52862E-11   0.26354E-22
   4    0.85595E-03   0.84958E-03   0.84958E-03   0.84958E-03   0.84958E-03
   5    0.83641E-03   0.81999E-03   0.81999E-03   0.81999E-03   0.81999E-03
   6    0.21435E-02   0.86157E-03   0.86144E-03   0.86145E-03   0.86144E-03
   7    0.13024E-01   0.13024E-01   0.13024E-01   0.13024E-01   0.13024E-01

                      ****   MASS-X1-PROFILE    ****
STAGE     COPPE-01      CHROM-01      MOLYB-01      COBAL-01      HYDRO-01
   1    0.16682E-61   0.16682E-61   0.16682E-61   0.16682E-61   0.10493E-05
   2    0.82843E-42   0.82843E-42   0.82843E-42   0.82843E-42   0.21777E-06
   3    0.26354E-22   0.26354E-22   0.26354E-22   0.26354E-22   0.35614E-06
   4    0.84958E-03   0.84958E-03   0.84958E-03   0.84958E-03   0.10542E-07
   5    0.81999E-03   0.81999E-03   0.81999E-03   0.81999E-03   0.77326E-11
   6    0.86144E-03   0.86144E-03   0.86144E-03   0.86144E-03   0.83604E-14
   7    0.13024E-01   0.13024E-01   0.13024E-01   0.13024E-01   0.66354E-17

                      ****   MASS-X2-PROFILE    ****
STAGE     1:4-B-01      WATER         DEXTR-01      CARBO-01      OLEIC-01
   1    0.99092       0.82162E-02   0.42626E-14   0.16703E-04   0.77425E-05
   2    0.99936       0.11864E-03   0.17903E-09   0.42434E-07   0.19627E-03
   3    0.99717       0.14670E-03   0.19719E-05   0.68659E-07   0.23963E-02
   4    0.93556       0.17072E-03   0.12777E-01   0.51097E-06   0.28532E-01
   5    0.93070       0.30305E-06   0.12343E-01   0.91541E-09   0.34766E-01
   6    0.76403       0.63248E-09   0.28701E-01   0.15118E-09   0.17135
   7    0.49220E-01   0.18208E-11   0.19582       0.86243E-10   0.40703

                      ****   MASS-X2-PROFILE    ****
STAGE     LYSIN-01      GLYCI-01      ISOLE-01      LEUCI-01      METHI-01
   1    0.11831E-10   0.16449E-13   0.22952E-61   0.22952E-61   0.22952E-61
   2    0.10235E-07   0.13362E-09   0.11398E-41   0.11398E-41   0.11398E-41
   3    0.39183E-05   0.44826E-06   0.36260E-22   0.36260E-22   0.36260E-22
   4    0.11718E-02   0.11693E-02   0.11689E-02   0.11689E-02   0.11689E-02
   5    0.11515E-02   0.11290E-02   0.11282E-02   0.11282E-02   0.11282E-02
   6    0.63145E-02   0.16627E-02   0.11852E-02   0.11852E-02   0.11852E-02
   7    0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01

                      ****   MASS-X2-PROFILE    ****
STAGE     L-PHE-01      THREO-01      TRYPT-01      TYROS-01      VALIN-01
   1    0.37749E-08   0.22952E-61   0.22952E-61   0.22952E-61   0.22952E-61
   2    0.41161E-06   0.11398E-41   0.11398E-41   0.11398E-41   0.11398E-41
   3    0.23283E-04   0.36260E-22   0.36260E-22   0.36260E-22   0.36260E-22
   4    0.11839E-02   0.11689E-02   0.11689E-02   0.11689E-02   0.11689E-02
   5    0.12346E-02   0.11282E-02   0.11282E-02   0.11282E-02   0.11282E-02
   6    0.71036E-02   0.11852E-02   0.11852E-02   0.11852E-02   0.11852E-02
   7    0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01   0.17919E-01

                      ****   MASS-X2-PROFILE    ****
STAGE     INOSI-01      NIACI-01      POTAS-01      MAGNE-01      CALCI-01
   1    0.73972E-22   0.83377E-03   0.19089E-17   0.49718E-32   0.30776E-45
   2    0.52215E-15   0.32662E-03   0.24351E-12   0.61687E-22   0.13213E-30
   3    0.87081E-09   0.24584E-03   0.15220E-07   0.26921E-12   0.13943E-16
   4    0.81181E-03   0.23978E-03   0.84959E-03   0.84958E-03   0.84958E-03
   5    0.78353E-03   0.14072E-03   0.82000E-03   0.81999E-03   0.81999E-03
   6    0.84032E-03   0.77832E-04   0.86579E-03   0.86144E-03   0.86144E-03
   7    0.12445E-01   0.83055E-05   0.13024E-01   0.13024E-01   0.13024E-01

                      ****   MASS-X2-PROFILE    ****
STAGE     SULFU-01      SODIU-01      IRON          ZINC          MANGA-01
   1    0.14472E-08   0.16704E-23   0.16682E-61   0.38175E-28   0.16682E-61
   2    0.17431E-06   0.25733E-16   0.82843E-42   0.23895E-19   0.82843E-42
   3    0.12284E-04   0.16480E-09   0.26354E-22   0.52862E-11   0.26354E-22
   4    0.85595E-03   0.84958E-03   0.84958E-03   0.84958E-03   0.84958E-03
   5    0.83641E-03   0.81999E-03   0.81999E-03   0.81999E-03   0.81999E-03
   6    0.21435E-02   0.86157E-03   0.86144E-03   0.86145E-03   0.86144E-03
   7    0.13024E-01   0.13024E-01   0.13024E-01   0.13024E-01   0.13024E-01

                      ****   MASS-X2-PROFILE    ****
STAGE     COPPE-01      CHROM-01      MOLYB-01      COBAL-01      HYDRO-01
```

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   1    0.16682E-61   0.16682E-61   0.16682E-61   0.16682E-61   0.10493E-05
   2    0.82843E-42   0.82843E-42   0.82843E-42   0.82843E-42   0.21777E-06
   3    0.26354E-22   0.26354E-22   0.26354E-22   0.26354E-22   0.35614E-06
   4    0.84958E-03   0.84958E-03   0.84958E-03   0.84958E-03   0.10542E-07
   5    0.81999E-03   0.81999E-03   0.81999E-03   0.81999E-03   0.77326E-11
   6    0.86144E-03   0.86144E-03   0.86144E-03   0.86144E-03   0.83604E-14
   7    0.13024E-01   0.13024E-01   0.13024E-01   0.13024E-01   0.66354E-17

                      ****   MASS-Y-PROFILE     ****
STAGE     1:4-B-01      WATER         DEXTR-01      CARBO-01      OLEIC-01
   1    0.39956E-02   0.98400       0.37842E-24   0.32084E-02   0.16014E-09
   2    0.83582       0.16157       0.35927E-14   0.51832E-03   0.65257E-05
   3    0.85150       0.14619       0.86997E-10   0.47276E-03   0.98228E-04
   4    0.86669       0.13115       0.98113E-06   0.47883E-03   0.11951E-02
   5    0.99741       0.18263E-03   0.29073E-05   0.54662E-06   0.21180E-02
   6    0.98993       0.32341E-06   0.14841E-04   0.97113E-09   0.97524E-02
   7    0.81466       0.67715E-09   0.16864E-01   0.15578E-09   0.15465

                      ****   MASS-Y-PROFILE     ****
STAGE     LYSIN-01      GLYCI-01      ISOLE-01      LEUCI-01      METHI-01
   1    0.54203E-17   0.66690E-21   0.19418E-80   0.19418E-80   0.19418E-80
   2    0.99718E-11   0.13864E-13   0.19345E-61   0.19345E-61   0.19345E-61
   3    0.49778E-08   0.64935E-10   0.55388E-42   0.55388E-42   0.55388E-42
   4    0.19496E-05   0.22303E-06   0.18041E-22   0.18041E-22   0.18041E-22
   5    0.30521E-05   0.37365E-06   0.11631E-22   0.11631E-22   0.11631E-22
   6    0.24817E-04   0.85703E-06   0.13629E-22   0.13629E-22   0.13629E-22
   7    0.54926E-02   0.51130E-03   0.21577E-21   0.21577E-21   0.21577E-21

                      ****   MASS-Y-PROFILE     ****
STAGE     L-PHE-01      THREO-01      TRYPT-01      TYROS-01      VALIN-01
   1    0.68295E-13   0.19418E-80   0.19418E-80   0.19418E-80   0.19418E-80
   2    0.31816E-08   0.19345E-61   0.19345E-61   0.19345E-61   0.19345E-61
   3    0.20141E-06   0.55388E-42   0.55388E-42   0.55388E-42   0.55388E-42
   4    0.11586E-04   0.18041E-22   0.18041E-22   0.18041E-22   0.18041E-22
   5    0.16017E-04   0.11631E-22   0.11631E-22   0.11631E-22   0.11631E-22
   6    0.11358E-03   0.13629E-22   0.13629E-22   0.13629E-22   0.13629E-22
   7    0.63375E-02   0.21577E-21   0.21577E-21   0.21577E-21   0.21577E-21

                      ****   MASS-Y-PROFILE     ****
STAGE     INOSI-01      NIACI-01      POTAS-01      MAGNE-01      CALCI-01
   1    0.13334E-34   0.36878E-04   0.18990E-25   0.26776E-46   0.26036E-64
   2    0.62346E-22   0.70852E-03   0.16088E-17   0.41904E-32   0.25939E-45
   3    0.25373E-15   0.47144E-03   0.11833E-12   0.29976E-22   0.64204E-31
   4    0.43327E-09   0.43898E-03   0.75727E-08   0.13394E-12   0.69375E-17
   5    0.14688E-08   0.25594E-03   0.10295E-07   0.27632E-12   0.22175E-16
   6    0.37309E-08   0.14962E-03   0.12263E-07   0.41407E-12   0.42332E-16
   7    0.18403E-04   0.82756E-04   0.46545E-05   0.63938E-09   0.25966E-12

                      ****   MASS-Y-PROFILE     ****
STAGE     SULFU-01      SODIU-01      IRON          ZINC          MANGA-01
   1    0.75244E-13   0.30592E-34   0.14113E-80   0.38454E-41   0.14113E-80
   2    0.12198E-08   0.14078E-23   0.14060E-61   0.32175E-28   0.14060E-61
   3    0.85236E-07   0.12505E-16   0.40256E-42   0.11611E-19   0.40256E-42
   4    0.61121E-05   0.81998E-10   0.13113E-22   0.26301E-11   0.13113E-22
   5    0.68132E-05   0.13669E-09   0.84537E-23   0.53605E-11   0.84537E-23
   6    0.17526E-04   0.18159E-09   0.99057E-23   0.79661E-11   0.99057E-23
   7    0.13729E-02   0.13510E-06   0.15682E-21   0.11444E-07   0.17941E-19

                      ****   MASS-Y-PROFILE     ****
STAGE     COPPE-01      CHROM-01      MOLYB-01      COBAL-01      HYDRO-01
   1    0.14113E-80   0.14113E-80   0.14113E-80   0.14113E-80   0.87612E-02
   2    0.14060E-61   0.14060E-61   0.14060E-61   0.14060E-61   0.13778E-02
   3    0.40256E-42   0.40256E-42   0.40256E-42   0.40256E-42   0.12746E-02
   4    0.13113E-22   0.13113E-22   0.13113E-22   0.13113E-22   0.27193E-04
   5    0.84537E-23   0.84537E-23   0.84537E-23   0.84537E-23   0.11277E-07
   6    0.99057E-23   0.99057E-23   0.99057E-23   0.99057E-23   0.82522E-11
   7    0.15682E-21   0.15682E-21   0.15682E-21   0.15682E-21   0.89520E-14
```

```
                      ****   VAPORIZATION EFF   ****
STAGE     1:4-B-01      WATER         DEXTR-01      CARBO-01      OLEIC-01
   1    0.70000       0.70000       0.70000       0.70000       0.70000
   2    0.70000       0.70000       0.70000       0.70000       0.70000
   3    0.60000       0.60000       0.60000       0.60000       0.60000
   4    0.60000       0.60000       0.60000       0.60000       0.60000
   5    0.60000       0.60000       0.60000       0.60000       0.60000
   6    0.50000       0.50000       0.50000       0.50000       0.50000
   7    0.50000       0.50000       0.50000       0.50000       0.50000

                      ****   VAPORIZATION EFF   ****
STAGE     LYSIN-01      GLYCI-01      ISOLE-01      LEUCI-01      METHI-01
   1    0.70000       0.70000       0.70000       0.70000       0.70000
   2    0.70000       0.70000       0.70000       0.70000       0.70000
   3    0.60000       0.60000       0.60000       0.60000       0.60000
   4    0.60000       0.60000       0.60000       0.60000       0.60000
   5    0.60000       0.60000       0.60000       0.60000       0.60000
   6    0.50000       0.50000       0.50000       0.50000       0.50000
   7    0.50000       0.50000       0.50000       0.50000       0.50000

                      ****   VAPORIZATION EFF   ****
STAGE     L-PHE-01      THREO-01      TRYPT-01      TYROS-01      VALIN-01
   1    0.70000       0.70000       0.70000       0.70000       0.70000
   2    0.70000       0.70000       0.70000       0.70000       0.70000
   3    0.60000       0.60000       0.60000       0.60000       0.60000
   4    0.60000       0.60000       0.60000       0.60000       0.60000
   5    0.60000       0.60000       0.60000       0.60000       0.60000
   6    0.50000       0.50000       0.50000       0.50000       0.50000
   7    0.50000       0.50000       0.50000       0.50000       0.50000

                      ****   VAPORIZATION EFF   ****
STAGE     INOSI-01      NIACI-01      POTAS-01      MAGNE-01      CALCI-01
   1    0.70000       0.70000       0.70000       0.70000       0.70000
   2    0.70000       0.70000       0.70000       0.70000       0.70000
   3    0.60000       0.60000       0.60000       0.60000       0.60000
   4    0.60000       0.60000       0.60000       0.60000       0.60000
   5    0.60000       0.60000       0.60000       0.60000       0.60000
   6    0.50000       0.50000       0.50000       0.50000       0.50000
   7    0.50000       0.50000       0.50000       0.50000       0.50000

                      ****   VAPORIZATION EFF   ****
STAGE     SULFU-01      SODIU-01      IRON          ZINC          MANGA-01
   1    0.70000       0.70000       0.70000       0.70000       0.70000
   2    0.70000       0.70000       0.70000       0.70000       0.70000
   3    0.60000       0.60000       0.60000       0.60000       0.60000
   4    0.60000       0.60000       0.60000       0.60000       0.60000
   5    0.60000       0.60000       0.60000       0.60000       0.60000
   6    0.50000       0.50000       0.50000       0.50000       0.50000
   7    0.50000       0.50000       0.50000       0.50000       0.50000

                      ****   VAPORIZATION EFF   ****
STAGE     COPPE-01      CHROM-01      MOLYB-01      COBAL-01      HYDRO-01
   1    0.70000       0.70000       0.70000       0.70000       0.70000
   2    0.70000       0.70000       0.70000       0.70000       0.70000
   3    0.60000       0.60000       0.60000       0.60000       0.60000
   4    0.60000       0.60000       0.60000       0.60000       0.60000
   5    0.60000       0.60000       0.60000       0.60000       0.60000
   6    0.50000       0.50000       0.50000       0.50000       0.50000
   7    0.50000       0.50000       0.50000       0.50000       0.50000

BLOCK:  DISTHX   MODEL: HEATX
-----------------------------
  HOT SIDE:
  ---------
  INLET STREAM:          HOTIN
  OUTLET STREAM:         OLETOLLE
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG
  COLD SIDE:
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INLET STREAM:          TOHX2
OUTLET STREAM:         DIST1IN
PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
 TOTAL BALANCE
    MOLE(LBMOL/HR)            322.302         322.302         0.00000
    MASS(LB/HR   )            16561.0         16561.0         0.00000
    ENTHALPY(BTU/HR  )      -0.496436E+08   -0.496436E+08    0.648656E-09

                       ***  INPUT DATA  ***

FLASH SPECS FOR HOT SIDE:
TWO    PHASE      FLASH
MAXIMUM NO. ITERATIONS                                 30
CONVERGENCE TOLERANCE                                   0.000100000

FLASH SPECS FOR COLD SIDE:
TWO    PHASE      FLASH
MAXIMUM NO. ITERATIONS                                 30
CONVERGENCE TOLERANCE                                   0.000100000

FLOW DIRECTION AND SPECIFICATION:
  COUNTERCURRENT   HEAT EXCHANGER
  SPECIFIED HOT OUTLET TEMP
  SPECIFIED VALUE               F                     120.0000
  LMTD CORRECTION FACTOR                                1.00000

PRESSURE SPECIFICATION:
  HOT  SIDE PRESSURE DROP        PSI                    0.0000
  COLD SIDE PRESSURE DROP        PSI                    0.0000

HEAT TRANSFER COEFFICIENT SPECIFICATION:
  HOT LIQUID    COLD LIQUID      BTU/HR-SQFT-R          149.6937
  HOT 2-PHASE   COLD LIQUID      BTU/HR-SQFT-R          149.6937
  HOT VAPOR     COLD LIQUID      BTU/HR-SQFT-R          149.6937
  HOT LIQUID    COLD 2-PHASE     BTU/HR-SQFT-R          149.6937
  HOT 2-PHASE   COLD 2-PHASE     BTU/HR-SQFT-R          149.6937
  HOT VAPOR     COLD 2-PHASE     BTU/HR-SQFT-R          149.6937
  HOT LIQUID    COLD VAPOR       BTU/HR-SQFT-R          149.6937
  HOT 2-PHASE   COLD VAPOR       BTU/HR-SQFT-R          149.6937
  HOT VAPOR     COLD VAPOR       BTU/HR-SQFT-R          149.6937

                     ***  OVERALL RESULTS  ***

STREAMS:
                --------------------------------------
                |                                    |
HOTIN     ----->|               HOT                  |-----> OLETOLLE
T=  3.9268D+02  |                                    |       T=  1.2000D+02
P=  2.4700D+01  |                                    |       P=  2.4700D+01
V=  0.0000D+00  |                                    |       V=  0.0000D+00
                |                                    |
DIST1IN   <-----|               COLD                 |<----- TOHX2
T=  1.1992D+02  |                                    |       T=  8.7695D+01
P=  3.0000D+01  |                                    |       P=  3.0000D+01
V=  0.0000D+00  |                                    |       V=  0.0000D+00
                --------------------------------------

DUTY AND AREA:
  CALCULATED HEAT DUTY           BTU/HR             277288.3332
  CALCULATED (REQUIRED) AREA     SQFT                   16.4346
  ACTUAL EXCHANGER AREA          SQFT                   16.4346
  PER CENT OVER-DESIGN                                   0.0000

HEAT TRANSFER COEFFICIENT:
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  AVERAGE COEFFICIENT (DIRTY)    BTU/HR-SQFT-R          149.6937
  UA (DIRTY)                     BTU/HR-R              2460.1505

LOG-MEAN TEMPERATURE DIFFERENCE:
  LMTD CORRECTION FACTOR                                 1.0000
  LMTD (CORRECTED)               F                     112.7119
  NUMBER OF SHELLS IN SERIES                               1

PRESSURE DROP:
  HOTSIDE, TOTAL                 PSI                     0.0000
  COLDSIDE, TOTAL                PSI                     0.0000

PRESSURE DROP PARAMETER:
  HOT SIDE:                                            0.0000
  COLD SIDE:                                           0.0000

                     ***  ZONE RESULTS  ***

TEMPERATURE LEAVING EACH ZONE:

                                  HOT
        -------------------------------------------------------------
        |                                                           |
HOTIN   |                         LIQ                               | OLETOLLE
------> |                                                           |------>
 392.7  |                                                           |  120.0
        |                                                           |
DIST1IN |                         LIQ                               | TOHX2
<------ |                                                           |<------
 119.9  |                                                           |   87.7
        |                                                           |
        -------------------------------------------------------------
                                  COLD

ZONE HEAT TRANSFER AND AREA:

ZONE       HEAT DUTY       AREA       LMTD       AVERAGE U       UA
           BTU/HR          SQFT        F         BTU/HR-SQFT-R   BTU/HR-R
  1     277288.307       16.4346     112.7119   149.6937        2460.1502

BLOCK:  FERMRXN1 MODEL: RSTOIC
------------------------------
  INLET STREAMS:         MOL2FERM    SOL2FERM    MED2FERM    WTR2FERM
  OUTLET STREAM:         FERMOUT
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                             IN           OUT       GENERATION   RELATIVE DIFF.
  TOTAL BALANCE
  MOLE(LBMOL/HR)          312.975       361.174       48.1985      0.157385E-15
  MASS(LB/HR   )          30546.6       30546.6                     0.00000
  ENTHALPY(BTU/HR  )   -0.422900E+08 -0.442639E+08                 0.445953E-01

                     ***  INPUT DATA  ***
  STOICHIOMETRY MATRIX:

   REACTION #   1:
    SUBSTREAM MIXED   :
    1:4-B-01   2.00    DEXTR-01  -1.00    CARBO-01   2.00    ETHYL-01  -1.00
    SUBSTREAM CISOLID :
    NO PARTICIPATING COMPONENTS


  REACTION CONVERSION SPECS: NUMBER=    1
    REACTION #   1:
    SUBSTREAM:MIXED    KEY COMP:DEXTR-01 CONV FRAC: 0.9500
```

```
 TWO    PHASE  TP  FLASH
 SPECIFIED TEMPERATURE F                                   86.0000
 SPECIFIED PRESSURE    PSIA                                17.4045
 MAXIMUM NO. ITERATIONS                                    30
 CONVERGENCE TOLERANCE                                      0.000100000
 SIMULTANEOUS REACTIONS
 GENERATE COMBUSTION REACTIONS FOR FEED SPECIES          NO

                        ***  RESULTS  ***
 OUTLET TEMPERATURE    F                                     86.000
 OUTLET PRESSURE       PSIA                                  17.405
 HEAT DUTY             BTU/HR                              -0.19740E+07
 VAPOR FRACTION                                              0.0000


 REACTION EXTENTS:

    REACTION          REACTION
    NUMBER            EXTENT
                      LBMOL/HR
    1                  24.099

 V-L PHASE EQUILIBRIUM :

    COMP             F(I)           X(I)           Y(I)           K(I)
    1:4-B-01        0.18923        0.18923        0.22348E-05    0.77810E-05
    WATER           0.37317        0.37317        0.32474E-01    0.57335E-01
    DEXTR-01        0.48935E-02    0.48935E-02    0.21396E-15    0.28807E-13
    CARBO-01        0.18595        0.18595        0.79021         2.7998
    LYSIN-01        0.55183E-03    0.55183E-03    0.24302E-11    0.29015E-08
    GLYCI-01        0.10747E-02    0.10747E-02    0.19183E-12    0.11760E-09
    ISOLE-01        0.61500E-03    0.61500E-03    0.14793E-81    0.15847E-78
    LEUCI-01        0.61500E-03    0.61500E-03    0.14793E-81    0.15847E-78
    METHI-01        0.54065E-03    0.54065E-03    0.13004E-81    0.15847E-78
    L-PHE-01        0.48836E-03    0.48836E-03    0.81413E-11    0.10984E-07
    THREO-01        0.67723E-03    0.67723E-03    0.16289E-81    0.15847E-78
    TRYPT-01        0.39501E-03    0.39501E-03    0.95012E-82    0.15847E-78
    TYROS-01        0.44523E-03    0.44523E-03    0.10709E-81    0.15847E-78
    VALIN-01        0.68864E-03    0.68864E-03    0.16564E-81    0.15847E-78
    INOSI-01        0.31099E-03    0.31099E-03    0.24094E-19    0.51046E-16
    NIACI-01        0.11716E-03    0.11716E-03    0.43295E-07    0.24346E-03
    POTAS-01        0.14996E-02    0.14996E-02    0.10068E-12    0.44232E-10
    MAGNE-01        0.24124E-02    0.24124E-02    0.90538E-19    0.24727E-16
    CALCI-01        0.14630E-02    0.14630E-02    0.50228E-25    0.22620E-22
    SULFU-01        0.18285E-02    0.18285E-02    0.61538E-09    0.22173E-06
    SODIU-01        0.25504E-02    0.25504E-02    0.30025E-15    0.77565E-13
    IRON            0.10499E-02    0.10499E-02    0.27362E-61    0.17171E-58
    ZINC            0.89668E-03    0.89668E-03    0.53527E-18    0.39330E-15
    MANGA-01        0.10673E-02    0.10673E-02    0.70292E-37    0.68018E-34
    COPPE-01        0.92270E-03    0.92270E-03    0.26087E-81    0.18627E-78
    CHROM-01        0.11277E-02    0.11277E-02    0.31882E-81    0.18627E-78
    MOLYB-01        0.61115E-03    0.61115E-03    0.31806E-90    0.34289E-87
    COBAL-01        0.99492E-03    0.99492E-03    0.28129E-81    0.18627E-78
    HYDRO-01        0.16081E-02    0.16081E-02    0.89869E-01     36.819
    ETHYL-01        0.22221        0.22221        0.87441E-01    0.25927

BLOCK:  FERMRXN2 MODEL: RSTOIC
------------------------------
 INLET STREAMS:         MOL2FER2    MED2FER2    WTR2FER2    SOL2FER2
 OUTLET STREAM:         FERMOUT2
 PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                     ***  MASS AND ENERGY BALANCE  ***
                             IN          OUT       GENERATION   RELATIVE DIFF.
 TOTAL BALANCE
```

```
 MOLE(LBMOL/HR)         312.975       361.174       48.1985      0.157385E-15
 MASS(LB/HR   )        30546.6       30546.6                      0.00000
 ENTHALPY(BTU/HR  )   -0.422900E+08 -0.442639E+08                0.445953E-01

                       ***  INPUT DATA  ***
 STOICHIOMETRY MATRIX:

  REACTION #   1:
   SUBSTREAM MIXED   :
   1:4-B-01   2.00    DEXTR-01  -1.00    CARBO-01   2.00    ETHYL-01  -1.00
   SUBSTREAM CISOLID :
   NO PARTICIPATING COMPONENTS


 REACTION CONVERSION SPECS: NUMBER=    1
   REACTION #   1:
   SUBSTREAM:MIXED    KEY COMP:DEXTR-01 CONV FRAC: 0.9500



 TWO    PHASE  TP  FLASH
 SPECIFIED TEMPERATURE F                                   86.0000
 SPECIFIED PRESSURE    PSIA                                17.4045
 MAXIMUM NO. ITERATIONS                                    30
 CONVERGENCE TOLERANCE                                      0.000100000
 SIMULTANEOUS REACTIONS
 GENERATE COMBUSTION REACTIONS FOR FEED SPECIES          NO

                        ***  RESULTS  ***
 OUTLET TEMPERATURE    F                                     86.000
 OUTLET PRESSURE       PSIA                                  17.405
 HEAT DUTY             BTU/HR                              -0.19740E+07
 VAPOR FRACTION                                              0.0000


 REACTION EXTENTS:

    REACTION          REACTION
    NUMBER            EXTENT
                      LBMOL/HR
    1                  24.099

 V-L PHASE EQUILIBRIUM :

    COMP             F(I)           X(I)           Y(I)           K(I)
    1:4-B-01        0.18923        0.18923        0.22348E-05    0.77810E-05
    WATER           0.37317        0.37317        0.32474E-01    0.57335E-01
    DEXTR-01        0.48935E-02    0.48935E-02    0.21396E-15    0.28807E-13
    CARBO-01        0.18595        0.18595        0.79021         2.7998
    LYSIN-01        0.55183E-03    0.55183E-03    0.24302E-11    0.29015E-08
    GLYCI-01        0.10747E-02    0.10747E-02    0.19183E-12    0.11760E-09
    ISOLE-01        0.61500E-03    0.61500E-03    0.14793E-81    0.15847E-78
    LEUCI-01        0.61500E-03    0.61500E-03    0.14793E-81    0.15847E-78
    METHI-01        0.54065E-03    0.54065E-03    0.13004E-81    0.15847E-78
    L-PHE-01        0.48836E-03    0.48836E-03    0.81413E-11    0.10984E-07
    THREO-01        0.67723E-03    0.67723E-03    0.16289E-81    0.15847E-78
    TRYPT-01        0.39501E-03    0.39501E-03    0.95012E-82    0.15847E-78
    TYROS-01        0.44523E-03    0.44523E-03    0.10709E-81    0.15847E-78
    VALIN-01        0.68864E-03    0.68864E-03    0.16564E-81    0.15847E-78
    INOSI-01        0.31099E-03    0.31099E-03    0.24094E-19    0.51046E-16
    NIACI-01        0.11716E-03    0.11716E-03    0.43295E-07    0.24346E-03
    POTAS-01        0.14996E-02    0.14996E-02    0.10068E-12    0.44232E-10
    MAGNE-01        0.24124E-02    0.24124E-02    0.90538E-19    0.24727E-16
    CALCI-01        0.14630E-02    0.14630E-02    0.50228E-25    0.22620E-22
    SULFU-01        0.18285E-02    0.18285E-02    0.61538E-09    0.22173E-06
    SODIU-01        0.25504E-02    0.25504E-02    0.30025E-15    0.77565E-13
```

```
     IRON             0.10499E-02    0.10499E-02    0.27362E-61    0.17171E-58
     ZINC             0.89668E-03    0.89668E-03    0.53527E-18    0.39330E-15
     MANGA-01         0.10673E-02    0.10673E-02    0.70292E-37    0.68018E-34
     COPPE-01         0.92270E-03    0.92270E-03    0.26087E-81    0.18627E-78
     CHROM-01         0.11277E-02    0.11277E-02    0.31882E-81    0.18627E-78
     MOLYB-01         0.61115E-03    0.61115E-03    0.31806E-90    0.34289E-87
     COBAL-01         0.99492E-03    0.99492E-03    0.28129E-81    0.18627E-78
     HYDRO-01         0.16081E-02    0.16081E-02    0.89869E-01     36.819
     ETHYL-01         0.22221        0.22221        0.87441E-01    0.25927

BLOCK:  FLASHVAP MODEL: FLASH2
------------------------------
  INLET STREAM:          CONDENSE
  OUTLET VAPOR STREAM:   VAPOROUT
  OUTLET LIQUID STREAM:  WATERNOV
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                     ***  MASS AND ENERGY BALANCE  ***
                                   IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            186.553         186.553         0.00000
      MASS(LB/HR   )            3393.23         3393.23        0.134016E-15
      ENTHALPY(BTU/HR  )      -0.228865E+08   -0.228865E+08     0.00000

                         ***  INPUT DATA  ***
  TWO    PHASE  TP  FLASH
  SPECIFIED TEMPERATURE F                                41.0000
  SPECIFIED PRESSURE    PSIA                              0.15000
  MAXIMUM NO. ITERATIONS                                 30
  CONVERGENCE TOLERANCE                                   0.000100000

                          ***  RESULTS  ***
  OUTLET TEMPERATURE    F                                  41.000
  OUTLET PRESSURE       PSIA                              0.15000
  HEAT DUTY             BTU/HR                           -0.47805E-08
  VAPOR FRACTION                                          0.32236E-01



  V-L PHASE EQUILIBRIUM :

     COMP              F(I)           X(I)           Y(I)           K(I)
     1:4-B-01         0.80643E-03    0.83323E-03    0.20183E-05    0.24223E-02
     WATER            0.99349        0.99855        0.84158        0.84280
     CARBO-01         0.13260E-02    0.54802E-03    0.24682E-01     45.040
     OLEIC-01         0.10312E-10    0.10656E-10    0.42237E-19    0.39640E-08
     L-PHE-01         0.75200E-14    0.77704E-14    0.12306E-17    0.15837E-03
     NIACI-01         0.54486E-05    0.56263E-05    0.11472E-06    0.20390E-01
     SULFU-01         0.42682E-13    0.44104E-13    0.76315E-19    0.17304E-05
     HYDRO-01         0.43707E-02    0.61515E-04    0.13374         2174.0

BLOCK:  LLE      MODEL: SEP2
----------------------------
  INLET STREAMS:         OLETOLLE    WTR2LLE
  OUTLET STREAMS:        OLEICPUR    LLEMIN
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                     ***  MASS AND ENERGY BALANCE  ***
                                   IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            199.134         199.134       -0.495764E-07
      MASS(LB/HR   )            5549.33         5549.34       -0.627375E-06
      ENTHALPY(BTU/HR  )      -0.253826E+08   -0.253581E+08   -0.965120E-03


                         ***  INPUT DATA  ***

  INLET PRESSURE:  MINIMUM OF INLET STREAM PRESSURES
```

```
 FLASH SPECS FOR STREAM OLEICPUR
 TWO    PHASE  TP  FLASH
 PRESSURE DROP         PSI                                  0.0
 MAXIMUM NO. ITERATIONS                                    30
 CONVERGENCE TOLERANCE                                      0.000100000

 FLASH SPECS FOR STREAM LLEMIN
 TWO    PHASE  TP  FLASH
 PRESSURE DROP         PSI                                  0.0
 MAXIMUM NO. ITERATIONS                                    30
 CONVERGENCE TOLERANCE                                      0.000100000

 SPLIT FRACTION
   SUBSTREAM= MIXED
     STREAM= OLEICPUR  CPT= 1:4-B-01  FRACTION=          0.0
                            WATER                        0.0
                            DEXTR-01                     0.0
                            CARBO-01                     0.0
                            OLEIC-01                     1.00000
                            LYSIN-01                     0.0
                            GLYCI-01                     0.0
                            ISOLE-01                     0.0
                            LEUCI-01                     0.0
                            METHI-01                     0.0
                            L-PHE-01                     0.0
                            THREO-01                     0.0
                            TRYPT-01                     0.0
                            TYROS-01                     0.0
                            VALIN-01                     0.0
                            INOSI-01                     0.0
                            NIACI-01                     0.0
                            POTAS-01                     0.0
                            MAGNE-01                     0.0
                            CALCI-01                     0.0
                            SULFU-01                     0.0
                            SODIU-01                     0.0
                            IRON                         0.0
                            ZINC                         0.0
                            MANGA-01                     0.0
                            COPPE-01                     0.0
                            CHROM-01                     0.0
                            MOLYB-01                     0.0
                            COBAL-01                     0.0
                            HYDRO-01                     0.0
                            ETHYL-01                     0.0
                            CELLU-01                     0.0
   SUBSTREAM= CISOLID
     STREAM= OLEICPUR  CPT= CELLU-01  FRACTION=          0.0


                         ***  RESULTS  ***

 HEAT DUTY             BTU/HR                                24502.

STREAM=   OLEICPUR   SUBSTREAM= MIXED
   COMPONENT = OLEIC-01  SPLIT FRACTION =          1.00000

STREAM=   LLEMIN     SUBSTREAM= MIXED
   COMPONENT = 1:4-B-01  SPLIT FRACTION =          1.00000
   COMPONENT = WATER     SPLIT FRACTION =          1.00000
   COMPONENT = DEXTR-01  SPLIT FRACTION =          1.00000
   COMPONENT = CARBO-01  SPLIT FRACTION =          1.00000
   COMPONENT = LYSIN-01  SPLIT FRACTION =          1.00000
   COMPONENT = GLYCI-01  SPLIT FRACTION =          1.00000
   COMPONENT = ISOLE-01  SPLIT FRACTION =          1.00000
   COMPONENT = LEUCI-01  SPLIT FRACTION =          1.00000
   COMPONENT = METHI-01  SPLIT FRACTION =          1.00000
```

```
    COMPONENT = L-PHE-01  SPLIT FRACTION =          1.00000
    COMPONENT = THREO-01  SPLIT FRACTION =          1.00000
    COMPONENT = TRYPT-01  SPLIT FRACTION =          1.00000
    COMPONENT = TYROS-01  SPLIT FRACTION =          1.00000
    COMPONENT = VALIN-01  SPLIT FRACTION =          1.00000
    COMPONENT = INOSI-01  SPLIT FRACTION =          1.00000
    COMPONENT = NIACI-01  SPLIT FRACTION =          1.00000
    COMPONENT = POTAS-01  SPLIT FRACTION =          1.00000
    COMPONENT = MAGNE-01  SPLIT FRACTION =          1.00000
    COMPONENT = CALCI-01  SPLIT FRACTION =          1.00000
    COMPONENT = SULFU-01  SPLIT FRACTION =          1.00000
    COMPONENT = SODIU-01  SPLIT FRACTION =          1.00000
    COMPONENT = IRON      SPLIT FRACTION =          1.00000
    COMPONENT = ZINC      SPLIT FRACTION =          1.00000
    COMPONENT = MANGA-01  SPLIT FRACTION =          1.00000
    COMPONENT = COPPE-01  SPLIT FRACTION =          1.00000
    COMPONENT = CHROM-01  SPLIT FRACTION =          1.00000
    COMPONENT = MOLYB-01  SPLIT FRACTION =          1.00000
    COMPONENT = COBAL-01  SPLIT FRACTION =          1.00000
    COMPONENT = HYDRO-01  SPLIT FRACTION =          1.00000

BLOCK:  LLEBOTVV MODEL: VALVE
-----------------------------
  INLET STREAM:         TOBOTVAL
  OUTLET STREAM:        TOMIXER
  PROPERTY OPTION SET:  NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           195.844         195.844         0.00000
      MASS(LB/HR   )           4620.04         4620.04         0.00000
      ENTHALPY(BTU/HR  )      -0.241922E+08   -0.241922E+08    0.910538E-09

                        ***  INPUT DATA  ***

   VALVE PRESSURE DROP       PSI                         25.0000
   VALVE FLOW COEF CALC.                                NO

                        FLASH SPECIFICATIONS:
   NPHASE                                                 2
   MAX NUMBER OF ITERATIONS                              30
   CONVERGENCE TOLERANCE                                  0.000100000

                        ***  RESULTS  ***

   VALVE OUTLET PRESSURE     PSIA                        17.4000

BLOCK:  LLETOPPU MODEL: PUMP
----------------------------
  INLET STREAM:         LLEMIN
  OUTLET STREAM:        TOBOTVAL
  PROPERTY OPTION SET:  NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           195.844         195.844         0.00000
      MASS(LB/HR   )           4620.04         4620.04         0.00000
      ENTHALPY(BTU/HR  )      -0.241926E+08   -0.241922E+08   -0.150384E-04

                        ***  INPUT DATA  ***
   OUTLET PRESSURE  PSIA                                 42.4000
   PUMP EFFICIENCY                                        0.70000
   DRIVER EFFICIENCY                                      1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
```

```
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                          30
   TOLERANCE                                              0.000100000

                        ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  CUFT/HR                         77.7632
   PRESSURE CHANGE  PSI                                  17.7000
   NPSH AVAILABLE    FT-LBF/LB                           59.0809
   FLUID POWER  HP                                        0.10010
   BRAKE POWER  HP                                        0.14300
   ELECTRICITY  KW                                        0.10664
   PUMP EFFICIENCY USED                                   0.70000
   NET WORK REQUIRED  HP                                  0.14300
   HEAD DEVELOPED FT-LBF/LB                              42.9007

BLOCK:  MEDSPLIT MODEL: FSPLIT
------------------------------
  INLET STREAM:         MEDIAPUM
  OUTLET STREAMS:       MEDFERM1    MEDFERM2
  PROPERTY OPTION SET:  NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           173.901         173.901         0.00000
      MASS(LB/HR   )           15055.0         15055.0         0.00000
      ENTHALPY(BTU/HR  )      -0.336451E+08   -0.336451E+08    0.151189E-08

                        ***  INPUT DATA  ***

 FRACTION OF FLOW               STRM=MEDFERM1 FRAC=         0.50000

                        ***  RESULTS  ***

 STREAM= MEDFERM1       SPLIT=          0.50000     KEY=  0    STREAM-ORDER=   1
         MEDFERM2                       0.50000           0                    2

BLOCK:  MEDVAL2  MODEL: VALVE
-----------------------------
  INLET STREAM:         MEDFERM2
  OUTLET STREAM:        MED2FER2
  PROPERTY OPTION SET:  NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           86.9506         86.9506         0.00000
      MASS(LB/HR   )           7527.48         7527.48         0.00000
      ENTHALPY(BTU/HR  )      -0.168225E+08   -0.168225E+08    0.528095E-10

                        ***  INPUT DATA  ***

   VALVE OUTLET PRESSURE     PSIA                        25.0000
   VALVE FLOW COEF CALC.                                NO

                        FLASH SPECIFICATIONS:
   NPHASE                                                 2
   MAX NUMBER OF ITERATIONS                              30
   CONVERGENCE TOLERANCE                                  0.000100000

                        ***  RESULTS  ***

   VALVE PRESSURE DROP       PSI                         17.4000

BLOCK:  MEDVALVE MODEL: VALVE
-----------------------------
  INLET STREAM:         MEDFERM1
  OUTLET STREAM:        MED2FERM
```

```
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           86.9506         86.9506         0.00000
      MASS(LB/HR   )           7527.48         7527.48         0.00000
      ENTHALPY(BTU/HR  )     -0.168225E+08   -0.168225E+08    0.213563E-10

                       ***  INPUT DATA  ***

   VALVE PRESSURE DROP       PSI                          25.0000
   VALVE FLOW COEF CALC.                                NO

                       FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                       ***  RESULTS  ***

   VALVE OUTLET PRESSURE     PSIA                         17.4000

BLOCK:  MIXER    MODEL: MIXER
-----------------------------
  INLET STREAMS:         TOMIX       OLEIC
  OUTLET STREAM:         TOHX2
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           303.707         303.707         0.00000
      MASS(LB/HR   )           14277.9         14277.9       -0.382198E-15
      ENTHALPY(BTU/HR  )     -0.468392E+08   -0.468392E+08    0.211785E-09

                       ***  INPUT DATA  ***
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                                  30
  CONVERGENCE TOLERANCE                                    0.000100000
  OUTLET PRESSURE   PSIA                                 30.0000

BLOCK:  MOL2VAL2 MODEL: VALVE
-----------------------------
  INLET STREAM:          MOLFERM2
  OUTLET STREAM:         MOL2FER2
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           45.7409         45.7409         0.00000
      MASS(LB/HR   )           7873.15         7873.15         0.00000
      ENTHALPY(BTU/HR  )     -0.132086E+08   -0.132086E+08   -0.903162E-11

                       ***  INPUT DATA  ***

   VALVE OUTLET PRESSURE     PSIA                         25.0000
   VALVE FLOW COEF CALC.                                NO

                       FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                       ***  RESULTS  ***

   VALVE PRESSURE DROP       PSI                          27.5000
```

```
BLOCK:  MOLSPLIT MODEL: FSPLIT
------------------------------
  INLET STREAM:          STERILEM
  OUTLET STREAMS:        MOLFERM1    MOLFERM2
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           91.4818         91.4818         0.00000
      MASS(LB/HR   )           15746.3         15746.3       -0.115519E-15
      ENTHALPY(BTU/HR  )     -0.264172E+08   -0.264172E+08     0.00000

                       ***  INPUT DATA  ***

 FRACTION OF FLOW                STRM=MOLFERM1 FRAC=         0.50000

                       ***  RESULTS  ***

 STREAM= MOLFERM1       SPLIT=          0.50000     KEY=  0    STREAM-ORDER=   1
         MOLFERM2                       0.50000           0                    2

BLOCK:  MOLVALVE MODEL: VALVE
-----------------------------
  INLET STREAM:          MOLFERM1
  OUTLET STREAM:         MOL2FERM
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           45.7409         45.7409         0.00000
      MASS(LB/HR   )           7873.15         7873.15         0.00000
      ENTHALPY(BTU/HR  )     -0.132086E+08   -0.132086E+08   -0.180101E-10

                       ***  INPUT DATA  ***

   VALVE PRESSURE DROP       PSI                          25.0000
   VALVE FLOW COEF CALC.                                NO

                       FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                       ***  RESULTS  ***

   VALVE OUTLET PRESSURE     PSIA                         27.5000

BLOCK:  NOVAP    MODEL: HEATER
------------------------------
  INLET STREAM:          BDODIST
  OUTLET STREAM:         BDO
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           98.5592         98.5592         0.00000
      MASS(LB/HR   )           8601.31         8601.31        0.211478E-15
      ENTHALPY(BTU/HR  )     -0.208995E+08   -0.210506E+08    0.717787E-02

                       ***  INPUT DATA  ***
  TWO    PHASE  PV  FLASH
  SPECIFIED PRESSURE                 PSIA                       0.074000
  VAPOR FRACTION                                                0.0
  MAXIMUM NO. ITERATIONS                                       30
```

```
  CONVERGENCE TOLERANCE                                          0.000100000



                         ***  RESULTS  ***
  OUTLET TEMPERATURE    F                                   49.852
  OUTLET PRESSURE       PSIA                               0.74000E-01
  HEAT DUTY             BTU/HR                            -0.15110E+06
  OUTLET VAPOR FRACTION                                     0.0000
  PRESSURE-DROP CORRELATION PARAMETER                      0.60482E+06



  V-L PHASE EQUILIBRIUM :

     COMP             F(I)           X(I)           Y(I)           K(I)
     1:4-B-01        0.95957        0.95957        0.26213E-03    0.27317E-03
     WATER           0.39802E-01    0.39802E-01    0.98928         24.855
     DEXTR-01        0.20649E-14    0.20649E-14    0.13741E-26    0.66550E-12
     CARBO-01        0.33122E-04    0.33122E-04    0.36953E-03     11.157
     OLEIC-01        0.23921E-05    0.23921E-05    0.15487E-11    0.64742E-06
     LYSIN-01        0.70629E-11    0.70629E-11    0.11272E-18    0.15959E-07
     GLYCI-01        0.19123E-13    0.19123E-13    0.24657E-22    0.12894E-08
     L-PHE-01        0.19943E-08    0.19943E-08    0.22338E-14    0.11201E-05
     NIACI-01        0.59104E-03    0.59104E-03    0.34359E-05    0.58133E-02
     SULFU-01        0.39388E-08    0.39388E-08    0.19006E-13    0.48254E-05
     HYDRO-01        0.25116E-05    0.25116E-05    0.10086E-01     4015.6

BLOCK:  OLESPLIT MODEL: FSPLIT
------------------------------
  INLET STREAM:          TOOLEMIX
  OUTLET STREAMS:        OLEICREC    OLPUROUT
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           3.28995         3.28995       -0.134983E-15
      MASS(LB/HR   )           929.303         929.303       -0.244671E-15
      ENTHALPY(BTU/HR  )      -0.116534E+07   -0.116534E+07    0.00000

                       ***  INPUT DATA  ***

 FRACTION OF FLOW                STRM=OLPUROUT FRAC=         0.20000

                        ***  RESULTS  ***

 STREAM= OLEICREC       SPLIT=          0.80000     KEY=  0    STREAM-ORDER=   2
         OLPUROUT                       0.20000           0                    1

BLOCK:  OLEVALVE MODEL: VALVE
-----------------------------
  INLET STREAM:          OLESPLIT
  OUTLET STREAM:         HOTIN
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           18.5950         18.5950         0.00000
      MASS(LB/HR   )           2283.14         2283.14         0.00000
      ENTHALPY(BTU/HR  )      -0.280441E+07   -0.280441E+07    0.178865E-11

                       ***  INPUT DATA  ***

   VALVE PRESSURE DROP       PSI                           25.0000
   VALVE FLOW COEF CALC.                                 NO
```

```
                         FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                         ***  RESULTS  ***

   VALVE OUTLET PRESSURE     PSIA                         24.7000

BLOCK:  OLMIXREC MODEL: MIXER
-----------------------------
  INLET STREAMS:         OLEICIN     OLEICREC
  OUTLET STREAM:         OLEIC
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           3.29045         3.29045         0.00000
      MASS(LB/HR   )           929.442         929.442         0.00000
      ENTHALPY(BTU/HR  )      -0.116426E+07   -0.116426E+07    0.848900E-10

                       ***  INPUT DATA  ***
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                                  30
  CONVERGENCE TOLERANCE                                    0.000100000
  OUTLET PRESSURE   PSIA                                  30.0000

BLOCK:  OLPUMPVA MODEL: VALVE
-----------------------------
  INLET STREAM:          2OLPVALV
  OUTLET STREAM:         TOOLEMIX
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           3.28995         3.28995         0.00000
      MASS(LB/HR   )           929.303         929.303         0.00000
      ENTHALPY(BTU/HR  )      -0.116534E+07   -0.116534E+07    0.141422E-10

                       ***  INPUT DATA  ***

   VALVE PRESSURE DROP       PSI                          25.0000
   VALVE FLOW COEF CALC.                                NO

                         FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                         ***  RESULTS  ***

   VALVE OUTLET PRESSURE     PSIA                         49.7000

BLOCK:  OLPVALVE MODEL: VALVE
-----------------------------
  INLET STREAM:          OLPUROUT
  OUTLET STREAM:         OLEICOUT
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)          0.657991        0.657991         0.00000
      MASS(LB/HR   )           185.861         185.861         0.00000
      ENTHALPY(BTU/HR  )      -233068.        -233068.         0.932326E-10
```

```
                      ***  INPUT DATA  ***

   VALVE PRESSURE DROP       PSI                          25.0000
   VALVE FLOW COEF CALC.                                NO

                      FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                      ***  RESULTS  ***

   VALVE OUTLET PRESSURE     PSIA                         24.7000

 BLOCK:  PRESVAVL MODEL: VALVE
 ----------------------------
   INLET STREAM:          SIDESTRE
   OUTLET STREAM:         TOBLWR
   PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                  ***  MASS AND ENERGY BALANCE  ***
                                IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           0.186740        0.186740         0.00000
      MASS(LB/HR   )            3.39663         3.39663        0.130744E-15
      ENTHALPY(BTU/HR  )       -19373.3        -19373.3        0.525106E-10

                      ***  INPUT DATA  ***

   VALVE PRESSURE DROP       PSI                          0.0
   VALVE FLOW COEF CALC.                                NO

                      FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                      ***  RESULTS  ***

   VALVE OUTLET PRESSURE     PSIA                          0.15000

 BLOCK:  PUMITUP  MODEL: PUMP
 ----------------------------
   INLET STREAM:          NOMEDIA
   OUTLET STREAM:         NOSOLHPR
   PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                  ***  MASS AND ENERGY BALANCE  ***
                                IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            300.417         300.417         0.00000
      MASS(LB/HR   )            13348.4         13348.4         0.00000
      ENTHALPY(BTU/HR  )      -0.456847E+08   -0.456749E+08   -0.214456E-03

                      ***  INPUT DATA  ***
   OUTLET PRESSURE  PSIA                                  99.7000
   DRIVER EFFICIENCY                                       1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                            30
   TOLERANCE                                               0.000100000

                       ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  CUFT/HR                         213.489
   PRESSURE CHANGE  PSI                                   85.0077
   NPSH AVAILABLE   FT-LBF/LB                             30.9263
   FLUID POWER  HP                                         1.31987
   BRAKE POWER  HP                                         3.85050
   ELECTRICITY  KW                                         2.87132
   PUMP EFFICIENCY USED                                    0.34278
   NET WORK REQUIRED  HP                                   3.85050
   HEAD DEVELOPED FT-LBF/LB                              195.779

 BLOCK:  PUMPIN1  MODEL: PUMP
 ----------------------------
   INLET STREAM:          WATERIN
   OUTLET STREAM:         WATERPUM
   PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                  ***  MASS AND ENERGY BALANCE  ***
                                IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            190.098         190.098         0.00000
      MASS(LB/HR   )            3424.66         3424.66         0.00000
      ENTHALPY(BTU/HR  )      -0.233197E+08   -0.233187E+08   -0.412330E-04

                      ***  INPUT DATA  ***
   OUTLET PRESSURE  PSIA                                  42.4000
   DRIVER EFFICIENCY                                       1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                            30
   TOLERANCE                                               0.000100000

                       ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  CUFT/HR                          55.4612
   PRESSURE CHANGE  PSI                                   27.7000
   NPSH AVAILABLE   FT-LBF/LB                             32.8441
   FLUID POWER  HP                                         0.11173
   BRAKE POWER  HP                                         0.37790
   ELECTRICITY  KW                                         0.28180
   PUMP EFFICIENCY USED                                    0.29566
   NET WORK REQUIRED  HP                                   0.37790
   HEAD DEVELOPED FT-LBF/LB                               64.5972

 BLOCK:  PUMPIN2  MODEL: PUMP
 ----------------------------
   INLET STREAM:          MEDIAIN
   OUTLET STREAM:         MEDIAPUM
   PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                  ***  MASS AND ENERGY BALANCE  ***
                                IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            173.901         173.901         0.00000
      MASS(LB/HR   )            15055.0         15055.0         0.00000
      ENTHALPY(BTU/HR  )      -0.336471E+08   -0.336451E+08   -0.599182E-04

                      ***  INPUT DATA  ***
   OUTLET PRESSURE  PSIA                                  42.4000
   PUMP EFFICIENCY                                         0.70000
   DRIVER EFFICIENCY                                       1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                            30
   TOLERANCE                                               0.000100000

                       ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  CUFT/HR                         275.279
   PRESSURE CHANGE  PSI                                   27.7041
```

```
   NPSH AVAILABLE   FT-LBF/LB                          26.9574
   FLUID POWER  HP                                      0.55464
   BRAKE POWER  HP                                      0.79235
   ELECTRICITY  KW                                      0.59085
   PUMP EFFICIENCY USED                                 0.70000
   NET WORK REQUIRED  HP                                0.79235
   HEAD DEVELOPED FT-LBF/LB                            72.9455

BLOCK:  PUMPIN3  MODEL: PUMP
----------------------------
  INLET STREAM:          MOLASSES
  OUTLET STREAM:         TOSTERIL
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            91.4818         91.4818         0.00000
      MASS(LB/HR   )            15746.3         15746.3         0.00000
      ENTHALPY(BTU/HR  )      -0.273678E+08   -0.273678E+08   -0.168536E-05

                       ***  INPUT DATA  ***
   OUTLET PRESSURE  PSIA                               52.5000
   PUMP EFFICIENCY                                      0.70000
   DRIVER EFFICIENCY                                    1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                         30
   TOLERANCE                                            0.000100000

                        ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  CUFT/HR                        7.75618
   PRESSURE CHANGE  PSI                                22.5000
   NPSH AVAILABLE   FT-LBF/LB                           3.66898
   FLUID POWER  HP                                      0.012692
   BRAKE POWER  HP                                      0.018131
   ELECTRICITY  KW                                      0.013521
   PUMP EFFICIENCY USED                                 0.70000
   NET WORK REQUIRED  HP                                0.018131
   HEAD DEVELOPED FT-LBF/LB                             2.75174

BLOCK:  PUMPIN4  MODEL: PUMP
----------------------------
  INLET STREAM:          OLEIN
  OUTLET STREAM:         OLEICIN
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           0.658485        0.658485         0.00000
      MASS(LB/HR   )            186.000         186.000         0.00000
      ENTHALPY(BTU/HR  )       -232118.        -231991.       -0.546169E-03

                       ***  INPUT DATA  ***
   OUTLET PRESSURE  PSIA                               74.7000
   DRIVER EFFICIENCY                                    1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                         30
   TOLERANCE                                            0.000100000

                        ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  CUFT/HR                        3.37587
```

```
   PRESSURE CHANGE  PSI                                60.0000
   NPSH AVAILABLE   FT-LBF/LB                          38.4196
   FLUID POWER  HP                                      0.014731
   BRAKE POWER  HP                                      0.049825
   ELECTRICITY  KW                                      0.037154
   PUMP EFFICIENCY USED                                 0.29566
   NET WORK REQUIRED  HP                                0.049825
   HEAD DEVELOPED FT-LBF/LB                           156.815

BLOCK:  PUMPWATE MODEL: PUMP
----------------------------
  INLET STREAM:          WATERNOV
  OUTLET STREAM:         WTRP30
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            180.539         180.539         0.00000
      MASS(LB/HR   )            3266.20         3266.20         0.417685E-15
      ENTHALPY(BTU/HR  )      -0.223016E+08   -0.223009E+08   -0.303008E-04

                       ***  INPUT DATA  ***
   OUTLET PRESSURE  PSIA                               49.7000
   PUMP EFFICIENCY                                      0.70000
   DRIVER EFFICIENCY                                    1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                         30
   TOLERANCE                                            0.000100000

                        ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  CUFT/HR                       51.6283
   PRESSURE CHANGE  PSI                                49.5500
   NPSH AVAILABLE   FT-LBF/LB                           0.0
   FLUID POWER  HP                                      0.18605
   BRAKE POWER  HP                                      0.26579
   ELECTRICITY  KW                                      0.19820
   PUMP EFFICIENCY USED                                 0.70000
   NET WORK REQUIRED  HP                                0.26579
   HEAD DEVELOPED FT-LBF/LB                           112.785

BLOCK:  SEP2GAS  MODEL: SEP2
----------------------------
  INLET STREAMS:         RXNDONE1    RXNDONE2
  OUTLET STREAMS:        CO2         WITHCELL
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            722.348         722.348         0.295905E-07
      MASS(LB/HR   )            61093.3         61093.2         0.551342E-07
      ENTHALPY(BTU/HR  )      -0.885278E+08   -0.864566E+08   -0.233965E-01


                       ***  INPUT DATA  ***

  INLET PRESSURE:  MINIMUM OF INLET STREAM PRESSURES

  FLASH SPECS FOR STREAM CO2
  TWO    PHASE  TP  FLASH
  PRESSURE DROP        PSI                               0.0
  MAXIMUM NO. ITERATIONS                                30
  CONVERGENCE TOLERANCE                                  0.000100000
```

```
 FLASH SPECS FOR STREAM WITHCELL
 TWO    PHASE  TP  FLASH
 PRESSURE DROP        PSI                              0.0
 MAXIMUM NO. ITERATIONS                               30
 CONVERGENCE TOLERANCE                                 0.000100000

 SPLIT FRACTION
   SUBSTREAM= MIXED
     STREAM= CO2       CPT= 1:4-B-01  FRACTION=          0.0
                            WATER                        0.0
                            DEXTR-01                     0.0
                            CARBO-01                     1.00000
                            OLEIC-01                     0.0
                            LYSIN-01                     0.0
                            GLYCI-01                     0.0
                            ISOLE-01                     0.0
                            LEUCI-01                     0.0
                            METHI-01                     0.0
                            L-PHE-01                     0.0
                            THREO-01                     0.0
                            TRYPT-01                     0.0
                            TYROS-01                     0.0
                            VALIN-01                     0.0
                            INOSI-01                     0.0
                            NIACI-01                     0.0
                            POTAS-01                     0.0
                            MAGNE-01                     0.0
                            CALCI-01                     0.0
                            SULFU-01                     0.0
                            SODIU-01                     0.0
                            IRON                         0.0
                            ZINC                         0.0
                            MANGA-01                     0.0
                            COPPE-01                     0.0
                            CHROM-01                     0.0
                            MOLYB-01                     0.0
                            COBAL-01                     0.0
                            HYDRO-01                     0.0
                            ETHYL-01                     0.0
                            CELLU-01                     0.0
   SUBSTREAM= CISOLID
     STREAM= CO2       CPT= CELLU-01  FRACTION=          0.0


                         ***  RESULTS  ***

 HEAT DUTY             BTU/HR                          0.20712E+07

STREAM=   CO2        SUBSTREAM= MIXED
   COMPONENT = CARBO-01  SPLIT FRACTION =         1.00000

STREAM=   WITHCELL   SUBSTREAM= MIXED
   COMPONENT = 1:4-B-01  SPLIT FRACTION =         1.00000
   COMPONENT = WATER     SPLIT FRACTION =         1.00000
   COMPONENT = DEXTR-01  SPLIT FRACTION =         1.00000
   COMPONENT = LYSIN-01  SPLIT FRACTION =         1.00000
   COMPONENT = GLYCI-01  SPLIT FRACTION =         1.00000
   COMPONENT = ISOLE-01  SPLIT FRACTION =         1.00000
   COMPONENT = LEUCI-01  SPLIT FRACTION =         1.00000
   COMPONENT = METHI-01  SPLIT FRACTION =         1.00000
   COMPONENT = L-PHE-01  SPLIT FRACTION =         1.00000
   COMPONENT = THREO-01  SPLIT FRACTION =         1.00000
   COMPONENT = TRYPT-01  SPLIT FRACTION =         1.00000
   COMPONENT = TYROS-01  SPLIT FRACTION =         1.00000
   COMPONENT = VALIN-01  SPLIT FRACTION =         1.00000
   COMPONENT = INOSI-01  SPLIT FRACTION =         1.00000
   COMPONENT = NIACI-01  SPLIT FRACTION =         1.00000
   COMPONENT = POTAS-01  SPLIT FRACTION =         1.00000
```

```
   COMPONENT = MAGNE-01  SPLIT FRACTION =         1.00000
   COMPONENT = CALCI-01  SPLIT FRACTION =         1.00000
   COMPONENT = SULFU-01  SPLIT FRACTION =         1.00000
   COMPONENT = SODIU-01  SPLIT FRACTION =         1.00000
   COMPONENT = IRON      SPLIT FRACTION =         1.00000
   COMPONENT = ZINC      SPLIT FRACTION =         1.00000
   COMPONENT = MANGA-01  SPLIT FRACTION =         1.00000
   COMPONENT = COPPE-01  SPLIT FRACTION =         1.00000
   COMPONENT = CHROM-01  SPLIT FRACTION =         1.00000
   COMPONENT = MOLYB-01  SPLIT FRACTION =         1.00000
   COMPONENT = COBAL-01  SPLIT FRACTION =         1.00000
   COMPONENT = HYDRO-01  SPLIT FRACTION =         1.00000
   COMPONENT = ETHYL-01  SPLIT FRACTION =         1.00000

STREAM=   WITHCELL   SUBSTREAM= CISOLID
   COMPONENT = CELLU-01  SPLIT FRACTION =         1.00000

BLOCK:  SEP2MED  MODEL: SEP2
----------------------------
  INLET STREAM:         WITHC02
  OUTLET STREAMS:       MEDIAOUT    NOMEDIA
  PROPERTY OPTION SET:  NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)           413.113         413.113        0.275195E-15
      MASS(LB/HR   )           23277.7         23277.7        0.781430E-15
      ENTHALPY(BTU/HR  )     -0.686957E+08   -0.689043E+08    0.302755E-02


                       ***  INPUT DATA  ***

  FLASH SPECS FOR STREAM MEDIAOUT
  TWO    PHASE  TP  FLASH
  PRESSURE DROP        PSI                              0.0
  MAXIMUM NO. ITERATIONS                               30
  CONVERGENCE TOLERANCE                                 0.000100000

  FLASH SPECS FOR STREAM NOMEDIA
  TWO    PHASE  TP  FLASH
  PRESSURE DROP        PSI                              0.0
  MAXIMUM NO. ITERATIONS                               30
  CONVERGENCE TOLERANCE                                 0.000100000

  SPLIT FRACTION
    SUBSTREAM= MIXED
      STREAM= MEDIAOUT  CPT= 1:4-B-01  FRACTION=          0.0
                             WATER                        0.0
                             DEXTR-01                     0.0
                             CARBO-01                     0.0
                             OLEIC-01                     0.0
                             LYSIN-01                     0.0
                             GLYCI-01                     0.0
                             ISOLE-01                     0.0
                             LEUCI-01                     0.0
                             METHI-01                     0.0
                             L-PHE-01                     0.0
                             THREO-01                     0.0
                             TRYPT-01                     0.0
                             TYROS-01                     0.0
                             VALIN-01                     0.0
                             INOSI-01                     0.0
                             NIACI-01                     0.0
                             POTAS-01                     0.0
                             MAGNE-01                     0.0
                             CALCI-01                     0.0
                             SULFU-01                     0.0
```

```
                             SODIU-01                     0.0
                             IRON                         0.0
                             ZINC                         0.0
                             MANGA-01                     0.0
                             COPPE-01                     0.0
                             CHROM-01                     0.0
                             MOLYB-01                     0.0
                             COBAL-01                     0.0
                             HYDRO-01                     0.0
                             ETHYL-01                     1.00000
                             CELLU-01                     0.0
     SUBSTREAM= CISOLID
       STREAM= MEDIAOUT  CPT= CELLU-01  FRACTION=         0.0


                          ***  RESULTS  ***

   HEAT DUTY             BTU/HR                            -0.20861E+06

  STREAM=   MEDIAOUT   SUBSTREAM= MIXED
     COMPONENT = ETHYL-01  SPLIT FRACTION =          1.00000

  STREAM=   NOMEDIA    SUBSTREAM= MIXED
     COMPONENT = 1:4-B-01  SPLIT FRACTION =          1.00000
     COMPONENT = WATER     SPLIT FRACTION =          1.00000
     COMPONENT = DEXTR-01  SPLIT FRACTION =          1.00000
     COMPONENT = CARBO-01  SPLIT FRACTION =          1.00000
     COMPONENT = LYSIN-01  SPLIT FRACTION =          1.00000
     COMPONENT = GLYCI-01  SPLIT FRACTION =          1.00000
     COMPONENT = ISOLE-01  SPLIT FRACTION =          1.00000
     COMPONENT = LEUCI-01  SPLIT FRACTION =          1.00000
     COMPONENT = METHI-01  SPLIT FRACTION =          1.00000
     COMPONENT = L-PHE-01  SPLIT FRACTION =          1.00000
     COMPONENT = THREO-01  SPLIT FRACTION =          1.00000
     COMPONENT = TRYPT-01  SPLIT FRACTION =          1.00000
     COMPONENT = TYROS-01  SPLIT FRACTION =          1.00000
     COMPONENT = VALIN-01  SPLIT FRACTION =          1.00000
     COMPONENT = INOSI-01  SPLIT FRACTION =          1.00000
     COMPONENT = NIACI-01  SPLIT FRACTION =          1.00000
     COMPONENT = POTAS-01  SPLIT FRACTION =          1.00000
     COMPONENT = MAGNE-01  SPLIT FRACTION =          1.00000
     COMPONENT = CALCI-01  SPLIT FRACTION =          1.00000
     COMPONENT = SULFU-01  SPLIT FRACTION =          1.00000
     COMPONENT = SODIU-01  SPLIT FRACTION =          1.00000
     COMPONENT = IRON      SPLIT FRACTION =          1.00000
     COMPONENT = ZINC      SPLIT FRACTION =          1.00000
     COMPONENT = MANGA-01  SPLIT FRACTION =          1.00000
     COMPONENT = COPPE-01  SPLIT FRACTION =          1.00000
     COMPONENT = CHROM-01  SPLIT FRACTION =          1.00000
     COMPONENT = MOLYB-01  SPLIT FRACTION =          1.00000
     COMPONENT = COBAL-01  SPLIT FRACTION =          1.00000
     COMPONENT = HYDRO-01  SPLIT FRACTION =          1.00000

 BLOCK:  SLDSPLIT MODEL: FSPLIT
 ------------------------------
   INLET STREAM:          SOLIDREC
   OUTLET STREAMS:        SLDFERM1    SLDFERM2
   PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                     ***  MASS AND ENERGY BALANCE  ***
                                   IN              OUT        RELATIVE DIFF.
    TOTAL BALANCE
       MOLE(LBMOL/HR)            170.470         170.470         0.00000
       MASS(LB/HR   )            26867.3         26867.3         0.00000
       ENTHALPY(BTU/HR  )      -0.119895E+07   -0.119895E+07     0.00000

                         ***  INPUT DATA  ***

 FRACTION OF FLOW                 STRM=SLDFERM1 FRAC=         0.50000

                          ***  RESULTS  ***

 STREAM= SLDFERM1       SPLIT=          0.50000     KEY=  0    STREAM-ORDER=   1
         SLDFERM2                       0.50000           0                    2

 BLOCK:  SLDVALVE MODEL: VALVE
 -----------------------------
   INLET STREAM:          SOLPURGE
   OUTLET STREAM:         SOLIDOUT
   PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                     ***  MASS AND ENERGY BALANCE  ***
                                   IN              OUT        RELATIVE DIFF.
    TOTAL BALANCE
       MOLE(LBMOL/HR)            42.6176         42.6176         0.00000
       MASS(LB/HR   )            6716.83         6716.83         0.00000
       ENTHALPY(BTU/HR  )       -299738.        -299737.       -0.130434E-06

                         ***  INPUT DATA  ***

    VALVE PRESSURE DROP       PSI                           25.0000
    VALVE FLOW COEF CALC.                                  NO

                         FLASH SPECIFICATIONS:
    NPHASE                                                   2
    MAX NUMBER OF ITERATIONS                                30
    CONVERGENCE TOLERANCE                                    0.000100000

                         ***  RESULTS  ***

    VALVE OUTLET PRESSURE     PSIA                          17.4000

 BLOCK:  SLURRYPU MODEL: PUMP
 ----------------------------
   INLET STREAM:          CELLSOLD
   OUTLET STREAM:         SLURRY
   PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                     ***  MASS AND ENERGY BALANCE  ***
                                   IN              OUT        RELATIVE DIFF.
    TOTAL BALANCE
       MOLE(LBMOL/HR)            213.088         213.088         0.00000
       MASS(LB/HR   )            33584.2         33584.2         0.00000
       ENTHALPY(BTU/HR  )      -0.149875E+07   -0.149869E+07   -0.416120E-04

                         ***  INPUT DATA  ***
    OUTLET PRESSURE  PSIA                                   42.4000
    PUMP EFFICIENCY                                          0.70000
    DRIVER EFFICIENCY                                        1.00000

    FLASH SPECIFICATIONS:
    LIQUID PHASE CALCULATION
    NO FLASH PERFORMED
    MAXIMUM NUMBER OF ITERATIONS                             30
    TOLERANCE                                                0.000100000

                          ***  RESULTS  ***
    VOLUMETRIC FLOW RATE  CUFT/HR                            8.51168
    PRESSURE CHANGE  PSI                                    27.7077
    NPSH AVAILABLE   FT-LBF/LB                              28.5476
    FLUID POWER  HP                                          0.017152
    BRAKE POWER  HP                                          0.024503
    ELECTRICITY  KW                                          0.018272
    PUMP EFFICIENCY USED                                     0.70000
    NET WORK REQUIRED  HP                                    0.024503
    HEAD DEVELOPED FT-LBF/LB                                65.9636
```

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BLOCK:  SOLIDPUM MODEL: PUMP
----------------------------
  INLET STREAM:          OLECOND
  OUTLET STREAM:         OLESPLIT
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            18.5950         18.5950         0.00000
      MASS(LB/HR   )            2283.14         2283.14         0.00000
      ENTHALPY(BTU/HR  )      -0.280494E+07   -0.280441E+07   -0.187667E-03

                       ***  INPUT DATA  ***
   OUTLET PRESSURE  PSIA                              49.7000
   PUMP EFFICIENCY                                     0.70000
   DRIVER EFFICIENCY                                   1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                        30
   TOLERANCE                                           0.000100000

                        ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  CUFT/HR                      40.4945
   PRESSURE CHANGE  PSI                               49.1778
   NPSH AVAILABLE   FT-LBF/LB                          0.0
   FLUID POWER  HP                                     0.14483
   BRAKE POWER  HP                                     0.20690
   ELECTRICITY  KW                                     0.15429
   PUMP EFFICIENCY USED                                0.70000
   NET WORK REQUIRED  HP                               0.20690
   HEAD DEVELOPED FT-LBF/LB                          125.602

BLOCK:  SOLPURGE MODEL: FSPLIT
------------------------------
  INLET STREAM:          SLURRY
  OUTLET STREAMS:        SOLIDREC    SOLPURGE
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            213.088         213.088         0.00000
      MASS(LB/HR   )            33584.2         33584.2         0.00000
      ENTHALPY(BTU/HR  )      -0.149869E+07   -0.149869E+07   -0.158743E-11

                       ***  INPUT DATA  ***

 FRACTION OF FLOW                STRM=SOLPURGE FRAC=         0.20000

                        ***  RESULTS  ***

 STREAM= SOLIDREC       SPLIT=          0.80000     KEY=  0    STREAM-ORDER=   2
         SOLPURGE                       0.20000           0                    1

BLOCK:  SOLVAL2  MODEL: VALVE
-----------------------------
  INLET STREAM:          SLDFERM2
  OUTLET STREAM:         SOL2FER2
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            85.2352         85.2352         0.00000
      MASS(LB/HR   )            13433.7         13433.7         0.00000
      ENTHALPY(BTU/HR  )       -599475.        -599475.        0.835387E-07

                       ***  INPUT DATA  ***

   VALVE OUTLET PRESSURE     PSIA                         25.0000
   VALVE FLOW COEF CALC.                                 NO

                       FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                       ***  RESULTS  ***

   VALVE PRESSURE DROP       PSI                          17.4000

BLOCK:  SOLVALVE MODEL: VALVE
-----------------------------
  INLET STREAM:          SLDFERM1
  OUTLET STREAM:         SOL2FERM
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            85.2352         85.2352         0.00000
      MASS(LB/HR   )            13433.7         13433.7         0.00000
      ENTHALPY(BTU/HR  )       -599475.        -599475.       -0.130434E-06

                       ***  INPUT DATA  ***

   VALVE PRESSURE DROP       PSI                          25.0000
   VALVE FLOW COEF CALC.                                 NO

                       FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                       ***  RESULTS  ***

   VALVE OUTLET PRESSURE     PSIA                         17.4000

BLOCK:  SPLIT    MODEL: FSPLIT
------------------------------
  INLET STREAM:          BLOWN
  OUTLET STREAMS:        ROCOMP      SIDESTRE
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            186.740         186.740         0.00000
      MASS(LB/HR   )            3396.63         3396.63         0.00000
      ENTHALPY(BTU/HR  )      -0.193733E+08   -0.193733E+08    0.525117E-10

                       ***  INPUT DATA  ***

 FRACTION OF FLOW                STRM=SIDESTRE FRAC=         0.00100000

                        ***  RESULTS  ***

 STREAM= ROCOMP         SPLIT=          0.99900     KEY=  0    STREAM-ORDER=   2
         SIDESTRE                       0.00100000        0                    1

BLOCK:  STERILIZ MODEL: HEATER
------------------------------
```

```
  INLET STREAM:          TOSTERIL
  OUTLET STREAM:         STERILEM
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            91.4818         91.4818         0.00000
      MASS(LB/HR   )            15746.3         15746.3         0.00000
      ENTHALPY(BTU/HR  )      -0.273678E+08   -0.264172E+08   -0.347347E-01

                       ***  INPUT DATA  ***
  TWO    PHASE  TP  FLASH
  SPECIFIED TEMPERATURE             F                      266.000
  SPECIFIED PRESSURE                PSIA                    52.5000
  MAXIMUM NO. ITERATIONS                                    30
  CONVERGENCE TOLERANCE                                      0.000100000



                        ***  RESULTS  ***
  OUTLET TEMPERATURE    F                                266.00
  OUTLET PRESSURE       PSIA                              52.500
  HEAT DUTY             BTU/HR                           0.95061E+06
  OUTLET VAPOR FRACTION                                   0.0000
  PRESSURE-DROP CORRELATION PARAMETER                     0.0000



  V-L PHASE EQUILIBRIUM :

     COMP             F(I)           X(I)           Y(I)           K(I)
     DEXTR-01         1.0000         1.0000         1.0000        0.24217E-06

BLOCK:  VENT     MODEL: FLASH2
------------------------------
  INLET STREAM:          FERMOUT
  OUTLET VAPOR STREAM:   CO2VENT
  OUTLET LIQUID STREAM:  RXNDONE1
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            361.174         361.174       -0.157385E-15
      MASS(LB/HR   )            30546.6         30546.6        0.476384E-15
      ENTHALPY(BTU/HR  )      -0.442639E+08   -0.442639E+08    0.168322E-15

                       ***  INPUT DATA  ***
  TWO    PHASE  TP  FLASH
  SPECIFIED TEMPERATURE F                               86.0000
  SPECIFIED PRESSURE    PSIA                            17.4045
  MAXIMUM NO. ITERATIONS                                30
  CONVERGENCE TOLERANCE                                  0.000100000

                        ***  RESULTS  ***
  OUTLET TEMPERATURE    F                                86.000
  OUTLET PRESSURE       PSIA                             17.405
  HEAT DUTY             BTU/HR                          -0.24729E-08
  VAPOR FRACTION                                         0.0000



  V-L PHASE EQUILIBRIUM :

     COMP             F(I)           X(I)           Y(I)           K(I)
     1:4-B-01        0.18923        0.18923        0.22348E-05    0.77810E-05
     WATER           0.37317        0.37317        0.32474E-01    0.57335E-01
```

```
     DEXTR-01         0.48935E-02    0.48935E-02    0.21396E-15    0.28807E-13
     CARBO-01         0.18595        0.18595        0.79021         2.7998
     LYSIN-01         0.55183E-03    0.55183E-03    0.24302E-11    0.29015E-08
     GLYCI-01         0.10747E-02    0.10747E-02    0.19183E-12    0.11760E-09
     ISOLE-01         0.61500E-03    0.61500E-03    0.14793E-81    0.15847E-78
     LEUCI-01         0.61500E-03    0.61500E-03    0.14793E-81    0.15847E-78
     METHI-01         0.54065E-03    0.54065E-03    0.13004E-81    0.15847E-78
     L-PHE-01         0.48836E-03    0.48836E-03    0.81413E-11    0.10984E-07
     THREO-01         0.67723E-03    0.67723E-03    0.16289E-81    0.15847E-78
     TRYPT-01         0.39501E-03    0.39501E-03    0.95012E-82    0.15847E-78
     TYROS-01         0.44523E-03    0.44523E-03    0.10709E-81    0.15847E-78
     VALIN-01         0.68864E-03    0.68864E-03    0.16564E-81    0.15847E-78
     INOSI-01         0.31099E-03    0.31099E-03    0.24094E-19    0.51046E-16
     NIACI-01         0.11716E-03    0.11716E-03    0.43295E-07    0.24346E-03
     POTAS-01         0.14996E-02    0.14996E-02    0.10068E-12    0.44232E-10
     MAGNE-01         0.24124E-02    0.24124E-02    0.90538E-19    0.24727E-16
     CALCI-01         0.14630E-02    0.14630E-02    0.50228E-25    0.22620E-22
     SULFU-01         0.18285E-02    0.18285E-02    0.61538E-09    0.22173E-06
     SODIU-01         0.25504E-02    0.25504E-02    0.30025E-15    0.77565E-13
     IRON             0.10499E-02    0.10499E-02    0.27362E-61    0.17171E-58
     ZINC             0.89668E-03    0.89668E-03    0.53527E-18    0.39330E-15
     MANGA-01         0.10673E-02    0.10673E-02    0.70292E-37    0.68018E-34
     COPPE-01         0.92270E-03    0.92270E-03    0.26087E-81    0.18627E-78
     CHROM-01         0.11277E-02    0.11277E-02    0.31882E-81    0.18627E-78
     MOLYB-01         0.61115E-03    0.61115E-03    0.31806E-90    0.34289E-87
     COBAL-01         0.99492E-03    0.99492E-03    0.28129E-81    0.18627E-78
     HYDRO-01         0.16081E-02    0.16081E-02    0.89869E-01     36.819
     ETHYL-01         0.22221        0.22221        0.87441E-01    0.25927

BLOCK:  VENT2    MODEL: FLASH2
------------------------------
  INLET STREAM:          FERMOUT2
  OUTLET VAPOR STREAM:   CO2VENT2
  OUTLET LIQUID STREAM:  RXNDONE2
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            361.174         361.174       -0.157385E-15
      MASS(LB/HR   )            30546.6         30546.6        0.476384E-15
      ENTHALPY(BTU/HR  )      -0.442639E+08   -0.442639E+08    0.168322E-15

                       ***  INPUT DATA  ***
  TWO    PHASE  TP  FLASH
  SPECIFIED TEMPERATURE F                               86.0000
  SPECIFIED PRESSURE    PSIA                            17.4045
  MAXIMUM NO. ITERATIONS                                30
  CONVERGENCE TOLERANCE                                  0.000100000

                        ***  RESULTS  ***
  OUTLET TEMPERATURE    F                                86.000
  OUTLET PRESSURE       PSIA                             17.405
  HEAT DUTY             BTU/HR                          -0.24729E-08
  VAPOR FRACTION                                         0.0000



  V-L PHASE EQUILIBRIUM :

     COMP             F(I)           X(I)           Y(I)           K(I)
     1:4-B-01        0.18923        0.18923        0.22348E-05    0.77810E-05
     WATER           0.37317        0.37317        0.32474E-01    0.57335E-01
     DEXTR-01        0.48935E-02    0.48935E-02    0.21396E-15    0.28807E-13
     CARBO-01        0.18595        0.18595        0.79021         2.7998
     LYSIN-01        0.55183E-03    0.55183E-03    0.24302E-11    0.29015E-08
     GLYCI-01        0.10747E-02    0.10747E-02    0.19183E-12    0.11760E-09
     ISOLE-01        0.61500E-03    0.61500E-03    0.14793E-81    0.15847E-78
```

```
    LEUCI-01         0.61500E-03    0.61500E-03    0.14793E-81    0.15847E-78
    METHI-01         0.54065E-03    0.54065E-03    0.13004E-81    0.15847E-78
    L-PHE-01         0.48836E-03    0.48836E-03    0.81413E-11    0.10984E-07
    THREO-01         0.67723E-03    0.67723E-03    0.16289E-81    0.15847E-78
    TRYPT-01         0.39501E-03    0.39501E-03    0.95012E-82    0.15847E-78
    TYROS-01         0.44523E-03    0.44523E-03    0.10709E-81    0.15847E-78
    VALIN-01         0.68864E-03    0.68864E-03    0.16564E-81    0.15847E-78
    INOSI-01         0.31099E-03    0.31099E-03    0.24094E-19    0.51046E-16
    NIACI-01         0.11716E-03    0.11716E-03    0.43295E-07    0.24346E-03
    POTAS-01         0.14996E-02    0.14996E-02    0.10068E-12    0.44232E-10
    MAGNE-01         0.24124E-02    0.24124E-02    0.90538E-19    0.24727E-16
    CALCI-01         0.14630E-02    0.14630E-02    0.50228E-25    0.22620E-22
    SULFU-01         0.18285E-02    0.18285E-02    0.61538E-09    0.22173E-06
    SODIU-01         0.25504E-02    0.25504E-02    0.30025E-15    0.77565E-13
    IRON             0.10499E-02    0.10499E-02    0.27362E-61    0.17171E-58
    ZINC             0.89668E-03    0.89668E-03    0.53527E-18    0.39330E-15
    MANGA-01         0.10673E-02    0.10673E-02    0.70292E-37    0.68018E-34
    COPPE-01         0.92270E-03    0.92270E-03    0.26087E-81    0.18627E-78
    CHROM-01         0.11277E-02    0.11277E-02    0.31882E-81    0.18627E-78
    MOLYB-01         0.61115E-03    0.61115E-03    0.31806E-90    0.34289E-87
    COBAL-01         0.99492E-03    0.99492E-03    0.28129E-81    0.18627E-78
    HYDRO-01         0.16081E-02    0.16081E-02    0.89869E-01     36.819
    ETHYL-01         0.22221        0.22221        0.87441E-01    0.25927

BLOCK:  VINMIXER MODEL: MIXER
-----------------------------
  INLET STREAMS:         SOLIDOUT    TOMIXER     MEDIAOUT
  OUTLET STREAM:         VINASSE
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            351.159         351.159         0.00000
      MASS(LB/HR   )            21266.2         21266.2         0.00000
      ENTHALPY(BTU/HR  )      -0.477115E+08   -0.477115E+08   -0.812620E-09

                        ***  INPUT DATA  ***
  TWO    PHASE      FLASH
  MAXIMUM NO. ITERATIONS                               30
  CONVERGENCE TOLERANCE                                  0.000100000
  OUTLET PRESSURE   PSIA                              39.7000

BLOCK:  WAT2VAL2 MODEL: VALVE
-----------------------------
  INLET STREAM:          WTRFERM2
  OUTLET STREAM:         WTR2FER2
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            95.0488         95.0488         0.00000
      MASS(LB/HR   )            1712.33         1712.33         0.00000
      ENTHALPY(BTU/HR  )      -0.116594E+08   -0.116594E+08   -0.113742E-08

                        ***  INPUT DATA  ***

   VALVE OUTLET PRESSURE     PSIA                         25.0000
   VALVE FLOW COEF CALC.                                 NO

                        FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                        ***  RESULTS  ***
```

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   VALVE PRESSURE DROP       PSI                          17.4000

BLOCK:  WATVALVE MODEL: VALVE
-----------------------------
  INLET STREAM:          WTRFERM1
  OUTLET STREAM:         WTR2FERM
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            95.0488         95.0488         0.00000
      MASS(LB/HR   )            1712.33         1712.33         0.00000
      ENTHALPY(BTU/HR  )      -0.116594E+08   -0.116594E+08   -0.340527E-09

                        ***  INPUT DATA  ***

   VALVE PRESSURE DROP       PSI                          25.0000
   VALVE FLOW COEF CALC.                                 NO

                        FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                        ***  RESULTS  ***

   VALVE OUTLET PRESSURE     PSIA                         17.4000

BLOCK:  WRMBDOVL MODEL: VALVE
-----------------------------
  INLET STREAM:          WARMBDO
  OUTLET STREAM:         BDOOUT
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            98.5592         98.5592         0.00000
      MASS(LB/HR   )            8601.31         8601.31         0.00000
      ENTHALPY(BTU/HR  )      -0.210483E+08   -0.210483E+08   -0.141577E-09

                        ***  INPUT DATA  ***

   VALVE PRESSURE DROP       PSI                          25.0000
   VALVE FLOW COEF CALC.                                 NO

                        FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                        ***  RESULTS  ***

   VALVE OUTLET PRESSURE     PSIA                         40.0000

BLOCK:  WTRSPLIT MODEL: FSPLIT
------------------------------
  INLET STREAM:          WATERPUM
  OUTLET STREAMS:        WTRFERM2    WTRFERM1
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                    ***  MASS AND ENERGY BALANCE  ***
                                  IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            190.098         190.098         0.00000
      MASS(LB/HR   )            3424.66         3424.66         0.00000
      ENTHALPY(BTU/HR  )      -0.233187E+08   -0.233187E+08   -0.521163E-08
```

```
                         ***  INPUT DATA  ***

 FRACTION OF FLOW                 STRM=WTRFERM2 FRAC=         0.50000

                          ***  RESULTS  ***

 STREAM= WTRFERM2       SPLIT=          0.50000     KEY=  0    STREAM-ORDER=   1
         WTRFERM1                       0.50000           0                    2

BLOCK:  WTRVALVE MODEL: VALVE
-----------------------------
  INLET STREAM:          WTRP30
  OUTLET STREAM:         WTR2LLE
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                     ***  MASS AND ENERGY BALANCE  ***
                                   IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            180.539         180.539         0.00000
      MASS(LB/HR   )            3266.20         3266.20         0.00000
      ENTHALPY(BTU/HR  )      -0.223009E+08   -0.223009E+08   -0.643342E-08

                         ***  INPUT DATA  ***

   VALVE PRESSURE DROP       PSI                          25.0000
   VALVE FLOW COEF CALC.                                 NO

                         FLASH SPECIFICATIONS:
   NPHASE                                                  2
   MAX NUMBER OF ITERATIONS                               30
   CONVERGENCE TOLERANCE                                   0.000100000

                         ***  RESULTS  ***

   VALVE OUTLET PRESSURE     PSIA                         24.7000

BLOCK:  ZEROVAP  MODEL: HEATER
------------------------------
  INLET STREAM:          OLESOLID
  OUTLET STREAM:         OLECOND
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                     ***  MASS AND ENERGY BALANCE  ***
                                   IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            18.5950         18.5950         0.00000
      MASS(LB/HR   )            2283.14         2283.14        0.398353E-15
      ENTHALPY(BTU/HR  )      -0.273178E+07   -0.280494E+07    0.260820E-01

                         ***  INPUT DATA  ***
  TWO    PHASE  PV  FLASH
  SPECIFIED PRESSURE                  PSIA                        0.52221
  VAPOR FRACTION                                                  0.0
  MAXIMUM NO. ITERATIONS                                         30
  CONVERGENCE TOLERANCE                                           0.000100000



                          ***  RESULTS  ***
  OUTLET TEMPERATURE    F                                   392.32
  OUTLET PRESSURE       PSIA                               0.52221
  HEAT DUTY             BTU/HR                             -73158.
  OUTLET VAPOR FRACTION                                     0.0000
  PRESSURE-DROP CORRELATION PARAMETER                      0.16668E+06
```

```
  V-L PHASE EQUILIBRIUM :

     COMP              F(I)           X(I)           Y(I)           K(I)
     1:4-B-01        0.67057E-01    0.67057E-01    0.94875         14.148
     WATER           0.12409E-10    0.12409E-10    0.59899E-08     482.69
     DEXTR-01        0.13346        0.13346        0.29687E-02    0.22244E-01
     CARBO-01        0.24058E-09    0.24058E-09    0.15671E-09    0.65142
     OLEIC-01        0.17693        0.17693        0.37843E-01    0.21389
     LYSIN-01        0.15050E-01    0.15050E-01    0.26245E-02    0.17439
     GLYCI-01        0.29309E-01    0.29309E-01    0.40440E-03    0.13798E-01
     ISOLE-01        0.16773E-01    0.16773E-01    0.61633E-79    0.36747E-77
     LEUCI-01        0.16773E-01    0.16773E-01    0.61633E-79    0.36747E-77
     METHI-01        0.14745E-01    0.14745E-01    0.54182E-79    0.36747E-77
     L-PHE-01        0.13319E-01    0.13319E-01    0.31482E-02    0.23637
     THREO-01        0.18470E-01    0.18470E-01    0.67870E-79    0.36747E-77
     TRYPT-01        0.10773E-01    0.10773E-01    0.39586E-79    0.36747E-77
     TYROS-01        0.12143E-01    0.12143E-01    0.44620E-79    0.36747E-77
     VALIN-01        0.18781E-01    0.18781E-01    0.69013E-79    0.36747E-77
     INOSI-01        0.84814E-02    0.84814E-02    0.20382E-05    0.24030E-03
     NIACI-01        0.82834E-05    0.82834E-05    0.78348E-04     9.4584
     POTAS-01        0.40899E-01    0.40899E-01    0.87093E-05    0.21294E-03
     MAGNE-01        0.65793E-01    0.65793E-01    0.10500E-08    0.15958E-07
     CALCI-01        0.39900E-01    0.39900E-01    0.14442E-12    0.36195E-11
     SULFU-01        0.49869E-01    0.49869E-01    0.41687E-02    0.83592E-01
     SODIU-01        0.69557E-01    0.69557E-01    0.32297E-06    0.46431E-05
     IRON            0.28634E-01    0.28634E-01    0.32780E-35    0.11447E-33
     ZINC            0.24455E-01    0.24455E-01    0.72622E-08    0.29696E-06
     MANGA-01        0.29107E-01    0.29107E-01    0.25525E-20    0.87686E-19
     COPPE-01        0.25164E-01    0.25164E-01    0.92898E-79    0.36917E-77
     CHROM-01        0.30754E-01    0.30754E-01    0.11353E-78    0.36917E-77
     MOLYB-01        0.16668E-01    0.16668E-01    0.91389E-55    0.54821E-53
     COBAL-01        0.27134E-01    0.27134E-01    0.10017E-78    0.36917E-77

BLOCK:  OLEPUMP  MODEL: PUMP
----------------------------
  INLET STREAM:          OLEICPUR
  OUTLET STREAM:         2OLPVALV
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                     ***  MASS AND ENERGY BALANCE  ***
                                   IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            3.28995         3.28995         0.00000
      MASS(LB/HR   )            929.303         929.303         0.00000
      ENTHALPY(BTU/HR  )      -0.116556E+07   -0.116534E+07   -0.190112E-03

                         ***  INPUT DATA  ***
   OUTLET PRESSURE  PSIA                                  74.7000
   PUMP EFFICIENCY                                         0.70000
   DRIVER EFFICIENCY                                       1.00000

   FLASH SPECIFICATIONS:
   LIQUID PHASE CALCULATION
   NO FLASH PERFORMED
   MAXIMUM NUMBER OF ITERATIONS                           30
   TOLERANCE                                               0.000100000

                          ***  RESULTS  ***
   VOLUMETRIC FLOW RATE  CUFT/HR                          16.7652
   PRESSURE CHANGE  PSI                                   50.0000
   NPSH AVAILABLE   FT-LBF/LB                             64.1667
   FLUID POWER  HP                                         0.060964
   BRAKE POWER  HP                                         0.087092
   ELECTRICITY  KW                                         0.064944
   PUMP EFFICIENCY USED                                    0.70000
   NET WORK REQUIRED  HP                                   0.087092
   HEAD DEVELOPED FT-LBF/LB                              129.892
```

```
BLOCK:  CFUGE    MODEL: CFUGE
------------------------------
  INLET STREAM:          2CFUGE
  OUTLET STREAMS:        NOSOLID     CELLSOLD
  PROPERTY OPTION SET:   NRTL-RK   RENON (NRTL) / REDLICH-KWONG

                   ***  MASS AND ENERGY BALANCE  ***
                                 IN              OUT        RELATIVE DIFF.
   TOTAL BALANCE
      MOLE(LBMOL/HR)            625.951         625.951         0.00000
      MASS(LB/HR   )            56850.8         56850.8         0.00000
      ENTHALPY(BTU/HR  )      -0.701520E+08   -0.701520E+08    0.658290E-08

                       ***  INPUT DATA  ***
  RATIO OF LIQ RADIUS TO RADIUS OF BOWL                   0.73800
  RATIO OF CAKE RADIUS TO RADIUS OF BOWL                  0.79000
  RATIO OF HEIGHT TO RADIUS OF BOWL                       0.95450
  CAKE RESISTANCE  FT/LB                            1,920,000.
  FILTER MEDIUM RESISTANCE  1/FT                          100.000
  MOISTURE CONTENT                                        MISSING
  POROSITY OF CAKE                                        0.45000
  PARTICLE SPHERICITY                                     0.75000
  AVERAGE PARTICLE DIAMETER  FT                           0.00065617
  SURFACE TENSION  DYNE/CM                               55.2554
  AVERAGE SOLID DENSITY  LB/CUFT                        374.689
  DRY SOLIDS FEED MASS FLOW RATE  LB/HR               33,069.3

                        ***  RESULTS  ***
  CALCULATED PARTICLE DIAMETER  FT                       0.00065617
  RESULTED MOISTURE CONTENT                              0.015569
  SELECTED BOWL RADIUS  FT                               0.50000
  REVOLUTION SPEED   RPM                              1,000.00
  BASKET HEIGHT  FT                                      0.47725
```

```
;
;Input Summary created by Aspen Plus Rel. 24.0 at 13:51:30 Tue Apr 5, 2011
;Directory S:\SchoolWork\CBE459  Filename c:\temp\~ap220.txt
;


DYNAMICS
    DYNAMICS RESULTS=ON

IN-UNITS ENG

DEF-STREAMS MIXCISLD ALL

SIM-OPTIONS OLD-DATABANK=YES

DESCRIPTION "
    Solids Simulation with English Units :
    F, psi, lb/hr, lbmol/hr, Btu/hr, cuft/hr.

    Property Method: None

    Flow basis for input: Mass
    "

DATABANKS PURE22  / AQUEOUS  / SOLIDS  / INORGANIC  /  &
        POLYMER  / SEGMENT  / NOASPENPCD

PROP-SOURCES PURE22  / AQUEOUS  / SOLIDS  / INORGANIC  /  &
        POLYMER  / SEGMENT

COMPONENTS
    1:4-B-01 C4H10O2-D2 /
    WATER H2O /
    DEXTR-01 C6H12O6 /
    CARBO-01 CO2 /
    OLEIC-01 C18H34O2 /
    LYSIN-01 C6H14N2O2 /
    GLYCI-01 C2H5NO2-D1 /
    ISOLE-01 C6H13NO2-I /
    LEUCI-01 C6H13NO2 /
    METHI-01 C5H11NO2S /
    L-PHE-01 C9H11NO2 /
    THREO-01 C4H9NO3 /
    TRYPT-01 C11H12N2O2 /
    TYROS-01 C9H11NO3 /
    VALIN-01 C5H11NO2 /
    INOSI-01 C6H12O6-D1 /
    NIACI-01 C6H5NO2-D1 /
    POTAS-01 K /
    MAGNE-01 MG /
    CALCI-01 CA /
    SULFU-01 S /
    SODIU-01 NA /
    IRON FE /
    ZINC ZN /
    MANGA-01 MN /
    COPPE-01 CU /
    CHROM-01 CR /
    MOLYB-01 MO /
    COBAL-01 COBALT /
    HYDRO-01 HCL /
    ETHYL-01 C4H8O2-3 /
    CELLU-01 CELLULOSE

FLOWSHEET
    BLOCK FERMRXN1 IN=MOL2FERM SOL2FERM MED2FERM WTR2FERM OUT= &
        FERMOUT
    BLOCK DIST1 IN=DIST1IN OUT=WTRVAPOR BDODIST OLESOLID
    BLOCK MIXER IN=TOMIX OLEIC OUT=TOHX2
    BLOCK VENT IN=FERMOUT OUT=CO2VENT RXNDONE1
    BLOCK CFUGE IN=2CFUGE OUT=NOSOLID CELLSOLD
    BLOCK PUMPIN3 IN=MOLASSES OUT=TOSTERIL
    BLOCK PUMPIN2 IN=MEDIAIN OUT=MEDIAPUM
    BLOCK PUMPIN1 IN=WATERIN OUT=WATERPUM
    BLOCK VINMIXER IN=SOLIDOUT TOMIXER MEDIAOUT OUT=VINASSE
    BLOCK OLMIXREC IN=OLEICIN OLEICREC OUT=OLEIC
    BLOCK OLESPLIT IN=TOOLEMIX OUT=OLEICREC OLPUROUT
    BLOCK SOLPURGE IN=SLURRY OUT=SOLIDREC SOLPURGE
    BLOCK CO2SPLIT IN=CO2 OUT=CO2DISSO CO2INRXN
    BLOCK SEP2GAS IN=RXNDONE1 RXNDONE2 OUT=CO2 WITHCELL
    BLOCK SEP2MED IN=WITHC02 OUT=MEDIAOUT NOMEDIA
    BLOCK CONDNSOR IN=ROCOMP OUT=CONDENSE
    BLOCK PUMPWATE IN=WATERNOV OUT=WTRP30
    BLOCK BDOPUMP IN=BDO OUT=WARMBDO
    BLOCK LLE IN=OLETOLLE WTR2LLE OUT=OLEICPUR LLEMIN
    BLOCK SOLIDPUM IN=OLECOND OUT=OLESPLIT
    BLOCK ZEROVAP IN=OLESOLID OUT=OLECOND
    BLOCK PUMITUP IN=NOMEDIA OUT=NOSOLHPR
    BLOCK DISTHX IN=HOTIN TOHX2 OUT=OLETOLLE DIST1IN
    BLOCK MOLVALVE IN=MOLFERM1 OUT=MOL2FERM
    BLOCK MEDVALVE IN=MEDFERM1 OUT=MED2FERM
    BLOCK WATVALVE IN=WTRFERM1 OUT=WTR2FERM
    BLOCK SOLVALVE IN=SLDFERM1 OUT=SOL2FERM
    BLOCK 2CNTPUMP IN=WITHCELL OUT=HPWCELL
    BLOCK CNTVALVE IN=HPWCELL OUT=2CFUGE
    BLOCK WRMBDOVL IN=WARMBDO OUT=BDOOUT
    BLOCK OLEVALVE IN=OLESPLIT OUT=HOTIN
    BLOCK OLPUMPVA IN=2OLPVALV OUT=TOOLEMIX
    BLOCK OLPVALVE IN=OLPUROUT OUT=OLEICOUT
    BLOCK ADDINCO2 IN=NOSOLID CO2DISSO OUT=WITHC02
```

```
    BLOCK LLETOPPU IN=LLEMIN OUT=TOBOTVAL
    BLOCK OLEPUMP IN=OLEICPUR OUT=2OLPVALV
    BLOCK LLEBOTVV IN=TOBOTVAL OUT=TOMIXER
    BLOCK SLURRYPU IN=CELLSOLD OUT=SLURRY
    BLOCK SLDVALVE IN=SOLPURGE OUT=SOLIDOUT
    BLOCK CFGVALVE IN=NOSOLHPR OUT=TOMIX
    BLOCK NOVAP IN=BDODIST OUT=BDO
    BLOCK FLASHVAP IN=CONDENSE OUT=VAPOROUT WATERNOV
    BLOCK WTRVALVE IN=WTRP30 OUT=WTR2LLE
    BLOCK STERILIZ IN=TOSTERIL OUT=STERILEM
    BLOCK PRESVAVL IN=SIDESTRE OUT=TOBLWR
    BLOCK SPLIT IN=BLOWN OUT=ROCOMP SIDESTRE
    BLOCK BLOWER IN=TOBLWR WTRVAPOR OUT=BLOWN
    BLOCK WTRSPLIT IN=WATERPUM OUT=WTRFERM2 WTRFERM1
    BLOCK MEDSPLIT IN=MEDIAPUM OUT=MEDFERM1 MEDFERM2
    BLOCK MOLSPLIT IN=STERILEM OUT=MOLFERM1 MOLFERM2
    BLOCK SLDSPLIT IN=SOLIDREC OUT=SLDFERM1 SLDFERM2
    BLOCK FERMRXN2 IN=MOL2FER2 MED2FER2 WTR2FER2 SOL2FER2 OUT= &
        FERMOUT2
    BLOCK VENT2 IN=FERMOUT2 OUT=CO2VENT2 RXNDONE2
    BLOCK PUMPIN4 IN=OLEIN OUT=OLEICIN
    BLOCK CO2EXIT IN=CO2INRXN CO2VENT2 CO2VENT DSLVCO2O OUT= &
        CO2TOATM
    BLOCK CO2COMP IN=VAPOROUT OUT=DSLVCO2O
    BLOCK WAT2VAL2 IN=WTRFERM2 OUT=WTR2FER2
    BLOCK MEDVAL2 IN=MEDFERM2 OUT=MED2FER2
    BLOCK MOL2VAL2 IN=MOLFERM2 OUT=MOL2FER2
    BLOCK SOLVAL2 IN=SLDFERM2 OUT=SOL2FER2

PROPERTIES NRTL-RK
    PROPERTIES NRTL / POLYNRTL / UNIQUAC

PROP-DATA PCES-1
    IN-UNITS ENG
    PROP-LIST RKTZRA / VLSTD
    PVAL DEXTR-01 .0715398735 / 2.673049051
    PVAL LYSIN-01 .1961662480 / 2.869755782
    PVAL GLYCI-01 .2269801180 / .9434410435
    PVAL L-PHE-01 .2112128620 / 2.876083075
    PVAL NIACI-01 .2353394780 / 2.070642696
    PVAL MAGNE-01 .2918287330 / 4.787029091
    PVAL MANGA-01 .2918596200 / 4.788020362
    PVAL COPPE-01 .2918596200 / 4.788020362
    PVAL CHROM-01 .2918596200 / 4.788020362
    PVAL MOLYB-01 .2918596200 / 4.788020362
    PVAL COBAL-01 .2918596200 / 4.788020362
    PVAL CELLU-01 .2918596200 / 4.788020362
    PROP-LIST VLSTD
    PVAL INOSI-01 1.929936513
    PVAL CALCI-01 .4693185532

PROP-DATA DHVLWT-1
    IN-UNITS ENG
    PROP-LIST DHVLWT
    PVAL INOSI-01 69383.03181 438.8000005 .3358157790  &
        -.1860484640 438.8000005

PROP-DATA KLDIP-1
    IN-UNITS ENG
    PROP-LIST KLDIP
    PVAL INOSI-01 -1.622711245 7.81423255E-3 -1.3509115E-5  &
        1.03016996E-8 -2.956333E-12 784.1299977 1055.029996
    PVAL MAGNE-01 -1.145883181 2.28001402E-3 -1.4723439E-6  &
        4.1270736E-10 -4.359307E-14 1990.129988 3104.329979
    PVAL CALCI-01 -.0733820551 2.33495546E-4 -1.0445689E-7  &
        1.9059933E-11 -1.315065E-15 2702.929982 5362.123961
    PVAL MANGA-01 -4.194043967 7.85893136E-3 -4.9575364E-6  &
        1.36450874E-9 -1.414532E-13 2060.329988 3104.329979
    PVAL COPPE-01 -3.899646973 7.30728106E-3 -4.6095468E-6  &
        1.26872834E-9 -1.315240E-13 2060.329988 3104.329979
    PVAL CHROM-01 -4.311063602 8.07820653E-3 -5.0958586E-6  &
        1.40258045E-9 -1.453999E-13 2060.329988 3104.329979
    PVAL MOLYB-01 -3.173726837 5.94702915E-3 -3.7514787E-6  &
        1.03255429E-9 -1.070408E-13 2060.329988 3104.329979
    PVAL COBAL-01 -4.049394842 7.58788337E-3 -4.7865551E-6  &
        1.31744797E-9 -1.365745E-13 2060.329988 3104.329979

PROP-DATA MULAND-1
    IN-UNITS ENG
    PROP-LIST MULAND
    PVAL GLYCI-01 169.6188260 -24686.52028 -21.16058700  &
        798.5299976 1359.751993
    PVAL INOSI-01 665.0384999 -99143.61401 -82.22983800  &
        784.1299977 1055.029996
    PVAL MAGNE-01 82.49628087 -20513.73620 -9.828118790  &
        1990.129988 3104.329979
    PVAL MANGA-01 86.74255549 -21829.17961 -10.25255770  &
        2060.329988 3104.329979
    PVAL COPPE-01 86.81533489 -21829.17961 -10.25255770  &
        2060.329988 3104.329979
    PVAL CHROM-01 86.71503619 -21829.17961 -10.25255770  &
        2060.329988 3104.329979
    PVAL MOLYB-01 87.02131429 -21829.17961 -10.25255770  &
        2060.329988 3104.329979
    PVAL COBAL-01 86.77765339 -21829.17961 -10.25255770  &
        2060.329988 3104.329979
    PVAL CELLU-01 87.28369039 -21829.17961 -10.25255770  &
        2060.329988 3104.329979

PROP-DATA SIGDIP-1
    IN-UNITS ENG
    PROP-LIST SIGDIP
    PVAL INOSI-01 252.2764310 1.222222220 -2.5631863E-9  &
        2.86171002E-9 -1.0929524E-9 784.1299977 1039.729996
    PVAL MAGNE-01 141.3279850 1.222222220 -1.7667850E-9  &
        1.97731990E-9 -7.904629E-10 1990.129988 3068.329979
    PVAL MANGA-01 157.4081400 1.222222220 -4.410621E-10  &
        4.9467696E-10 -1.969408E-10 2060.329988 3068.329979
    PVAL COPPE-01 157.4081400 1.222222220 -4.410621E-10  &
        4.9467696E-10 -1.969408E-10 2060.329988 3068.329979
    PVAL CHROM-01 157.4081400 1.222222220 -4.410621E-10  &
        4.9467696E-10 -1.969408E-10 2060.329988 3068.329979
    PVAL MOLYB-01 157.4081400 1.222222220 -4.410621E-10  &
        4.9467696E-10 -1.969408E-10 2060.329988 3068.329979
    PVAL COBAL-01 157.4081400 1.222222220 -4.410621E-10  &
        4.9467696E-10 -1.969408E-10 2060.329988 3068.329979

PROP-DATA NRTL-1
    IN-UNITS ENG
    PROP-LIST NRTL
    BPVAL 1:4-B-01 WATER 0.0 997.9138720 .4700000000 0.0 0.0  &
        0.0 212.1800023 222.8000022
    BPVAL WATER 1:4-B-01 0.0 1386.073789 .4700000000 0.0 0.0  &
        0.0 212.1800023 222.8000022
    BPVAL 1:4-B-01 DEXTR-01 0.0 658.4954293 .3000000000 0.0  &
        0.0 0.0 77.00000338 77.00000338
    BPVAL DEXTR-01 1:4-B-01 0.0 -301.3552344 .3000000000 0.0  &
        0.0 0.0 77.00000338 77.00000338
    BPVAL 1:4-B-01 CARBO-01 0.0 4406.683447 .3000000000 0.0  &
        0.0 0.0 77.00000338 77.00000338
    BPVAL CARBO-01 1:4-B-01 0.0 -1799.182541 .3000000000 0.0  &
        0.0 0.0 77.00000338 77.00000338
    BPVAL 1:4-B-01 OLEIC-01 0.0 1589.165139 .3000000000 0.0  &
        0.0 0.0 77.00000338 77.00000338
    BPVAL OLEIC-01 1:4-B-01 0.0 441.3420361 .3000000000 0.0  &
        0.0 0.0 77.00000338 77.00000338
```

```
BPVAL 1:4-B-01 LYSIN-01 0.0 544.8235060 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL LYSIN-01 1:4-B-01 0.0 -1084.083372 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL 1:4-B-01 GLYCI-01 0.0 -1388.769913 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL GLYCI-01 1:4-B-01 0.0 2635.218573 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL 1:4-B-01 L-PHE-01 0.0 -681.4751327 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL L-PHE-01 1:4-B-01 0.0 915.3482129 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL 1:4-B-01 INOSI-01 0.0 831.2266716 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL INOSI-01 1:4-B-01 0.0 -308.1533519 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL 1:4-B-01 NIACI-01 0.0 533.0818001 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL NIACI-01 1:4-B-01 0.0 187.0192659 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL 1:4-B-01 ETHYL-01 0.0 82.39646850 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL ETHYL-01 1:4-B-01 0.0 1177.568596 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL WATER DEXTR-01 0.0 -638.4037593 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL DEXTR-01 WATER 0.0 343.2181347 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL WATER CARBO-01 10.06400000 -5882.642953 .2000000000  &
    0.0 0.0 0.0 32.00000374 392.0000009
BPVAL CARBO-01 WATER 10.06400000 -5882.642953 .2000000000  &
    0.0 0.0 0.0 32.00000374 392.0000009
BPVAL WATER OLEIC-01 0.0 8961.942348 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL OLEIC-01 WATER 0.0 967.0927477 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL WATER LYSIN-01 0.0 -17.45581209 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL LYSIN-01 WATER 0.0 -348.1439966 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL WATER GLYCI-01 0.0 -1458.187239 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL GLYCI-01 WATER 0.0 2944.048206 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL WATER L-PHE-01 0.0 3276.316936 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL L-PHE-01 WATER 0.0 -267.0737829 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL WATER INOSI-01 0.0 -1241.186282 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL INOSI-01 WATER 0.0 2157.215707 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL WATER NIACI-01 0.0 2277.956844 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL NIACI-01 WATER 0.0 -411.1685265 .3000000000 0.0 0.0  &
    0.0 77.00000338 77.00000338
BPVAL WATER ETHYL-01 9.463200000 -3070.229375 .2000000000  &
    0.0 0.0 0.0 32.00000374 158.7200027
BPVAL ETHYL-01 WATER -3.719800000 2315.048921 .2000000000  &
    0.0 0.0 0.0 32.00000374 158.7200027
BPVAL DEXTR-01 CARBO-01 0.0 8598.307629 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL CARBO-01 DEXTR-01 0.0 -2402.179199 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL DEXTR-01 OLEIC-01 0.0 3950.370562 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL OLEIC-01 DEXTR-01 0.0 4167.525567 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
```

```
BPVAL DEXTR-01 LYSIN-01 0.0 -705.2884126 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL LYSIN-01 DEXTR-01 0.0 -411.2201235 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL DEXTR-01 GLYCI-01 0.0 -1120.539328 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL GLYCI-01 DEXTR-01 0.0 -19.28295183 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL DEXTR-01 L-PHE-01 0.0 347.3649242 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL L-PHE-01 DEXTR-01 0.0 -203.5814456 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL DEXTR-01 INOSI-01 0.0 191.1185877 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL INOSI-01 DEXTR-01 0.0 -156.6104654 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL DEXTR-01 NIACI-01 0.0 255.2237116 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL NIACI-01 DEXTR-01 0.0 514.7442409 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL DEXTR-01 ETHYL-01 0.0 407.1247923 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL ETHYL-01 DEXTR-01 0.0 3495.096602 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL CARBO-01 OLEIC-01 0.0 -4849.927863 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL OLEIC-01 CARBO-01 0.0 53999.99957 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL CARBO-01 LYSIN-01 0.0 -1906.951521 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL LYSIN-01 CARBO-01 0.0 5025.977798 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL CARBO-01 GLYCI-01 0.0 7.367513737 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL GLYCI-01 CARBO-01 0.0 -62.78602612 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL CARBO-01 L-PHE-01 0.0 -2013.167342 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL L-PHE-01 CARBO-01 0.0 5622.544287 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL CARBO-01 INOSI-01 0.0 -2323.060127 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL INOSI-01 CARBO-01 0.0 7928.052057 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL CARBO-01 NIACI-01 0.0 1197.876585 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL NIACI-01 CARBO-01 0.0 -669.8756448 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL CARBO-01 ETHYL-01 0.0 -1288.711091 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL ETHYL-01 CARBO-01 0.0 2353.712237 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL OLEIC-01 LYSIN-01 0.0 273.4860236 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL LYSIN-01 OLEIC-01 0.0 1165.457633 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL OLEIC-01 GLYCI-01 0.0 424.2981404 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL GLYCI-01 OLEIC-01 0.0 2900.589097 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL OLEIC-01 L-PHE-01 0.0 111.7890322 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL L-PHE-01 OLEIC-01 0.0 1044.320863 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL OLEIC-01 INOSI-01 0.0 5453.020486 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL INOSI-01 OLEIC-01 0.0 4757.479000 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
```

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BPVAL OLEIC-01 NIACI-01 0.0 235.2334157 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL NIACI-01 OLEIC-01 0.0 1972.529516 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL OLEIC-01 ETHYL-01 0.0 -680.2604982 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL ETHYL-01 OLEIC-01 0.0 1269.442343 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL LYSIN-01 GLYCI-01 0.0 -387.5938871 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL GLYCI-01 LYSIN-01 0.0 638.3286687 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL LYSIN-01 L-PHE-01 0.0 181.2393094 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL L-PHE-01 LYSIN-01 0.0 -84.14378897 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL LYSIN-01 INOSI-01 0.0 -278.7917666 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL INOSI-01 LYSIN-01 0.0 -752.1699738 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL LYSIN-01 NIACI-01 0.0 -154.6238029 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL NIACI-01 LYSIN-01 0.0 407.7482313 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL LYSIN-01 ETHYL-01 0.0 -678.3302520 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL ETHYL-01 LYSIN-01 0.0 1484.819347 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL GLYCI-01 L-PHE-01 0.0 709.9275291 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL L-PHE-01 GLYCI-01 0.0 -276.7398854 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL GLYCI-01 INOSI-01 0.0 112.7863242 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL INOSI-01 GLYCI-01 0.0 -1206.976175 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL GLYCI-01 NIACI-01 0.0 -5.217836664 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL NIACI-01 GLYCI-01 0.0 -43.81903027 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL GLYCI-01 ETHYL-01 0.0 -258.5535585 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL ETHYL-01 GLYCI-01 0.0 1183.850555 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL L-PHE-01 INOSI-01 0.0 405.6268540 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL INOSI-01 L-PHE-01 0.0 21.57353425 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL L-PHE-01 NIACI-01 0.0 -404.8761190 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL NIACI-01 L-PHE-01 0.0 781.4769183 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL L-PHE-01 ETHYL-01 0.0 -817.5274081 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL ETHYL-01 L-PHE-01 0.0 1564.374460 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL INOSI-01 NIACI-01 0.0 289.3262971 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL NIACI-01 INOSI-01 0.0 844.3621318 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL INOSI-01 ETHYL-01 0.0 685.9562183 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL ETHYL-01 INOSI-01 0.0 4428.316729 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL NIACI-01 ETHYL-01 0.0 -97.14936612 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
BPVAL ETHYL-01 NIACI-01 0.0 1738.185889 .3000000000 0.0  &
    0.0 0.0 77.00000338 77.00000338
```

```
PROP-DATA UNIQ-1
    IN-UNITS ENG
    PROP-LIST UNIQ
    BPVAL 1:4-B-01 WATER 0.0 -1235.539610 0.0 0.0 212.1800023  &
        222.8000022 0.0
    BPVAL WATER 1:4-B-01 0.0 249.0355780 0.0 0.0 212.1800023  &
        222.8000022 0.0
    BPVAL WATER ETHYL-01 -2.053200000 955.8599324 0.0 0.0  &
        32.00000374 158.7200027 0.0
    BPVAL ETHYL-01 WATER 2.721400000 -2183.200543 0.0 0.0  &
        32.00000374 158.7200027 0.0

PROP-SET ALL-SUBS VOLFLMX MASSVFRA MASSSFRA RHOMX MASSFLOW  &
        TEMP PRES UNITS='lb/cuft' SUBSTREAM=ALL
;  "Entire Stream Flows, Density, Phase Frac, T, P"


PROP-SET TXPORT RHOMX MUMX SIGMAMX UNITS='lb/cuft'  &
        SUBSTREAM=MIXED PHASE=V L
;  "Density, viscosity, and surface tension"


STREAM MEDIAIN
    SUBSTREAM MIXED TEMP=30. <C> PRES=1. <atm>
    MASS-FLOW LYSIN-01 18640.8 <gm/hr> / GLYCI-01  &
        18640.8 <gm/hr> / ISOLE-01 18640.8 <gm/hr> / LEUCI-01  &
        18640.8 <gm/hr> / METHI-01 18640.8 <gm/hr> / L-PHE-01  &
        18640.8 <gm/hr> / THREO-01 18640.8 <gm/hr> / TRYPT-01  &
        18640.8 <gm/hr> / TYROS-01 18640.8 <gm/hr> / VALIN-01  &
        18640.8 <gm/hr> / INOSI-01 12945.92 <gm/hr> / NIACI-01  &
        3332.99 <gm/hr> / POTAS-01 13548.36 <gm/hr> / MAGNE-01  &
        13548.36 <gm/hr> / CALCI-01 13548.36 <gm/hr> / SULFU-01  &
        13548.36 <gm/hr> / SODIU-01 13548.36 <gm/hr> / IRON  &
        13548.36 <gm/hr> / ZINC 13548.36 <gm/hr> / MANGA-01  &
        13548.36 <gm/hr> / COPPE-01 13548.36 <gm/hr> / CHROM-01  &
        13548.36 <gm/hr> / MOLYB-01 13548.36 <gm/hr> / COBAL-01  &
        13548.36 <gm/hr> / HYDRO-01 13548.36 <gm/hr> / ETHYL-01  &
        6450000. <gm/hr>
    SUBSTREAM CISOLID TEMP=30. <C> PRES=1. <atm>

STREAM MOLASSES
    SUBSTREAM MIXED TEMP=30. <C> PRES=30.
    MASS-FLOW DEXTR-01 4142400. <gm/hr>
    SUBSTREAM CISOLID TEMP=30. <C> PRES=30.
    MASS-FLOW CELLU-01 3000000. <gm/hr>

STREAM OLEIN
    SUBSTREAM MIXED TEMP=300. <K> PRES=14.7
    MASS-FLOW OLEIC-01 186.

STREAM WATERIN
    SUBSTREAM MIXED TEMP=30. <C> PRES=14.7
    MASS-FLOW WATER 1553400. <gm/hr>

BLOCK ADDINCO2 MIXER

BLOCK BLOWER MIXER
    PARAM PRES=0.15

BLOCK CO2EXIT MIXER

BLOCK MIXER MIXER
    PARAM PRES=30. T-EST=300. <K>

BLOCK OLMIXREC MIXER
    PARAM PRES=30. T-EST=300. <K>
```

```
BLOCK VINMIXER MIXER
    PARAM PRES=39.7 T-EST=30. <C>

BLOCK CO2SPLIT FSPLIT
    FRAC CO2DISSO 0.0026

BLOCK MEDSPLIT FSPLIT
    FRAC MEDFERM1 0.5

BLOCK MOLSPLIT FSPLIT
    FRAC MOLFERM1 0.5

BLOCK OLESPLIT FSPLIT
    FRAC OLPUROUT 0.2

BLOCK SLDSPLIT FSPLIT
    FRAC SLDFERM1 0.5

BLOCK SOLPURGE FSPLIT
    FRAC SOLPURGE 0.2

BLOCK SPLIT FSPLIT
    FRAC SIDESTRE 0.001

BLOCK WTRSPLIT FSPLIT
    FRAC WTRFERM2 0.5

BLOCK LLE SEP2
    FRAC STREAM=OLEICPUR SUBSTREAM=MIXED COMPS=1:4-B-01 WATER  &
        DEXTR-01 CARBO-01 OLEIC-01 LYSIN-01 GLYCI-01 ISOLE-01  &
        LEUCI-01 METHI-01 L-PHE-01 THREO-01 TRYPT-01 TYROS-01  &
        VALIN-01 INOSI-01 NIACI-01 POTAS-01 MAGNE-01 CALCI-01  &
        SULFU-01 SODIU-01 IRON ZINC MANGA-01 COPPE-01 CHROM-01  &
        MOLYB-01 COBAL-01 HYDRO-01 ETHYL-01 CELLU-01 FRACS=0.  &
        0. 0. 0. 1. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0.  &
        0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0.  &
        0.
    FRAC STREAM=OLEICPUR SUBSTREAM=CISOLID COMPS=CELLU-01  &
        FRACS=0.

BLOCK SEP2GAS SEP2
    FRAC STREAM=CO2 SUBSTREAM=MIXED COMPS=1:4-B-01 WATER  &
        DEXTR-01 CARBO-01 OLEIC-01 LYSIN-01 GLYCI-01 ISOLE-01  &
        LEUCI-01 METHI-01 L-PHE-01 THREO-01 TRYPT-01 TYROS-01  &
        VALIN-01 INOSI-01 NIACI-01 POTAS-01 MAGNE-01 CALCI-01  &
        SULFU-01 SODIU-01 IRON ZINC MANGA-01 COPPE-01 CHROM-01  &
        MOLYB-01 COBAL-01 HYDRO-01 ETHYL-01 CELLU-01 FRACS=0.  &
        0. 0. 1. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0.  &
        0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0.  &
        0.
    FRAC STREAM=CO2 SUBSTREAM=CISOLID COMPS=CELLU-01 FRACS=0.

BLOCK SEP2MED SEP2
    FRAC STREAM=MEDIAOUT SUBSTREAM=MIXED COMPS=1:4-B-01 WATER  &
        DEXTR-01 CARBO-01 OLEIC-01 LYSIN-01 GLYCI-01 ISOLE-01  &
        LEUCI-01 METHI-01 L-PHE-01 THREO-01 TRYPT-01 TYROS-01  &
        VALIN-01 INOSI-01 NIACI-01 POTAS-01 MAGNE-01 CALCI-01  &
        SULFU-01 SODIU-01 IRON ZINC MANGA-01 COPPE-01 CHROM-01  &
        MOLYB-01 COBAL-01 HYDRO-01 ETHYL-01 CELLU-01 FRACS=0.  &
        0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0.  &
        0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 1.  &
        0.
    FRAC STREAM=MEDIAOUT SUBSTREAM=CISOLID COMPS=CELLU-01  &
        FRACS=0.

BLOCK CONDNSOR HEATER
    PARAM TEMP=41. PRES=0.15 NPHASE=2
    BLOCK-OPTION FREE-WATER=NO
```

```
BLOCK NOVAP HEATER
    PARAM PRES=0.074 VFRAC=0.

BLOCK STERILIZ HEATER
    PARAM TEMP=130. <C> PRES=52.5

BLOCK ZEROVAP HEATER
    PARAM PRES=0.631 VFRAC=0.

BLOCK FLASHVAP FLASH2
    PARAM TEMP=41. PRES=0.15

BLOCK VENT FLASH2
    PARAM TEMP=30. <C> PRES=1.2 <bar>

BLOCK VENT2 FLASH2
    PARAM TEMP=30. <C> PRES=1.2 <bar>

BLOCK DISTHX HEATX
    PARAM T-HOT=120. U-OPTION=PHASE F-OPTION=CONSTANT  &
        CALC-METHOD=SHORTCUT
    FEEDS HOT=HOTIN COLD=TOHX2
    PRODUCTS HOT=OLETOLLE COLD=DIST1IN
    HOT-SIDE DP-OPTION=CONSTANT
    COLD-SIDE DP-OPTION=CONSTANT

BLOCK DIST1 RADFRAC
    PARAM NSTAGE=7 NPHASE=3
    COL-CONFIG CONDENSER=PARTIAL-V-L REBOILER=KETTLE
    RATESEP-ENAB CALC-MODE=EQUILIBRIUM
    FEEDS DIST1IN 4
    PRODUCTS OLESOLID 7 L / WTRVAPOR 1 V / BDODIST 1 L
    P-SPEC 1 0.05
    COL-SPECS DP-STAGE=0.11 MASS-RDV=0.2828 MASS-D=12000.  &
        MASS-RR=0.8 DP-COND=0.005
    SC-REFLUX OPTION=0
    STAGE-EFF 1 0.7 / 2 0.7 / 3 0.6 / 4 0.6 / 5 0.6 /  &
        6 0.5 / 7 0.5
    L2-COMPS OLEIC-01
    L2-STAGES 2 4
    BLOCK-OPTION FREE-WATER=NO

BLOCK FERMRXN1 RSTOIC
    PARAM TEMP=30. <C> PRES=1.2 <bar>
    STOIC 1 MIXED ETHYL-01 -1. / DEXTR-01 -1. / 1:4-B-01 2. / &
        CARBO-01 2.
    CONV 1 MIXED DEXTR-01 0.95

BLOCK FERMRXN2 RSTOIC
    PARAM TEMP=30. <C> PRES=1.2 <bar>
    STOIC 1 MIXED DEXTR-01 -1. / ETHYL-01 -1. / 1:4-B-01 2. / &
        CARBO-01 2.
    CONV 1 MIXED DEXTR-01 0.95

BLOCK 2CNTPUMP PUMP
    PARAM PRES=39.7 EFF=0.7

BLOCK BDOPUMP PUMP
    PARAM PRES=65. EFF=0.7

BLOCK LLETOPPU PUMP
    PARAM PRES=42.4 EFF=0.7

BLOCK OLEPUMP PUMP
    PARAM PRES=74.7 EFF=0.7

BLOCK PUMITUP PUMP
```

```
    PARAM PRES=99.7

BLOCK PUMPIN1 PUMP
    PARAM PRES=42.4 <psi>

BLOCK PUMPIN2 PUMP
    PARAM PRES=42.4 <psi> EFF=0.7

BLOCK PUMPIN3 PUMP
    PARAM PRES=52.5 <psi> EFF=0.7

BLOCK PUMPIN4 PUMP
    PARAM PRES=74.7

BLOCK PUMPWATE PUMP
    PARAM PRES=49.7 EFF=0.7

BLOCK SLURRYPU PUMP
    PARAM PRES=42.4 EFF=0.7

BLOCK SOLIDPUM PUMP
    PARAM PRES=49.7 EFF=0.7

BLOCK CO2COMP MCOMPR
    PARAM NSTAGE=2 TYPE=ISENTROPIC PRES=1.2 <bar>
    FEEDS VAPOROUT 1
    PRODUCTS DSLVCO2O 2
    COOLER-SPECS 1 TEMP=200.

BLOCK CFUGE CFUGE
    CENTRIFUGES DIAM=1. REVS=1000.
    CAKE-PROPS CAKE-RES=1920000. MEDIUM-RES=100.  &
        DIAM-PART=0.02 <cm>

BLOCK CFGVALVE VALVE
    PARAM P-DROP=25.

BLOCK CNTVALVE VALVE
    PARAM P-DROP=25.

BLOCK LLEBOTVV VALVE
    PARAM P-DROP=25.

BLOCK MEDVAL2 VALVE
    PARAM P-OUT=25.

BLOCK MEDVALVE VALVE
    PARAM P-DROP=25.

BLOCK MOL2VAL2 VALVE
    PARAM P-OUT=25.

BLOCK MOLVALVE VALVE
    PARAM P-DROP=25.

BLOCK OLEVALVE VALVE
    PARAM P-DROP=25.

BLOCK OLPUMPVA VALVE
    PARAM P-DROP=25.

BLOCK OLPVALVE VALVE
    PARAM P-DROP=25.

BLOCK PRESVAVL VALVE
    PARAM P-DROP=0.

BLOCK SLDVALVE VALVE
```

```
    PARAM P-DROP=25.

BLOCK SOLVAL2 VALVE
    PARAM P-OUT=25.

BLOCK SOLVALVE VALVE
    PARAM P-DROP=25.

BLOCK WAT2VAL2 VALVE
    PARAM P-OUT=25.

BLOCK WATVALVE VALVE
    PARAM P-DROP=25.

BLOCK WRMBDOVL VALVE
    PARAM P-DROP=25.

BLOCK WTRVALVE VALVE
    PARAM P-DROP=25.

DESIGN-SPEC BDOPUR
    DEFINE WATERIMP MASS-FRAC STREAM=BDO SUBSTREAM=MIXED  &
        COMPONENT=1:4-B-01
    SPEC "WATERIMP" TO ".991"
    TOL-SPEC ".001"
    VARY BLOCK-VAR BLOCK=DIST1 VARIABLE=MASS-RDV  &
        SENTENCE=COL-SPECS
    LIMITS "0.1" ".4" STEP-SIZE=0.01

DESIGN-SPEC OLEICPUR
    DEFINE WATERIMP MASS-FRAC STREAM=OLESOLID SUBSTREAM=MIXED  &
        COMPONENT=1:4-B-01
    SPEC "WATERIMP" TO "0.05"
    TOL-SPEC "0.01"
    VARY BLOCK-VAR BLOCK=DIST1 VARIABLE=MASS-D  &
        SENTENCE=COL-SPECS
    LIMITS "11000" "13000"

DESIGN-SPEC REBTEMP
    DEFINE REBOTEMP BLOCK-VAR BLOCK=DIST1 VARIABLE=TEMP  &
        SENTENCE=PROFILE ID1=7
    SPEC "REBOTEMP" TO "450"
    TOL-SPEC "1"
    VARY BLOCK-VAR BLOCK=DIST1 VARIABLE=PRES SENTENCE=P-SPEC  &
        ID1=1
    LIMITS "0.001" "14.7"

EO-CONV-OPTI

CALCULATOR CNDPDROP
    DEFINE CONDPRES BLOCK-VAR BLOCK=DIST1 VARIABLE=PRES  &
        SENTENCE=P-SPEC ID1=1
    DEFINE CONDDROP BLOCK-VAR BLOCK=DIST1 VARIABLE=DP-COND  &
        SENTENCE=COL-SPECS
F     CONDDROP = 0.1 * CONDPRES
    EXECUTE BEFORE BLOCK DIST1

CALCULATOR CONDPRES
    DEFINE PRES BLOCK-VAR BLOCK=ZEROVAP VARIABLE=PRES  &
        SENTENCE=PARAM
    DEFINE BLOCKP BLOCK-VAR BLOCK=DIST1 VARIABLE=PRES  &
        SENTENCE=PROFILE ID1=5
F     PRES=BLOCKP
    EXECUTE AFTER BLOCK DIST1

CONV-OPTIONS
    PARAM TEAR-METHOD=BROYDEN
```

```
BLOCK-REPORT NEWPAGE

STREAM-REPOR WIDE MOLEFLOW MASSFLOW MOLEFRAC MASSFRAC  &
        PROPERTIES=ALL-SUBS TXPORT

PROPERTY-REP PCES
;
;
;
;
;
```

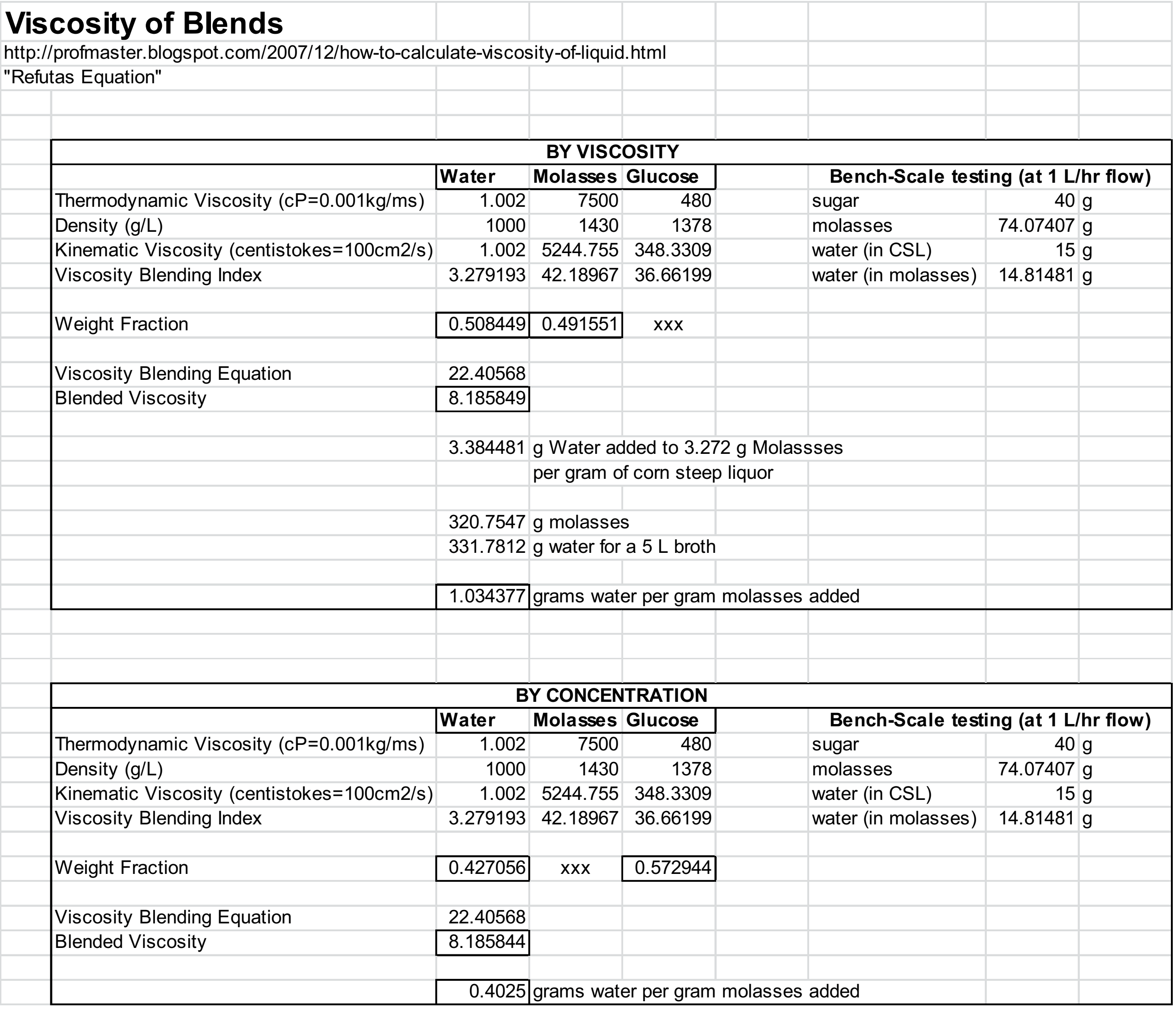
Appendix B: Calculations

Horsepower Calculations

# of Units	Unit Number	Unit Name	Mass Flow (lb/hr)	Density (lb/cuft)	Pressure Difference (psia)	Head (ft)	Horsepower (hp)	HP for units		
1	B-103	Compressor	-	-	-	-	25	25		
1	B-102	Distillation Tower Blower	-	-	-	-	81.46	81.46		
1	C-100	Centrifuge	-	-	-	-	26.49	26.49		
2	P-101	Molasses Pump (before storage)	16911.9	89.3	15.3	15.77	4.31	8.62		
6	P-103	Molasses Pump (after storage)	5637.3	89.3	22.5	16.13	1.47	8.82		
2	P-105	Media Feed Pump	16455.3	54.5	27.5	17.26	4.59	9.18		
2	P-106	Water Feed Pump	3424.7	61.7	27.7	17.01	0.94	1.88		
2	P-100	Oleic Feed Pump	186.0	55.1	60	34.87	0.10	0.21		
28	P-###	Generic Pump (est.)	6613.9	81.2	30	16.65	1.78	49.85		
1		Packaged Boiler (per 1/10 months)	-	-	-	-	0.30	0.3		
1		Water Sterilization Skid	-	-	-	-	3.00	3		
							TOTAL -->	214.81	hp	
								160.25	kW	
								160.25	kWh	in 1 hour

Constants		
g	32	ft/s^2
	33000	(ft.lb/min)/hp
	60	min/hr
	12	in/ft
	32.174	lbf/lbm
	7.481	gal/cuft

Viscosity



Viscosity of Blends

http://profmaster.blogspot.com/2007/12/how-to-calculate-viscosity-of-liquid.html

"Refutas Equation"

BY VISCOSITY

	Water	Molasses	Glucose		Bench-Scale testing (at 1 L/hr flow)		
Thermodynamic Viscosity (cP=0.001kg/ms)	1.002	7500	480		sugar	40	g
Density (g/L)	1000	1430	1378		molasses	74.07407	g
Kinematic Viscosity (centistokes=100cm2/s)	1.002	5244.755	348.3309		water (in CSL)	15	g
Viscosity Blending Index	3.279193	42.18967	36.66199		water (in molasses)	14.81481	g
Weight Fraction	0.508449	0.491551	xxx				
Viscosity Blending Equation	22.40568						
Blended Viscosity	8.185849						
	3.384481	g Water added to 3.272 g Molassses					
		per gram of corn steep liquor					
	320.7547	g molasses					
	331.7812	g water for a 5 L broth					
	1.034377	grams water per gram molasses added					

BY CONCENTRATION

	Water	Molasses	Glucose		Bench-Scale testing (at 1 L/hr flow)		
Thermodynamic Viscosity (cP=0.001kg/ms)	1.002	7500	480		sugar	40	g
Density (g/L)	1000	1430	1378		molasses	74.07407	g
Kinematic Viscosity (centistokes=100cm2/s)	1.002	5244.755	348.3309		water (in CSL)	15	g
Viscosity Blending Index	3.279193	42.18967	36.66199		water (in molasses)	14.81481	g
Weight Fraction	0.427056	xxx	0.572944				
Viscosity Blending Equation	22.40568						
Blended Viscosity	8.185844						
	0.4025	grams water per gram molasses added					

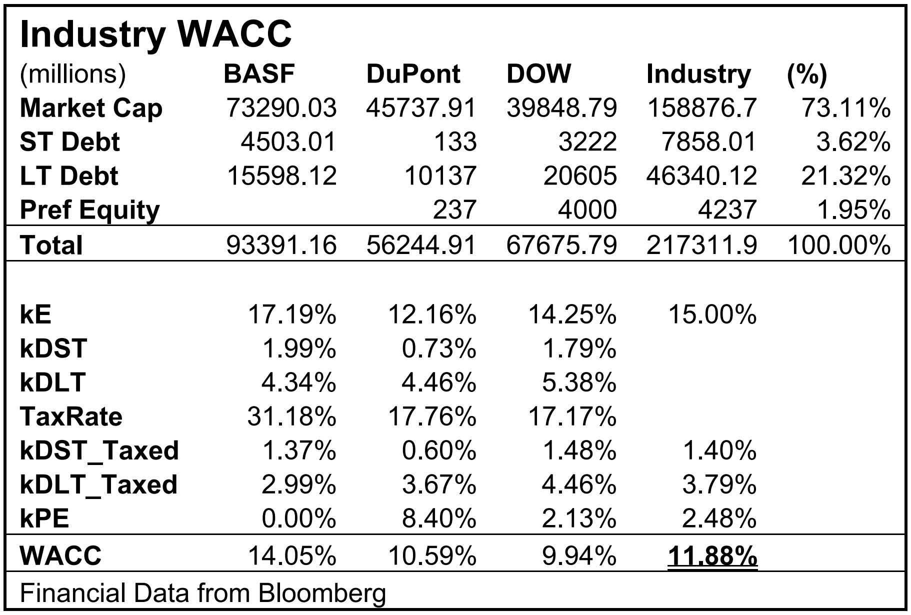
Materials Costing

Material Inputs

All prices in Thousands

Feed Streams	grams/hr	lbs/hr	tons/hr	tons/yr	Price/ton	Cost
Molasses						
Continuous	7671111	16911.9	8.4560	58853	$ 0.070000	$ 4,120
Seed		7212.79	3.6064	866	$ 0.070000	$ 61
Oleic Acid						
Continuous		186	0.0930	647	$ 1.270059	$ 822
Cornsteep Liquor						
Continuous	3.10E+06	6.83E+03	3.4172	23783	$ 0.050000	$ 1,189
Seed		608.58	0.3043	73	$ 0.050000	$ 4
Carbon Dioxide						
Continuous		Negligible		Negligible	$ -	$ -
Seed		Negligible		Negligible	$ -	$ -
Water						
Continuous		3425.247	1.7126	11920	$ 0.000018	$ 0
Seed		Negligible		Negligible	$ 0.00	$ -
Startup Cost						
Molasses		16911.9	8.455952	299.4816	$ 0.070000	$ 21
Oleic Acid		186	0.093	3.29375	$ 1.270059	$ 4
Cornsteep Liquor		6.83E+03	3.417165	121.0246	$ 0	$ 6
Carbon Dioxide		Negligible			$ -	$ -
Water		3425.247	1.712624	60.65542	$ 0	$ 0
Annual Feed Stream Cost						$ 6,195
Utilities						
Cooling Water						
Steam						
Electricity						
Annual Utilities Cost						
Other						
Cost for Initial GMO Cells						
Treatment of Waste Oleic Acid		186	0.093	647	$ 0.06	$ 38.84
Treatment of Unsterile Water		4050	2.025	14094	$ 0.0600	$ 845.64

Cost of Capital



Industry WACC

(millions)	**BASF**	**DuPont**	**DOW**	**Industry**	**(%)**
Market Cap	73290.03	45737.91	39848.79	158876.7	73.11%
ST Debt	4503.01	133	3222	7858.01	3.62%
LT Debt	15598.12	10137	20605	46340.12	21.32%
Pref Equity		237	4000	4237	1.95%
Total	93391.16	56244.91	67675.79	217311.9	100.00%
kE	17.19%	12.16%	14.25%	15.00%	
kDST	1.99%	0.73%	1.79%		
kDLT	4.34%	4.46%	5.38%		
TaxRate	31.18%	17.76%	17.17%		
kDST_Taxed	1.37%	0.60%	1.48%	1.40%	
kDLT_Taxed	2.99%	3.67%	4.46%	3.79%	
kPE	0.00%	8.40%	2.13%	2.48%	
WACC	14.05%	10.59%	9.94%	**11.88%**	

Financial Data from Bloomberg

Appendix C: Emails with Consultants

Email Correspondence #1

Outgoing Email on Thu, Feb. 17, 2011 at 1:28 AM

Dear Mr. Tieri,

Thank you again for meeting with us on Tuesday. We were wondering if you had the information on the nutrients required for the *E. coli*, and what the best source for them would be.

We will email Mr. Vrana regarding the decanter as well.

Thank you again!

Sincerely,

Gabe Fernando
Somil Shah
Erinn Bibolet

Email Response on Sat, Feb 19, 2011 at 8:51 AM

Gabe, Somil, & Erinn,

I apologize for delayed response. I spoke with some colleagues about potential nutrient sources which could be used to support the bacterial nutritional requirements, in place of Corn Steep Liquor, as we discussed on Tuesday. Yeast extract was suggested by several resources, as a reasonable alternative to CSL, and available in Brazil. If you have difficulty finding commercial pricing for yeast extract, in the US or Brazil, you can assume a price of ~$4/kg in 2008, and index/adjust appropriately.

I will continue to pursue information about E.coli nutrient requirements, but wanted you to have this info. Do any of the patents or other information (white papers, journal articles, etc.) reference a specific quantity of CSL or other nutrients added to the fermentations, even if it did not specify the nutrients consumed? Do any of the patent examples reference fermentation broth nutrient loading or addition rate and relate the condition to fermentation productivity?

Please do not hesitate to contact me with any additional questions.

Steve

Email Correspondence #2

Outgoing Email on Thu, Feb. 17, 2011 at 1:59 AM

Dear Mr. Vrana,

Our senior design team met with you last Tuesday (the 8th) to discuss our Renewable 1,4 Butanediol project. This week, Mr. Tieri referred us to you for information about decanters and Liquid-Liquid Extraction. We modified our process from the last time we met, and now we are using Oleic Acid to carry away minerals and other solutes that would affect the purity of our product (while the rest—water and BDO—are boiled and distilled). I have attached a draft of our process for your convenience.

However, we want to recover as much of the Oleic Acid as possible while using water to carry away the solutes. In doing so, we hope to minimize the amount of Oleic Acid we have to feed the system, as well as minimize the amount of Oleic Acid-nutrient mixture we are releasing to the environment. Rather, we would prefer to make it a water-nutrient mixture that could be recycled or utilized elsewhere. We tried using a Liquid-Liquid extractor in ASPEN, but it seems that the solutes are staying within the Oleic Acid phase and not transferring over to the water phase. Would you have any recommendations for us? We picked Oleic Acid since it had a high boiling point and would be able to separate well from water. Are there other compounds we should be using, or are there other operations we should be using?

Thank you very much!

Sincerely,

Gabe Fernando
Somil Shah
Erinn Bibolet

Email Response on Thu, Feb 17, 2011 at 8:21 AM

Well, my first suggestion would be to not believe everything that Aspen (or any other computer software) tells you. Despite Watson's performance on Jeopardy, most computers are fast ... but stupid. They do not understand engineering, only how to do calculations fast. For the extraction, the salts will obviously go with the aqueous phase and not the organic phase. Unless you use electrolyte thermodynamics in Aspen, it will do a terrible job of predicting how electrolyte components partition between the phases. And I do not recommend using electrolyte thermo in Aspen if you can avoid it. So I would use a black box separation (SEP2) in Aspen to do the mass balance of the decanter. (I suspect you do not need an expensive counter-current extractor, as oleic and water are pretty immiscible, I believe. A decanter will be much more cost effective) You'd tell the SEP2 block where each component goes, using the solubility of water in oleic and vice versa at your operating temperature, and telling it where you know the ions will go. (Prof. Fabiano can help you with a SEP2 block if you need it, but you can probably figure out how to make it work.) There are rules of thumb to use for sizing decanters that deal with the velocity of droplets and allowing them enough time to separate based on the density difference between the two phases, etc. If you can't find anything, feel free to ask again for a specific reference.

A question for you - do you really want to boil the entire fermentation broth in the first distillation column, leaving the salts and oleic behind? How much energy will be used in that operation, how much will the column cost, and can you afford it? You will likely want to run the column at vacuum so the reboiler temperature is reasonable, based on the steam pressure available to you, which of course makes the column even bigger. I don't necessarily know the answer to those questions, but suggest you think about them. If distillation is too expensive, are there other less energy- or capital-intensive ways to do the separation? I see you're boiling all the water from the fermentation broth again in the BDO column - again, can you afford that? If not, what options might you have to reduce the energy consumption between the two columns, rather than boiling all the water twice?

Hope this helps more than it confuses the matter. Please let Steve or me know if you have other questions or if something in this doesn't make sense.

Bruce

Email Response on Sat, Feb 19, 2011 at 2:37 PM

Gabe, Somil, & Erinn,

In addition to the suggestions Bruce provided with respect to reconsidering taking all of the water overhead in the 1st distillation column, I wanted to suggest the use of live steam addition to the first column as the heat source. In addition to eliminating the column reboiler (hopefully), it may be possible to have a two phase bottoms product oleic and water, which you can decant directly (possibly using a Flash3 block & Sep if that doesn't work) to recycle the oleic phase and produce the aqueous nutrients/minerals stream we discussed.

Hope this helps,

Steve

Appendix D: MSDS

MSDS Number: **B5740** * * * * * *Effective Date:* ***09/15/09*** * * * * * *Supercedes:* ***07/03/07***

MSDS Material Safety Data Sheet

From: Mallinckrodt Baker, Inc.
222 Red School Lane
Phillipsburg, NJ 08865

J.T.Baker

24 Hour Emergency Telephone: 908-859-2151
CHEMTREC: 1-800-424-9300

National Response in Canada
CANUTEC: 613-996-6666

Outside U.S. And Canada
Chemtrec: 703-527-3887

NOTE: CHEMTREC, CANUTEC and National Response Center emergency numbers to be used only in the event of chemical emergencies involving a spill, leak, fire, exposure or accident involving chemicals.

All non-emergency questions should be directed to Customer Service (1-800-582-2537) for assistance.

1,4-Butanediol

1. Product Identification

Synonyms: 1,4-Butylene Glycol; 1,4-Dihydroxybutane; 1,4-Tetramethylene Glycol
CAS No.: 110-63-4
Molecular Weight: 90.12
Chemical Formula: HO(CH2)4OH
Product Codes: D570

2. Composition/Information on Ingredients

Ingredient	CAS No	Percent	Hazardous
1,4-Butanediol	110-63-4	90 - 100%	Yes

3. Hazards Identification

Emergency Overview

CAUTION! MAY BE HARMFUL IF SWALLOWED. MAY CAUSE IRRITATION TO SKIN, EYES, AND RESPIRATORY TRACT. MAY AFFECT KIDNEYS.

SAF-T-DATA$^{(tm)}$ Ratings (Provided here for your convenience)

Health Rating: 2 - Moderate (Life)
Flammability Rating: 1 - Slight
Reactivity Rating: 1 - Slight
Contact Rating: 1 - Slight
Lab Protective Equip: GOGGLES; LAB COAT; VENT HOOD; PROPER GLOVES
Storage Color Code: Green (General Storage)

Potential Health Effects

Inhalation:
A nonvolatile liquid - vapor inhalation is unlikely. Inhalation of high vapor concentrations may cause respiratory tract irritation and have a narcotic effect with symptoms of drunkenness.
Ingestion:
Large oral dose may produce symptoms of narcosis, incoordination, and kidney damage.
Skin Contact:
No adverse health effects expected. Prolonged contact may cause slight irritation.
Eye Contact:
May cause mild irritation, possible reddening.
Chronic Exposure:
No information found.
Aggravation of Pre-existing Conditions:
No information found.

4. First Aid Measures

Inhalation:
Remove to fresh air. Get medical attention for any breathing difficulty.
Ingestion:
Induce vomiting immediately as directed by medical personnel. Never give anything by mouth to an unconscious person.
Skin Contact:
Immediately flush skin with plenty of water for at least 15 minutes. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention if irritation develops.
Eye Contact:
Immediately flush eyes with plenty of water for at least 15 minutes, lifting upper and lower eyelids occasionally. Get medical attention if irritation persists.

5. Fire Fighting Measures

Fire:
Flash point: 121C (250F) OC
Autoignition temperature: 402.5C (756F)
Slight fire hazard when exposed to heat or flame.
Hazardous Combustion Products: Highly flammable tetrahydrofuran may be produced.
Explosion:
Not considered to be an explosion hazard.
Fire Extinguishing Media:
Use alcohol foam, dry chemical or carbon dioxide. (Water may be ineffective.) Water or foam may cause frothing. Direct stream of water can scatter and spread flames. Water spray may be used to keep fire exposed containers cool. Do not allow water runoff to enter sewers or waterways.
Special Information:
In the event of a fire, wear full protective clothing and NIOSH-approved self-contained breathing apparatus with full facepiece operated in the pressure demand or other positive pressure mode.

6. Accidental Release Measures

Ventilate area of leak or spill. Remove all sources of ignition. Wear appropriate personal protective equipment as specified in Section 8. Isolate hazard area. Keep unnecessary and unprotected personnel from entering. Contain and recover liquid when possible. Use non-sparking tools and equipment. Collect liquid in an appropriate container or absorb with an inert material (e. g., vermiculite, dry sand, earth), and place in a chemical waste container. Do not use combustible materials, such as saw dust. Do not flush to sewer!

7. Handling and Storage

Keep in a tightly closed container. Store in a cool, dry, ventilated area away from sources of heat or ignition. Protect against physical damage. Store separately from reactive or combustible materials, and out of direct sunlight. Containers of this material may be hazardous when empty since they retain product residues (vapors, liquid); observe all warnings and precautions listed for the product.

8. Exposure Controls/Personal Protection

Airborne Exposure Limits:
None established.
Ventilation System:
In general, dilution ventilation is a satisfactory health hazard control for this substance. However, if conditions of use create discomfort to the worker, a local exhaust system should be considered.
Personal Respirators (NIOSH Approved):
For conditions of use where exposure to the substance is apparent and engineering controls are not feasble, consult an industrial hygienist. For emergencies, or instances where the exposure levels are not known, use a full-facepiece positive-pressure, air-supplied respirator. WARNING: Air purifying respirators do not protect workers in oxygen-deficient atmospheres.
Skin Protection:
Wear protective gloves and clean body-covering clothing.
Eye Protection:
Use chemical safety goggles and/or a full face shield where splashing is possible. Maintain eye wash fountain and quick-drench facilities in work area.

9. Physical and Chemical Properties

Appearance:
Colorless viscous liquid.
Odor:
Odorless.
Solubility:
Complete (100%)
Specific Gravity:
1.0171 @ 20C/4C
pH:
No information found.
% Volatiles by volume @ 21C (70F):
N/A
Boiling Point:
230C (446F)
Melting Point:

20.1C (68F)
Vapor Density (Air=1):
3.1
Vapor Pressure (mm Hg):
0.01 @ 25C (77F)
Evaporation Rate (BuAc=1):
Nonvolatile.

10. Stability and Reactivity

Stability:
Stable under ordinary conditions of use and storage.
Hazardous Decomposition Products:
When heated to decomposition, emits tetrahydrofuran and carbon oxides.
Hazardous Polymerization:
Will not occur.
Incompatibilities:
1,4-Butanediol incompatibilities include strong inorganic oxidizers, nitric acid, strong hydrogen peroxide. Forms highly flammable tetrahydrofuran when heated in presence of sulfuric acid.
Conditions to Avoid:
Heat, flames, ignition sources and incompatibles.

11. Toxicological Information

For 1,4-Butanediol: LD50 oral rat: 1525 mg/kg. Investigated as a tumorigen and mutagen.

```
--------\Cancer Lists\------------------------------------------------------
                                       ---NTP Carcinogen---
Ingredient                             Known    Anticipated    IARC Category
------------------------------------   -----    -----------    -------------
1,4-Butanediol (110-63-4)               No          No             None
```

12. Ecological Information

Environmental Fate:
When released into the soil, this material may biodegrade to a moderate extent. When released into water, this material may biodegrade to a moderate extent. When released into water, this material is not expected to evaporate significantly. This material is not expected to significantly bioaccumulate. When released into the air, this material is expected to be readily degraded by reaction with photochemically produced hydroxyl radicals.
Environmental Toxicity:
No information found.

13. Disposal Considerations

Whatever cannot be saved for recovery or recycling should be managed in an appropriate and approved waste disposal facility. Processing, use or contamination of this product may change the waste management options. State and local disposal regulations may differ from federal disposal regulations. Dispose of container and unused contents in accordance with federal, state and local requirements.

14. Transport Information

Not regulated.

---

15. Regulatory Information

```
  --------\Chemical Inventory Status - Part 1\---------------------------------
  Ingredient                                       TSCA  EC   Japan  Australia
  -----------------------------------------------  ----  ---  -----  ---------
  1,4-Butanediol (110-63-4)                         Yes  Yes   Yes      Yes

  --------\Chemical Inventory Status - Part 2\---------------------------------
                                                          --Canada--
  Ingredient                                       Korea  DSL   NDSL  Phil.
  -----------------------------------------------  -----  ---   ----  -----
  1,4-Butanediol (110-63-4)                         Yes   Yes   No     Yes

  --------\Federal, State & International Regulations - Part 1\----------------
                                             -SARA 302-    ------SARA 313------
  Ingredient                                 RQ    TPQ     List  Chemical Catg.
  -----------------------------------------  ---   -----   ----  --------------
  1,4-Butanediol (110-63-4)                  No    No      No         No

  --------\Federal, State & International Regulations - Part 2\----------------
                                                       -RCRA-    -TSCA-
  Ingredient                                 CERCLA     261.33     8(d)
  -----------------------------------------  ------     ------    ------
  1,4-Butanediol (110-63-4)                  No         No         No


Chemical Weapons Convention:  No     TSCA 12(b):  No     CDTA:  No
SARA 311/312:  Acute: Yes      Chronic: No   Fire: No  Pressure: No
Reactivity: No          (Pure / Liquid)
```

Australian Hazchem Code: None allocated.
Poison Schedule: None allocated.
WHMIS:
This MSDS has been prepared according to the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all of the information required by the CPR.

---

16. Other Information

NFPA Ratings: Health: **1** Flammability: **1** Reactivity: **0**
Label Hazard Warning:
CAUTION! MAY BE HARMFUL IF SWALLOWED. MAY CAUSE IRRITATION TO SKIN, EYES, AND RESPIRATORY TRACT. MAY AFFECT KIDNEYS.
Label Precautions:
Avoid contact with eyes, skin and clothing.
Wash thoroughly after handling.
Avoid breathing mist.
Keep container closed.
Use with adequate ventilation.
Label First Aid:
If swallowed, induce vomiting immediately as directed by medical personnel. Never give anything by mouth to an unconscious person. If inhaled, remove to fresh air. Get medical attention for any breathing difficulty. In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes. Get medical attention if

irritation develops or persists.

Product Use:

Laboratory Reagent.

Revision Information:

No Changes.

Disclaimer:

****

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****

Prepared by: Environmental Health & Safety
Phone Number: (314) 654-1600 (U.S.A.)

SIGMA-ALDRICH

sigma-aldrich.com

Material Safety Data Sheet

Version 4.0
Revision Date 07/21/2010
Print Date 04/04/2011

1. PRODUCT AND COMPANY IDENTIFICATION

Product name	:	Corn steep liquor
Product Number	:	C4648
Brand	:	Sigma
Company	:	Sigma-Aldrich 3050 Spruce Street SAINT LOUIS MO 63103 USA
Telephone	:	+1 800-325-5832
Fax	:	+1 800-325-5052
Emergency Phone #	:	(314) 776-6555

2. HAZARDS IDENTIFICATION

Emergency Overview

OSHA Hazards
No known OSHA hazards

HMIS Classification

Health hazard:	0
Flammability:	0
Physical hazards:	0

NFPA Rating

Health hazard:	0
Fire:	0
Reactivity Hazard:	0

Potential Health Effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.
Ingestion	May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

CAS-No.	EC-No.	Index-No.	Concentration
Corn steep liquor			
66071-94-1	266-113-4	-	-

4. FIRST AID MEASURES

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact
Wash off with soap and plenty of water.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for fire-fighters
Wear self contained breathing apparatus for fire fighting if necessary.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Avoid breathing vapors, mist or gas.

Environmental precautions
Do not let product enter drains.

Methods and materials for containment and cleaning up
Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Precautions for safe handling
Normal measures for preventive fire protection.

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Respiratory protection not required. For nuisance exposures use type OV/AG (US) or type ABEK (EU EN 14387) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
General industrial hygiene practice.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	liquid

Safety data

pH	no data available
Melting point	no data available

Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Water solubility	no data available

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10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Conditions to avoid
no data available

Materials to avoid
Strong oxidizing agents

Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Nature of decomposition products not known.

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11. TOXICOLOGICAL INFORMATION

Acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC:	No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP:	No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity
no data available

Specific target organ toxicity - single exposure (GHS)
no data available

Specific target organ toxicity - repeated exposure (GHS)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.

Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Additional Information

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

OSHA Hazards
No known OSHA hazards

DSL Status
All components of this product are on the Canadian DSL list.

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
SARA 313: This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
No SARA Hazards

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Corn steep liquor	66071-94-1	

New Jersey Right To Know Components

	CAS-No.	Revision Date
Corn steep liquor	66071-94-1	

California Prop. 65 Components

This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Co., shall not be held liable for any damage resulting from handling or from contact with the above product. See reverse side of invoice or packing slip for additional terms and conditions of sale.

MATERIAL SAFETY DATA SHEET

SECTION I - PRODUCT IDENTIFICATION

Product name:
BLACKSTRAP MOLASSES
FEEDGRADE MOLASSES
BBO MOLASSES

Manufacturer's Name:	Australian Molasses Trading Limited, Brisbane, Queensland
Imported By:	Agri-feeds Ltd, Tasman Quay, Mt Maunganui
Phone:	+64 7 574 0840

Chemical Name & Synonyms:	Inverted syrup from the juice of sugar cane.
Chemical family:	Sugars

SECTION II – HAZARDOUS INGREDIENTS

None

WHMIS RATING Rating: 0 = None - - - 4 = Extreme

Health 0 Flammability 1 Reactivity 0

Product is generally considered safe for human consumption. (GRAS)

SECTION III - PHYSICAL DATA

Physical state	Viscous liquid		Odours & Appearance: Fruity sweet, brown, clear viscous liquid.
Vapour pressure	Not determined		
pH	5.1	Vapour density	Not applicable
Evaporation rate	Not determined	Specific gravity	1.4
Freezing point	Less than minus 18°C	Boiling point	107° C
Solubility in water		Highly soluble	

SECTION IV - FIRE & EXPLOSION HAZARD DATA

Flammability		Will burn only under conditions of extreme heat.	
Means of extinction		Water	
Flashpoint	Not determined	Auto-ignition temperature	60° C
Flammable limits		Not determined	
Special fire fighting procedures		Use NIOH approved self-contained breathing apparatus	
Unusual fire 7 explosion hazards		Material may spontaneously decompose at temperatures >60°C	

SECTION 5 - REACTIVITY DATA

Product is stable

Incompatibility with other substances	None
Conditions to avoid	Heat over 60° C. Keep container vented, too allow release of CO2 produced by natural yeast in product

SECTION 6 - TOXICOLOGICAL PROPERTIES

None

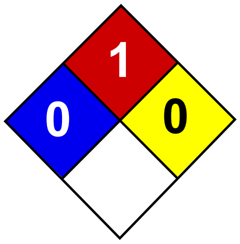
SECTION 7 - PREVENTIVE MEASURES

Storage	Keep container vented, to allow release of CO2 produced by natural yeast in product.
Personal protective equipment	Not required

Product is generally considered as safe for human consumption. (GRAS)

SECTION 8 - SPILL OR LEAK PROCEDURES

Wash with hot water. If large quantities are to be flushed into drains follow local regulations.



Health	1
Fire	1
Reactivity	0
Personal Protection	J

Material Safety Data Sheet
Oleic acid MSDS

Section 1: Chemical Product and Company Identification

Product Name: Oleic acid

Catalog Codes: SLO1078, SLO1318

CAS#: 112-80-1

RTECS: RG2275000

TSCA: TSCA 8(b) inventory: Oleic acid

CI#: Not available.

Synonym: 9-Octadecenoic acid

Chemical Name: (Z)-9-Octadecenoic Acid

Chemical Formula: $C18H34O2$

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call:
1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Oleic acid	112-80-1	100

Toxicological Data on Ingredients: Oleic acid: ORAL (LD50): Acute: 25000 mg/kg [Rat]. 28000 mg/kg [Mouse].

Section 3: Hazards Identification

Potential Acute Health Effects: Slightly hazardous in case of skin contact (irritant, permeator), of eye contact (irritant), of ingestion, of inhalation.

Potential Chronic Health Effects:
CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Not available. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available. Repeated or prolonged exposure is not known to aggravate medical condition.

Section 4: First Aid Measures

Eye Contact:
Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.

Skin Contact:

In case of contact, immediately flush skin with plenty of water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.

Serious Skin Contact:
Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek medical attention.

Inhalation:
If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.

Serious Inhalation: Not available.

Ingestion:
Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms appear.

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: May be combustible at high temperature.

Auto-Ignition Temperature: 363°C (685.4°F)

Flash Points: CLOSED CUP: 188.89°C (372°F). OPEN CUP: 198.89°C (390°F) - 218.33 C (425 F).

Flammable Limits: Not available.

Products of Combustion: These products are carbon oxides (CO, CO2).

Fire Hazards in Presence of Various Substances:
Slightly flammable to flammable in presence of heat. Non-flammable in presence of shocks.

Explosion Hazards in Presence of Various Substances:
Risks of explosion of the product in presence of mechanical impact: Not available. Risks of explosion of the product in presence of static discharge: Not available.

Fire Fighting Media and Instructions:
SMALL FIRE: Use DRY chemical powder. LARGE FIRE: Use water spray, fog or foam. Do not use water jet.

Special Remarks on Fire Hazards: Not available.

Special Remarks on Explosion Hazards: Not available.

Section 6: Accidental Release Measures

Small Spill: Absorb with an inert material and put the spilled material in an appropriate waste disposal.

Large Spill:
If the product is in its solid form: Use a shovel to put the material into a convenient waste disposal container. If the product is in its liquid form: Absorb with an inert material and put the spilled material in an appropriate waste disposal. Finish cleaning by spreading water on the contaminated surface and allow to evacuate through the sanitary system.

Section 7: Handling and Storage

Precautions:
Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not ingest. Do not breathe gas/fumes/ vapor/spray. Wear suitable protective clothing. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents.

Storage:
Keep container tightly closed. Keep container in a cool, well-ventilated area. Sensitive to light. Store in light-resistant containers. Air Sensitive

Section 8: Exposure Controls/Personal Protection

Engineering Controls:
Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that eyewash stations and safety showers are proximal to the work-station location.

Personal Protection: Splash goggles. Lab coat. Gloves.

Personal Protection in Case of a Large Spill:
Splash goggles. Full suit. Boots. Gloves. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

Exposure Limits: Not available.

Section 9: Physical and Chemical Properties

Physical state and appearance: Liquid.

Odor: Peculiar Lard-Like odor

Taste: Not available.

Molecular Weight: 282.47 g/mole

Color: Colorless to light yellow.

pH (1% soln/water): Not applicable.

Boiling Point: 286.11°C (547°F)

Melting Point: 16.3°C (61.3°F)

Critical Temperature: Not available.

Specific Gravity: 0.895 (Water = 1)

Vapor Pressure: Not available.

Vapor Density: 9.7(Air = 1)

Volatility: Not available.

Odor Threshold: Not available.

Water/Oil Dist. Coeff.: Not available.

Ionicity (in Water): Not available.

Dispersion Properties: See solubility in water, methanol, diethyl ether, acetone.

Solubility:
Soluble in methanol, diethyl ether, acetone. Insoluble in cold water. Soluble in chloroform, most organic solvents, benzene, alcohol, carbon tetrachloride, and fixed and volatile oils.

Section 10: Stability and Reactivity Data

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Excess heat

Incompatibility with various substances: Reactive with oxidizing agents.

Corrosivity: Non-corrosive in presence of glass.

Special Remarks on Reactivity:
Air and light sensitive. On exposure to air, especially when impure, it oxidizes and acquires a yellow to brown color and rancid odor. Also incompatible with perchloric acid, and powdered aluminum.

Special Remarks on Corrosivity: Not available.

Polymerization: Will not occur.

Section 11: Toxicological Information

Routes of Entry: Absorbed through skin. Eye contact.

Toxicity to Animals: Acute oral toxicity (LD50): 25000 mg/kg [Rat].

Chronic Effects on Humans: Not available.

Other Toxic Effects on Humans: Slightly hazardous in case of skin contact (irritant, permeator), of ingestion, of inhalation.

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans:
Human: passes the placental barrier, detected in maternal milk. May cause cancer based on animal test data. No human data found. May affect genetic material (mutagenic).

Special Remarks on other Toxic Effects on Humans:
Acute Potential Health Effects: Skin: Causes skin irritation. Eyes: May cause eye irritation. Ingestion: May cause digestive tract irritation. It is expected to be a low hazard for usual industrial handling. Inhalation: May cause respiratory tract irritation. It is expected to be a low hazard to usual industrial handling. Note: According the Registery of Toxic Effects of Chemicals, when Oleic acid was administered to rats and mice through intravenous injection, behavior and respiration were affected.

Section 12: Ecological Information

Ecotoxicity: Not available.

BOD5 and COD: Not available.

Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

Toxicity of the Products of Biodegradation: The product itself and its products of degradation are not toxic.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14: Transport Information

DOT Classification: Not a DOT controlled material (United States).

Identification: Not applicable.

Special Provisions for Transport: Not applicable.

Section 15: Other Regulatory Information

Federal and State Regulations:
Rhode Island RTK hazardous substances: Oleic acid Pennsylvania RTK: Oleic acid TSCA 8(b) inventory: Oleic acid

Other Regulations: EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

Other Classifications:

WHMIS (Canada): Not controlled under WHMIS (Canada).

DSCL (EEC):
R36/38- Irritating to eyes and skin. S24/25- Avoid contact with skin and eyes. S28- After contact with skin, wash immediately with plenty of water. S35- This material and its container must be disposed of in a safe way. S37- Wear suitable gloves.

HMIS (U.S.A.):

Health Hazard: 1

Fire Hazard: 1

Reactivity: 0

Personal Protection: j

National Fire Protection Association (U.S.A.):

Health: 0

Flammability: 1

Reactivity: 0

Specific hazard:

Protective Equipment:
Gloves. Lab coat. Not applicable. Splash goggles.

Section 16: Other Information

References: Not available.

Other Special Considerations: Not available.

Created: 10/11/2005 01:35 PM

Last Updated: 11/01/2010 12:00 PM

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MATERIAL SAFETY DATA SHEET

BioDunder

SECTION 1: IDENTIFICATION OF THE MATERIAL AND SUPPLIER

Product Name:	**BioDunder**
Other Names:	Molasses Dunder, Dunder, Vinasse
Product Codes/Trade Names:	Suplamite, DunDust, Suplaflo, BioDunder™
Recommended Use:	BioDunder co-products are licensed by the Department of Environmental Resource Management (Queensland) for beneficial re-use as a fertiliser, animal feed and dust suppressant purposes.
Applicable In:	Australia
Supplier:	Sucrogen BioEthanol Pty Ltd (ABN 85 009 660 191)
Address:	Bruce Highway, Sarina, QLD, 4737, Australia
Telephone:	+61 7 4940 9822
Email Address:	agservices@sucrogen.com
Web Site:	www.sucrogen.com
Facsimile:	+61 7 4956 2147
Emergency Phone Number:	000 Fire Brigade and Police (available in Australia only)
Poisons Information Centre:	13 11 26 (available in Australia only)

This Material Safety Data Sheet (MSDS) is issued by the Supplier in accordance with National Standards and Guidelines from Safe Work Australia (SWA – formerly ASCC/NOHSC). The information in it must not be altered, deleted or added to. The Supplier will not accept any responsibility for any changes made to its MSDS by any other person or organization. The Supplier will issue a new MSDS when there is a change in product specifications and/or Standards, Codes, Guidelines, or Regulations.

SECTION 2: HAZARD IDENTIFICATION

STATEMENT OF HAZARDOUS NATURE: Classified as **Non Hazardous** according to the Approved Criteria For Classifying Hazardous Substances [NOHSC:1008] 3rd Edition.

BioDunder is classified as **Non Dangerous** according to the Australian Code for the Transport of Dangerous Goods by Road and Rail.

SECTION 3: COMPOSITION / INFORMATION ON INGREDIENTS

Chemical Name:	**Synonyms**	**Proportion:**	**CAS Number:**
Protein	-	5% - 35% (DWB)	-
Ash	-	11% - 65% (DWB)	-
Carbohydrates	-	5 - 25% w/v	-
Glycerol	-	1 - 6 % w/v	56-81-5
Water	-	30 - 80% w/v	7732-18-5

BioDunder is a co-product of the molasses based ethanol manufacturing process and contains vegetable matter with traces of potassium, sodium, nitrogen, calcium, magnesium, phosphorus and sulphur.

SECTION 4: FIRST AID MEASURES

Swallowed:	Unlikely in the industrial situation. Give water to drink to dilute stomach contents. Do not induce vomiting.
Eyes:	Flush thoroughly with flowing water for at least 15 minutes. If symptoms/irritation persist, seek medical attention.
Skin:	Wash with soap and water.
Inhaled:	Remove to fresh air.
First Aid Facilities:	No special requirements.
Advice to Doctor:	Treat symptomatically.

SECTION 5: FIRE FIGHTING MEASURES

Flammability:	Product will not burn. No specific requirements.
Suitable extinguishing media:	Product will not burn. If there is a fire in the surrounding area use suitable extinguishing media for the surrounding material.
Hazards from combustion products:	None - product will not burn.
Special protective precautions and equipment for fire fighters:	As required for fire in surrounding materials.
HAZCHEM Code:	Not applicable

SECTION 6: ACCIDENTAL RELEASE MEASURES

Emergency Procedure:	See Disposal Considerations.
Containment Procedure:	Bund and pump into suitable drum for re-use. For dust suppressant applications, refer to permit number ENBU00824808 for BioDunder issued by the Department of Environment and Resource Management in Queensland (approval of resource for beneficial use).
Clean Up Procedure:	Large spills - hose down but avoid this product getting into drains, sewers and waterways because of high BOD load and low pH. For dust suppressant applications, refer to permit number ENBU00824808 for BioDunder issued by the Department of Environment and Resource Management in Queensland (approval of resource for beneficial use).

SECTION 7: HANDLING AND STORAGE

Handling:	Transported by bulk tanker. For dust suppressant applications, refer to permit number ENBU00824808 for BioDunder issued by the Department of Environment and Resource Management in Queensland (approval of resource for beneficial use).
Storage:	For dust suppressant applications, refer to permit number ENBU00824808 for BioDunder issued by the Department of Environment and Resource Management in Queensland (approval of resource for beneficial use).
Incompatibilities:	None

SECTION 8: EXPOSURE CONTROLS / PERSONAL PROTECTION

Exposure Standards:	National Occupational Exposure Standard (NES), Safe Work Australia (formerly ASCC/NOHSC). No exposure standard is applicable to this non-hazardous product.
Notes:	Sucrogen AgServices recommendation: Keep exposure to aerosols and mists as low as practicable and avoid skin contact.
Biological Limit Values:	No biological limit allocated.
ENGINEERING CONTROLS	
☐ **Ventilation:**	All work with BioDunder should be carried out in such a way as to minimise skin contact with the product and avoid generation of liquid aerosol (mist) where persons can inhale it. For applications where there is a risk of exposure to mists, please refer to Respiratory Protection requirements below.
☐ **Special Consideration for Repair &/or Maintenance of Contaminated Equipment:**	Recommendations on exposure control and Personal Protection should be followed.
PERSONAL PROTECTION	
☐ **Personal Hygiene**	Wash hands before eating, drinking, using the toilet or smoking.
☐ **Skin Protection:**	Wear Loose comfortable clothing. Wear PVC or rubber gloves AS 2161. Wash work clothes regularly.
☐ **Eye Protection:**	Goggles or safety glasses (AS 1336 and AS/NZS 1337) and a face shield should be worn if there is a risk of splash.
☐ **Respiratory Protection:**	If an aerosol or mist is generated, wear a respirator for acid gases conforming to AS/NZS 1715 and 1716.
☐ **Thermal Protection:**	None should be needed under normal circumstances.
☐ **Smoking & Other Dusts**	Inhalation of airborne particles from other sources, including those from cigarette smoke, may increase the risk of lung disease. Sucrogen AgServices recommends that all storage and work areas should be non-smoking zones, and other airborne contaminants be kept to a minimum.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	Dark brown-black, viscous liquid
Odour:	Sharp organic odour like molasses
pH, at stated concentration:	4.0 - 4.5
Vapour Pressure:	Not applicable - does not vapourise
Vapour Density:	Not applicable - does not vapourise
Boiling Point/range (°C):	~ 100°C
Freezing/Melting Point (°C):	Not determined
Solubility:	Partially soluble in water.
Specific Gravity (H_2O = 1):	1.15 (typical)
FLAMMABLE MATERIALS	
☐ **Flash Point:**	Not applicable

☐ **Flash Point Method:**	Not applicable
☐ **Flammable (Explosive) Limit - Upper:**	Not applicable
☐ **Flammable (Explosive) Limit - Lower:**	Not applicable
☐ **Autoignition Temperature:**	Does not auto-ignite
ADDITIONAL PROPERTIES	
☐ **Evaporation Rate:**	Not determined
☐ **Molecular Weight:**	Not determined
☐ **Volatile Organic Compounds Content (VOC):** (as specified by the Green Building Council of Australia)	Nil
☐ **% Volatiles:**	0%

SECTION 10: STABILITY AND REACTIVITY

Chemical Stability:	Product is stable and will not polymerize.
Incompatible Materials:	None known.
Conditions to avoid:	None known.
Hazardous Decomposition Products:	None known.
Hazardous Reactions:	None known.

SECTION 11: TOXICOLOGICAL INFORMATION

Toxicological Data:

The health effects listed below are related to the acid pH of the product (3.3 - 4.0) and potential for mild irritation of skin and mucous membranes.

Health effects information is based on reported effects in use from overseas and Australian reports.

Effects: Acute

Swallowed:	Unlikely under normal conditions of use, but could cause abdominal discomfort and nausea, and throat irritation.
Eyes:	Can irritate the eyes and cause watering and redness.
Skin:	Can cause skin irritation if in repeated or prolonged contact with the skin.
Inhaled:	Can be temporarily irritating to the respiratory system if aerosol mist breathed in.

Effects: Chronic

Repeated skin contact may result in chronic dermatitis with redness and dryness of the skin.

SECTION 12: ECOLOGICAL INFORMATION

Eco-toxicity:	Product has no significant ecotoxicity.
Persistence and Degradability:	Biodegradeable.
Mobility:	Biodegradeable.

SECTION 13: DISPOSAL CONSIDERATIONS

Large spills - hose down but avoid this product getting into drains, sewers and waterways because of high BOD load and low pH. Bund and pump into suitable drum for reuse.

For dust suppressant applications, refer to permit number ENBU00824808 for BioDunder issued by the Department of Environment and Resource Management in Queensland (approval of resource for beneficial use).

SECTION 14: TRANSPORT INFORMATION

Proper Shipping Name:	Niot applicable
UN number:	Not applicable
DG Class:	Not applicable
Subsidiary Risk 1:	Not applicable
Packaging Group:	Not applicable
HAZCHEM code:	Not applicable
Marine Pollutant:	No
Special Precautions for User:	None

ADDITIONAL TRANSPORT REQUIREMENTS:

For dust suppressant applications, refer to permit number ENBU00824808 for BioDunder issued by the Department of Environment and Resource Management in Queensland (approval of resource for beneficial use).

SECTION 15: REGULATORY INFORMATION

Poisons Schedule:	Not scheduled
Other:	Approval for BioDunder for beneficial use by Department of Environmental Resource Management Queensland Government including as a dust suppressant, stock feed and a fertiliser. Permit Number ENBU00824808.

SECTION 16: OTHER INFORMATION

For further information on this product, please contact:

Sucrogen BioEthanol Pty Ltd (ABN 85 009 660 191)
Bruce Highway, Sarina, QLD, 4737, Australia
Phone: +61 7 4940 9822
Fax: +61 7 4956 2147

ADDITIONAL INFORMATION

Australian Standards References:

AS 1020	The Control of Undesirable Static Electricity.
AS 1076	Code of Practice for selection, installation and maintenance of electrical apparatus and associated equipment for use in explosive atmospheres (other than mining applications) – Parts 1 to 13
AS/NZS 1336	Recommended Practices for Occupational Eye Protection
AS/NZS 1715	Selection, Use and Maintenance of Respiratory Protective Devices
AS/NZS 1716	Respiratory Protective Devices
AS 1940	The Storage and Handling of Flammable and Combustible Liquids
AS 2161	Industrial Safety Gloves and Mittens (excluding electrical and medical gloves)
AS 2380	Electrical equipment for explosive atmospheres – Explosion Protection Techniques (Parts 1 to 9)
AS 3000	Electrical installations (known as the Australian/New Zealand Wiring Rules).

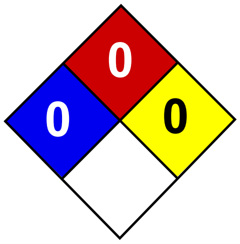
Other References:

NOHSC:2011(2003)	National Code of Practice for the Preparation of Material Safety Data Sheets 2nd Edition, April 2003, National Occupational Health and Safety Commission.
NOHSC; 2012 (1994)	National Code of Practice for the Labelling of Workplace Substances, March 1994, Australian Government Publishing Service, Canberra.
NES	National Occupational Exposure Standards for Workplace Atmospheric Contaminants (NES), Safe Work Australia (formerly ASCC/NOHSC) 1995 as amended.
ADG Code	Australian Dangerous Goods Code 7th Edition.
Permit ENBU00824808	Approval of resource for beneficial use for BioDunder issued by the Department of Environment and Resource Management in Queensland. A copy of this permit can be obtained by contacting Sucrogen BioEthanol.

AUTHORISATION

Reason for Issue:	New Sucrogen format & 5 Yearly Review
Authorised by:	Quality & Technical Manager and Sucrogen Legal
Date of Issue:	28 July 2010

END OF MSDS



Health	0
Fire	0
Reactivity	0
Personal Protection	A

Material Safety Data Sheet

Water MSDS

Section 1: Chemical Product and Company Identification

Product Name: Water

Catalog Codes: SLW1063

CAS#: 7732-18-5

RTECS: ZC0110000

TSCA: TSCA 8(b) inventory: Water

CI#: Not available.

Synonym: Dihydrogen oxide

Chemical Name: Water

Chemical Formula: H2O

Contact Information:

Sciencelab.com, Inc.
14025 Smith Rd.
Houston, Texas 77396

US Sales: **1-800-901-7247**
International Sales: **1-281-441-4400**

Order Online: ScienceLab.com

CHEMTREC (24HR Emergency Telephone), call:
1-800-424-9300

International CHEMTREC, call: 1-703-527-3887

For non-emergency assistance, call: 1-281-441-4400

Section 2: Composition and Information on Ingredients

Composition:

Name	CAS #	% by Weight
Water	7732-18-5	100

Toxicological Data on Ingredients: Not applicable.

Section 3: Hazards Identification

Potential Acute Health Effects:
Non-corrosive for skin. Non-irritant for skin. Non-sensitizer for skin. Non-permeator by skin. Non-irritating to the eyes. Non-hazardous in case of ingestion. Non-hazardous in case of inhalation. Non-irritant for lungs. Non-sensitizer for lungs. Non-corrosive to the eyes. Non-corrosive for lungs.

Potential Chronic Health Effects:
Non-corrosive for skin. Non-irritant for skin. Non-sensitizer for skin. Non-permeator by skin. Non-irritating to the eyes. Non-hazardous in case of ingestion. Non-hazardous in case of inhalation. Non-irritant for lungs. Non-sensitizer for lungs. CARCINOGENIC EFFECTS: Not available. MUTAGENIC EFFECTS: Not available. TERATOGENIC EFFECTS: Not available. DEVELOPMENTAL TOXICITY: Not available.

Section 4: First Aid Measures

Eye Contact: Not applicable.

Skin Contact: Not applicable.

Serious Skin Contact: Not available.

Inhalation: Not applicable.

Serious Inhalation: Not available.

Ingestion: Not Applicable

Serious Ingestion: Not available.

Section 5: Fire and Explosion Data

Flammability of the Product: Non-flammable.

Auto-Ignition Temperature: Not applicable.

Flash Points: Not applicable.

Flammable Limits: Not applicable.

Products of Combustion: Not available.

Fire Hazards in Presence of Various Substances: Not applicable.

Explosion Hazards in Presence of Various Substances: Not Applicable

Fire Fighting Media and Instructions: Not applicable.

Special Remarks on Fire Hazards: Not available.

Special Remarks on Explosion Hazards: Not available.

Section 6: Accidental Release Measures

Small Spill: Mop up, or absorb with an inert dry material and place in an appropriate waste disposal container.

Large Spill: Absorb with an inert material and put the spilled material in an appropriate waste disposal.

Section 7: Handling and Storage

Precautions: No specific safety phrase has been found applicable for this product.

Storage: Not applicable.

Section 8: Exposure Controls/Personal Protection

Engineering Controls: Not Applicable

Personal Protection: Safety glasses. Lab coat.

Personal Protection in Case of a Large Spill: Not Applicable

Exposure Limits: Not available.

Section 9: Physical and Chemical Properties

Physical state and appearance: Liquid.

Odor: Odorless.

Taste: Not available.

Molecular Weight: 18.02 g/mole

Color: Colorless.

pH (1% soln/water): 7 [Neutral.]

Boiling Point: 100°C (212°F)

Melting Point: Not available.

Critical Temperature: Not available.

Specific Gravity: 1 (Water = 1)

Vapor Pressure: 2.3 kPa (@ 20°C)

Vapor Density: 0.62 (Air = 1)

Volatility: Not available.

Odor Threshold: Not available.

Water/Oil Dist. Coeff.: Not available.

Ionicity (in Water): Not available.

Dispersion Properties: Not applicable

Solubility: Not Applicable

Section 10: Stability and Reactivity Data

Stability: The product is stable.

Instability Temperature: Not available.

Conditions of Instability: Not available.

Incompatibility with various substances: Not available.

Corrosivity: Not available.

Special Remarks on Reactivity: Not available.

Special Remarks on Corrosivity: Not available.

Polymerization: Will not occur.

Section 11: Toxicological Information

Routes of Entry: Absorbed through skin. Eye contact.

Toxicity to Animals:
LD50: [Rat] - Route: oral; Dose: > 90 ml/kg LC50: Not available.

Chronic Effects on Humans: Not available.

Other Toxic Effects on Humans:
Non-corrosive for skin. Non-irritant for skin. Non-sensitizer for skin. Non-permeator by skin. Non-hazardous in case of ingestion. Non-hazardous in case of inhalation. Non-irritant for lungs. Non-sensitizer for lungs. Non-corrosive to the eyes. Non-corrosive for lungs.

Special Remarks on Toxicity to Animals: Not available.

Special Remarks on Chronic Effects on Humans: Not available.

Special Remarks on other Toxic Effects on Humans: Not available.

Section 12: Ecological Information

Ecotoxicity: Not available.

BOD5 and COD: Not available.

Products of Biodegradation:
Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.

Toxicity of the Products of Biodegradation: The product itself and its products of degradation are not toxic.

Special Remarks on the Products of Biodegradation: Not available.

Section 13: Disposal Considerations

Waste Disposal:
Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14: Transport Information

DOT Classification: Not a DOT controlled material (United States).

Identification: Not applicable.

Special Provisions for Transport: Not applicable.

Section 15: Other Regulatory Information

Federal and State Regulations: TSCA 8(b) inventory: Water

Other Regulations: EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

Other Classifications:

WHMIS (Canada): Not controlled under WHMIS (Canada).

DSCL (EEC):
This product is not classified according to the EU regulations. Not applicable.

HMIS (U.S.A.):

Health Hazard: 0

Fire Hazard: 0

Reactivity: 0

Personal Protection: a

National Fire Protection Association (U.S.A.):

Health: 0

Flammability: 0

Reactivity: 0

Specific hazard:

Protective Equipment:
Not applicable. Lab coat. Not applicable. Safety glasses.

Section 16: Other Information

References: Not available.

Other Special Considerations: Not available.

Created: 10/10/2005 08:33 PM

Last Updated: 11/01/2010 12:00 PM

Appendix E: Patent Applications

US 20090075351A1

(19) **United States**

(12) **Patent Application Publication** (10) **Pub. No.: US 2009/0075351 A1**
Burk et al. (43) **Pub. Date: Mar. 19, 2009**

(54) **COMPOSITIONS AND METHODS FOR THE BIOSYNTHESIS OF 1,4-BUTANEDIOL AND ITS PRECURSORS**

(76) Inventors: **Mark J. Burk**, San Diego, CA (US); **Stephen J. Van Dien**, Encinitas, CA (US); **Anthony P. Burgard**, Bellefonte, PA (US); **Wei Niu**, San Diego, CA (US)

Correspondence Address:
MCDERMOTT, WILL & EMERY
11682 EL CAMINO REAL, SUITE 400
SAN DIEGO, CA 92130-2047 (US)

(21) Appl. No.: **12/049,256**

(22) Filed: **Mar. 14, 2008**

Related U.S. Application Data

(60) Provisional application No. 60/918,463, filed on Mar. 16, 2007.

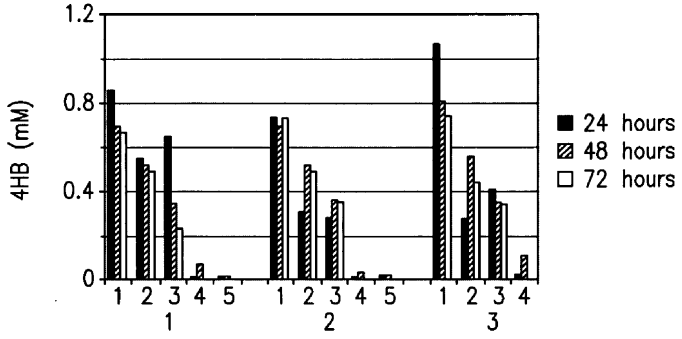
Publication Classification

(51) **Int. Cl.**
C12N 1/21 (2006.01)
C12P 7/52 (2006.01)
C12P 7/18 (2006.01)

(52) **U.S. Cl.** **435/141**; 435/252.33; 435/158

(57) **ABSTRACT**

The invention provides a non-naturally occurring microbial biocatalyst including a microbial organism having a 4-hydroxybutanoic acid (4-HB) biosynthetic pathway having at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, or α-ketoglutarate decarboxylase, wherein the exogenous nucleic acid is expressed in sufficient amounts to produce monomeric 4-hydroxybutanoic acid (4-HB). Also provided is a non-naturally occurring microbial biocatalyst including a microbial organism having 4-hydroxybutanoic acid (4-HB) and 1,4-butanediol (BDO) biosynthetic pathways, the pathways include at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, 4-hydroxybutyrate:CoA transferase, 4-butyrate kinase, phosphotransbutyrylase, α-ketoglutarate decarboxylase, aldehyde dehydrogenase, alcohol dehydrogenase or an aldehyde/alcohol dehydrogenase, wherein the exogenous nucleic acid is expressed in sufficient amounts to produce 1,4-butanediol (BDO). Additionally provided is a method for the production of 4-HB. The method includes culturing a non-naturally occurring microbial organism having a 4-hydroxybutanoic acid (4-HB) biosynthetic pathway including at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase or α-ketoglutarate decarboxylase under substantially anaerobic conditions for a sufficient period of time to produce monomeric 4-hydroxybutanoic acid (4-HB). Further provided is a method for the production of BDO. The method includes culturing a non-naturally occurring microbial biocatalyst, comprising a microbial organism having 4-hydroxybutanoic acid (4-HB) and 1,4-butanediol (BDO) biosynthetic pathways, the pathways including at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, 4-hydroxybutyrate:CoA transferase, 4-hydroxybutyrate kinase, phosphotranshydroxybutyrylase, α-ketoglutarate decarboxylase, aldehyde dehydrogenase, alcohol dehydrogenase or an aldehyde/alcohol dehydrogenase for a sufficient period of time to produce 1,4-butanediol (BDO). The 4-HB and/or BDO products can be secreted into the culture medium.



Patent Application Publication **Mar. 19, 2009 Sheet 1 of 14** **US 2009/0075351 A1**

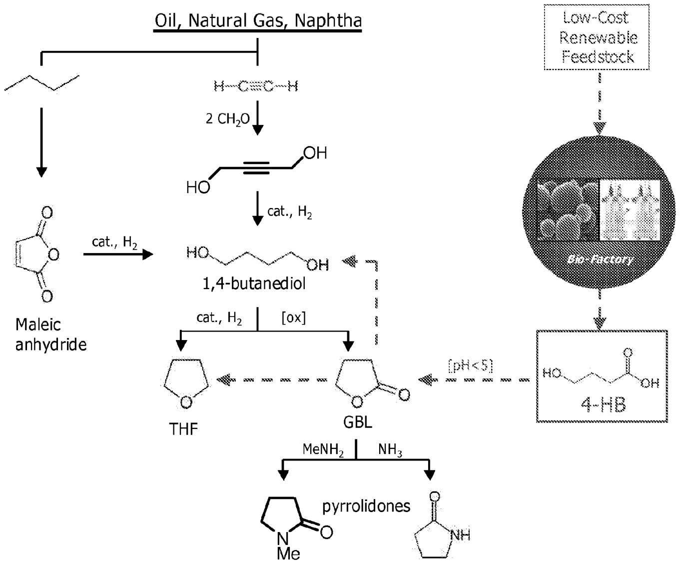


FIGURE 1

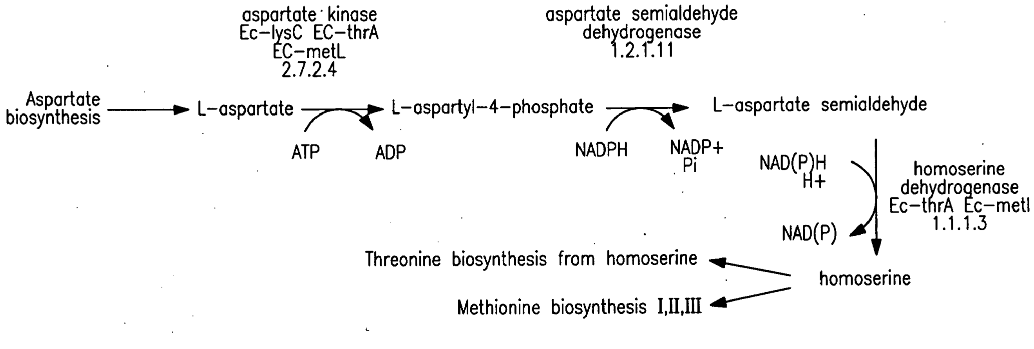


FIG. 3

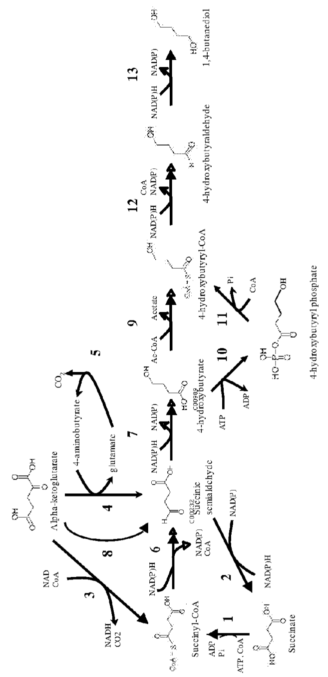


FIGURE 2

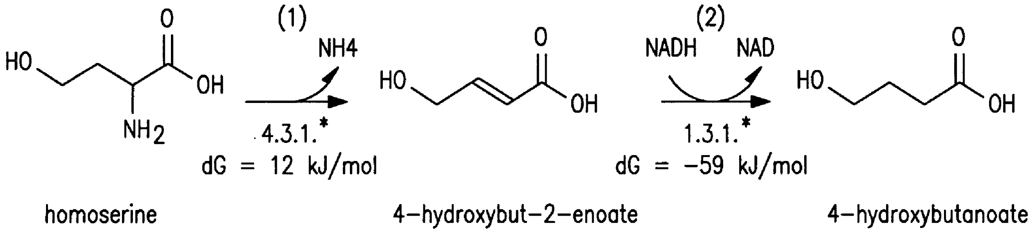

(1)
NH4
4.3.1.*
dG = 12 kJ/mol
(2)
NADH
NAD
1.3.1.*
dG = −59 kJ/mol
HO
O
OH
NH2
homoserine
4−hydroxybut−2−enoate
4−hydroxybutanoate

FIG. 4

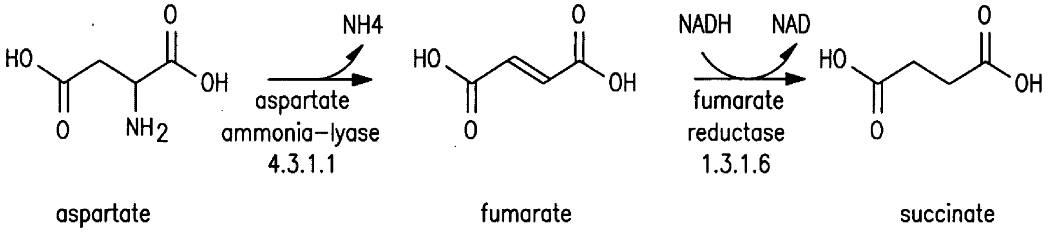

NH4
aspartate
ammonia−lyase
4.3.1.1
NADH
NAD
fumarate
reductase
1.3.1.6
HO
O
OH
NH2
aspartate
fumarate
succinate

FIG. 5

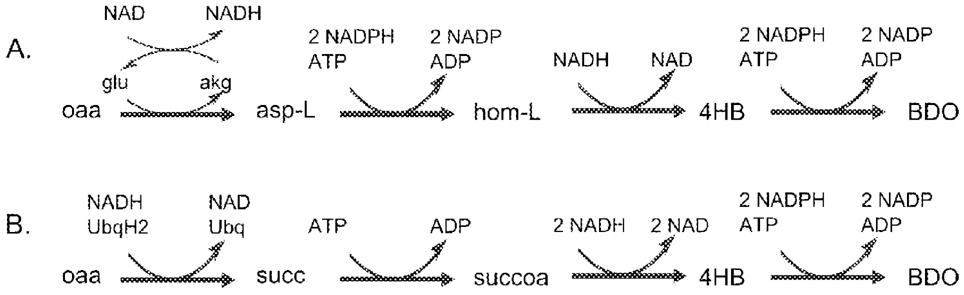


FIGURE 6

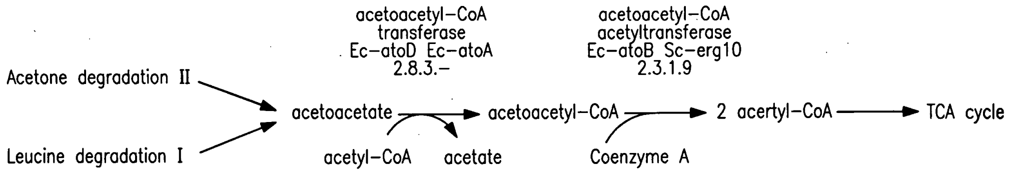


FIG. 7

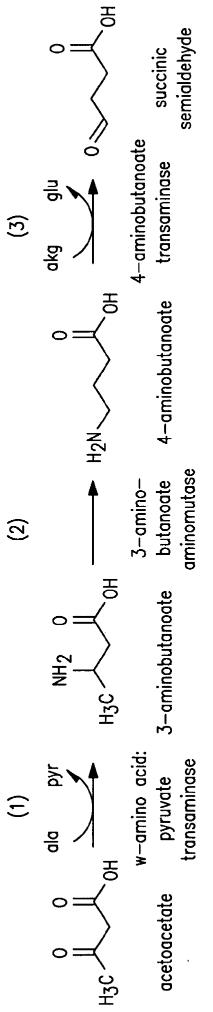


FIG. 8

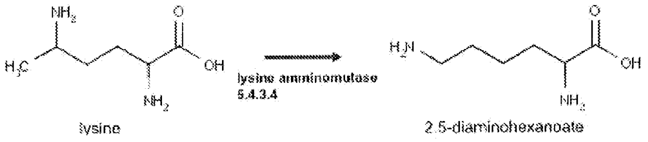


FIGURE 9

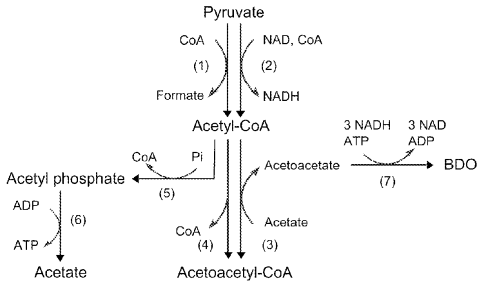


FIGURE 10

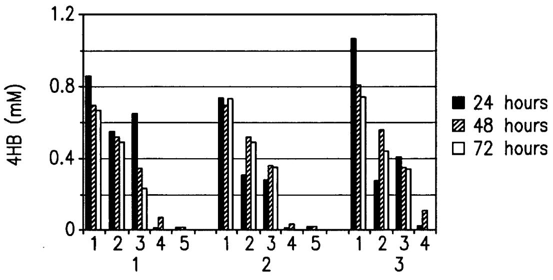


FIG. 11(a)

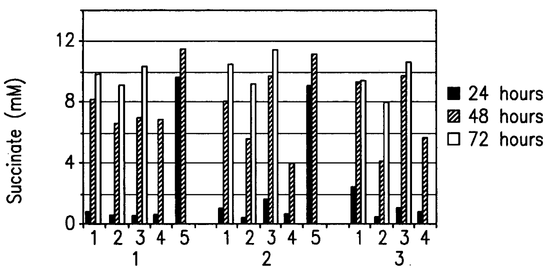


FIG. 11(b)

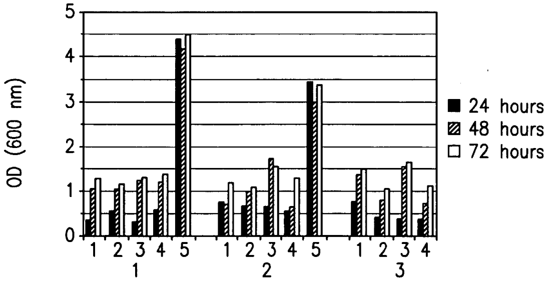


FIG. 11(c)

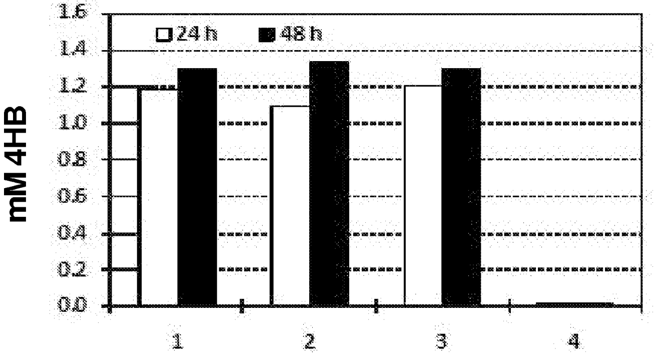


FIGURE 12

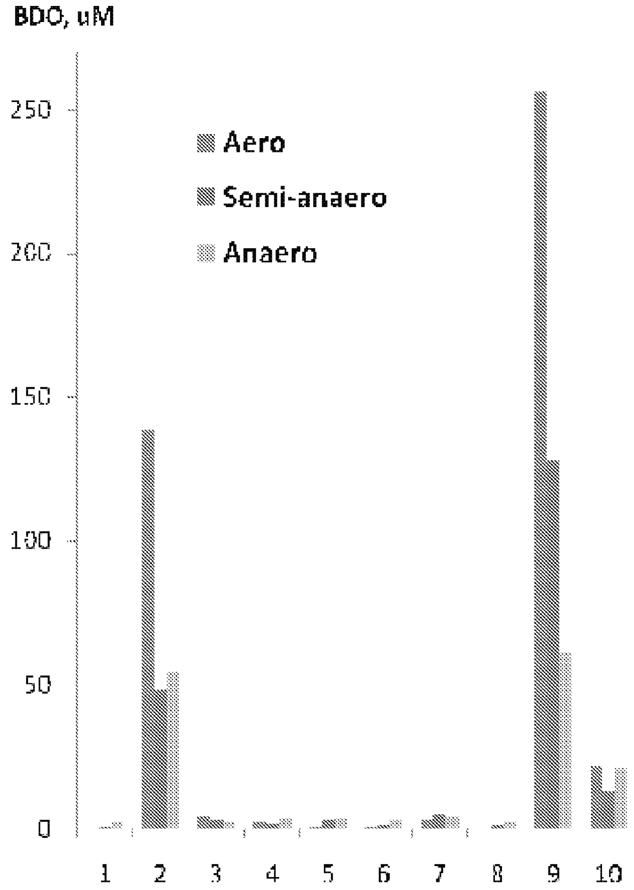


FIGURE 13

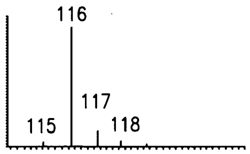


FIG. 14(a)

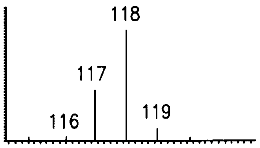


FIG. 14(b)

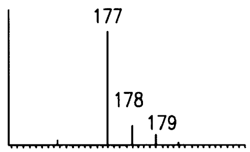


FIG. 14(c)

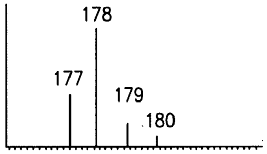


FIG. 14(d)

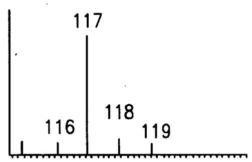


FIG. 14(e)

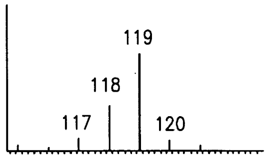


FIG. 14(f)

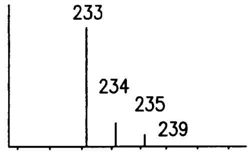


FIG. 14(g)

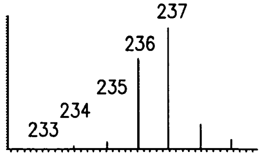


FIG. 14(h)

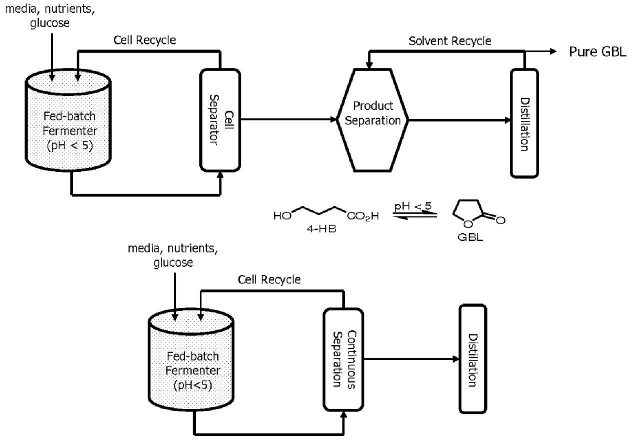


FIGURE 15

COMPOSITIONS AND METHODS FOR THE BIOSYNTHESIS OF 1,4-BUTANEDIOL AND ITS PRECURSORS

[0001] This application claims the benefit of priority of U.S. Provisional application Ser. No. 60/918,463, filed Mar. 16, 2007, the entire contents of which are incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] This invention relates generally to in silico design of organisms and, more particularly to organisms having 1,4-butanediol biosynthesis capability.

[0003] The compound 4-hydroxybutanoic acid (4-hydroxybutanoate, 4-hydroxybutyrate, 4-HB) is a 4-carbon carboxylic acid that has industrial potential as a building block for various commodity and specialty chemicals. In particular, 4-HB has the potential to serve as a new entry point into the 1,4-butanediol family of chemicals, which includes solvents, resins, polymer precursors, and specialty chemicals. 1,4-Butanediol (BDO) is a polymer intermediate and industrial solvent with a global market of about 3 billion lb/year. BDO is currently produced from petrochemical precursors, primarily acetylene, maleic anhydride, and propylene oxide.

[0004] For example, acetylene is reacted with 2 molecules of formaldehyde in the Reppe synthesis reaction (Kroschwitz and Grant, *Encyclopedia of Chem. Tech.*, John Wiley and Sons, Inc., New York (1999)), followed by catalytic hydrogenation to form 1,4-butanediol. It has been estimated that 90% of the acetylene produced in the U.S. is consumed for butanediol production. Alternatively, it can be formed by esterification and catalytic hydrogenation of maleic anhydride, which is derived from butane. Downstream, butanediol can be further transformed; for example, by oxidation to □-butyrolactone, which can be further converted to pyrrolidone and N-methyl-pyrrolidone, or hydrogenolysis to tetrahydrofuran (FIG. **1**). These compounds have varied uses as polymer intermediates, solvents, and additives, and have a combined market of nearly 2 billion lb/year.

[0005] It is desirable to develop a method for production of these chemicals by alternative means that not only substitute renewable for petroleum-based feedstocks, and also use less energy- and capital-intensive processes. The Department of Energy has proposed 1,4-diacids, and particularly succinic acid, as key biologically-produced intermediates for the manufacture of the butanediol family of products (DOE Report, "Top Value-Added Chemicals from Biomass", 2004). However, succinic acid is costly to isolate and purify and requires high temperatures and pressures for catalytic reduction to butanediol.

[0006] Thus, there exists a need for alternative means for effectively producing commercial quantities of 1,4-butanediol and its chemical precursors. The present invention satisfies this need and provides related advantages as well.

SUMMARY OF THE INVENTION

[0007] The invention provides a non-naturally occurring microbial biocatalyst including a microbial organism having a 4-hydroxybutanoic acid (4-HB) biosynthetic pathway having at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, or α-ketoglutarate decarboxylase, wherein the exogenous nucleic acid is expressed in sufficient amounts to produce monomeric 4-hydroxybutanoic acid (4-HB). Also provided is a non-naturally occurring microbial biocatalyst including a microbial organism having 4-hydroxybutanoic acid (4-HB) and 1,4-butanediol (BDO) biosynthetic pathways, the pathways include at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, 4-hydroxybutyrate:CoA transferase, 4-butyrate kinase, phosphotransbutyrylase, α-ketoglutarate decarboxylase, aldehyde dehydrogenase, alcohol dehydrogenase or an aldehyde/alcohol dehydrogenase, wherein the exogenous nucleic acid is expressed in sufficient amounts to produce 1,4-butanediol (BDO). Additionally provided is a method for the production of 4-HB. The method includes culturing a non-naturally occurring microbial organism having a 4-hydroxybutanoic acid (4-HB) biosynthetic pathway including at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase or α-ketoglutarate decarboxylase under substantially anaerobic conditions for a sufficient period of time to produce monomeric 4-hydroxybutanoic acid (4-HB). Further provided is a method for the production of BDO. The method includes culturing a non-naturally occurring microbial biocatalyst, comprising a microbial organism having 4-hydroxybutanoic acid (4-HB) and 1,4-butanediol (BDO) biosynthetic pathways, the pathways including at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, 4-hydroxybutyrate:CoA transferase, 4-hydroxybutyrate kinase, phosphotranshydroxybutyrylase, α-ketoglutarate decarboxylase, aldehyde dehydrogenase, alcohol dehydrogenase or an aldehyde/alcohol dehydrogenase for a sufficient period of time to produce 1,4-butanediol (BDO). The 4-HB and/or BDO products can be secreted into the culture medium.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] FIG. **1** is a schematic diagram showing an entry point of 4-hydroxybutanoic acid (4-HB) into the product pipeline of the 1,4-butanediol (BDO) family of chemicals, and comparison with chemical synthesis routes from petrochemical feedstocks. Solid black arrows show chemical synthesis routes; dashed blue arrows show a biosynthetic route to 4-HB and subsequent conversion steps to BDO family chemicals.

[0009] FIG. **2** is a schematic diagram showing biochemical pathways to 4-hydroxybutyurate (4-HB) and to 1,4-butanediol production. The first 5 steps are endogenous to *E. coli*, while the remainder can be expressed heterologously. Enzymes catalyzing the biosynthetic reactions are: (1) succinyl-CoA synthetase; (2) CoA-independent succinic semialdehyde dehydrogenase; (3) α-ketoglutarate dehydrogenase; (4) glutamate:succinate semialdehyde transaminase; (5) glutamate decarboxylase; (6) CoA-dependent succinic semialdehyde dehydrogenase; (7) 4-hydroxybutanoate dehydrogenase; (8) α-ketoglutarate decarboxylase; (9) 4-hydroxybutyryl CoA:acetyl-CoA transferase; (10) butyrate kinase; (11) phosphotransbutyrylase; (12) aldehyde dehydrogenase; (13) alcohol dehydrogenase.

[0010] FIG. **3** is a schematic diagram showing homoserine biosynthesis in *E. coli*.

[0011] FIG. **4** shows a schematic diagram of a predicted homoserine biopathway from L-homoserine to 4-HB. Step **1** is a deduced ammonia-lyase (EC class 4.3.1) with an estimated ΔrxnG of 12 kJ/mol. Step **2** is a deduced oxidoreductase (EC class 1.3.1) with an estimated ΔrxnG of −59 kJ/mol.

[0012] FIG. **5** shows a schematic diagram for the endogenous *E. coli* pathway for aspartate conversion to succinate via fumarate. This pathway exhibits similar chemistry to the predicted homoserine biopathway.

[0013] FIG. **6** shows a schematic diagram illustrating the parallels between (A) homoserine and (B) succinyl-CoA biosynthetic pathways to BDO.

[0014] FIG. **7** is a schematic diagram showing biochemical pathways to acetoacetate in *E. coli.*

[0015] FIG. **8** is a schematic diagram showing a biochemical pathway from acetoacetate to BDO via succinic semialdehyde.

[0016] FIG. **9** is a schematic diagram showing a reaction scheme of D-lysine-5,6-amminomutase.

[0017] FIG. **10** is a schematic diagram showing a pathway to acetoacetate from acetyl-CoA. Enzymes are: (1) pyruvate formate-lyase, (2) pyruvate dehydrogenase, (3) acetyl-CoA: acetoacetyl-CoA transferase, (4) acetyl-CoA C-acetyltransferase, (5) phosphotransacetylase, and (6) Acetate kinase. Enzyme 7 represents the multistep acetoacetate to BDO pathway in FIG. **8**.

[0018] FIG. **11** shows the production of 4-HB in glucose minimal medium using *E. coli* strains harboring plasmids expressing various combinations of 4-HB pathway genes. (a) 4-HB concentration in culture broth; (b) succinate concentration in culture broth; (c) culture OD, measured at 600 nm. Clusters of bars represent the 24 hour, 48 hour, and 72 hour (if measured) timepoints. The codes along the x-axis indicate the strain/plasmid combination used. The first index refers to the host strain: 1, MG1655 $lacI^Q$; 2, MG1655 ΔgabD $lacI^Q$; 3, MG1655 ΔgabD ΔaldA $lacI^Q$. The second index refers to the plasmid combination used: 1, pZE13-0004-0035 and pZA33-0036; 2, pZE13-0004-0035 and pZA33-0010n; 3, pZE13-0004-0008 and pZA33-0036; 4, pZE13-0004-0008 and pZA33-0010n; 5, Control vectors pZE13 and pZA33.

[0019] FIG. **12** shows the production of 4-HB from glucose in *E. coli* strains expressing α-ketoglutarate decarboxylase from *Mycobacterium tuberculosis*. Strains 1-3 contain pZE13-0032 and pZA33-0036. Strain 4 expresses only the empty vectors pZE13 and pZA33. Host strains are as follows: 1 and 4, MG1655 $lacI^Q$; 2, MG1655 ΔgabD $lacI^Q$; 3, MG1655 ΔgabD ΔaldA $lacI^Q$. The bars refer to concentration at 24 and 48 hours.

[0020] FIG. **13** shows the production of BDO from 10 mM 4-HB in recombinant *E. coli* strains. Numbered positions correspond to experiments with MG1655 $lacI^Q$ containing pZA33-0024, expressing cat2 from *P. gingivalis*, and the following genes expressed on pZE13: 1, none (control); 2, 0002; 3, 0003; 4, 0003n; 5, 0011; 6, 0013; 7, 0023; 8, 0025; 9, 0008n; 10, 0035. Gene numbers are defined in Table 6. For each position, the bars refer to aerobic, microaerobic, and anaerobic conditions, respectively. Microaerobic conditions were created by sealing the culture tubes but not evacuating them.

[0021] FIG. **14** shows the mass spectrum of 4-HB and BDO produced by MG1655 $lacI^Q$ pZE13-0004-0035-0002 pZA33-0034-0036 grown in M9 minimal medium supplemented with 4 g/L unlabeled glucose (a, c, e, and g) uniformly labeled ^{13}C-glucose (b, d, f, and h). (a) and (b), mass 116 characteristic fragment of derivatized BDO, containing 2 carbon atoms; (c) and (d), mass 177 characteristic fragment of derivatized BDO, containing 1 carbon atom; (e) and (f), mass 117 characteristic fragment of derivatized 4-HB, containing 2 carbon atoms; (g) and (h), mass 233 characteristic fragment of derivatized 4-HB, containing 4 carbon atoms.

[0022] FIG. **15** is a schematic process flow diagram of bioprocesses for the production of γ-butyrolactone. Panel (a) illustrates fed-batch fermentation with batch separation and panel (b) illustrates fed-batch fermentation with continuous separation.

DETAILED DESCRIPTION OF THE INVENTION

[0023] This invention is directed to the design and production of cells and organisms having biosynthetic production capabilities for 4-hydroxybutanoic acid (4-HB), γ-butyrolactone and 1,4-butanediol. In one embodiment, the invention utilizes in silico stoichiometric models of *Escherichia coli* metabolism that identify metabolic designs for biosynthetic production of 4-hydroxybutanoic acid (4-HB) and 1,4-butanediol (BDO). The results described herein indicate that metabolic pathways can be designed and recombinantly engineered to achieve the biosynthesis of 4-HB and downstream products such as 1,4-butanediol in *Escherichia coli* and other cells or organisms. Biosynthetic production of 4-HB, for example, for the in silico designs can be confirmed by construction of strains having the designed metabolic genotype. These metabolically engineered cells or organisms also can be subjected to adaptive evolution to further augment 4-HB biosynthesis, including under conditions approaching theoretical maximum growth.

[0024] In certain embodiments, the 4-HB biosynthesis characteristics of the designed strains make them genetically stable and particularly useful in continuous bioprocesses. Separate strain design strategies were identified with incorporation of different non-native or heterologous reaction capabilities into *E. coli* leading to 4-HB and 1,4-butanediol producing metabolic pathways from either CoA-independent succinic semialdehyde dehydrogenase, succinyl-CoA synthetase and CoA-dependent succinic semialdehyde dehydrogenase, or glutamate:succinic semialdehyde transaminase. In silico metabolic designs were identified that resulted in the biosynthesis of 4-HB in both *E. coli* and yeast species from each of these metabolic pathways. The 1,4-butanediol intermediate γ-butyrolactone can be generated in culture by spontaneous cyclization under conditions at pH<7.5, particularly under acidic conditions, such as below pH 5.5, for example, pH<7, pH<6.5, pH<6, and particularly at pH<5.5 or lower.

[0025] Strains identified via the computational component of the platform can be put into actual production by genetically engineering any of the predicted metabolic alterations which lead to the biosynthetic production of 4-HB, 1,4-butanediol or other intermediate and/or downstream products. In yet a further embodiment, strains exhibiting biosynthetic production of these compounds can be further subjected to adaptive evolution to further augment product biosynthesis. The levels of product biosynthesis yield following adaptive evolution also can be predicted by the computational component of the system.

[0026] In other specific embodiments, microbial organisms were constructed to express a 4-HB biosynthetic pathway encoding the enzymatic steps from succinate to 4-HB and to 4-HB-CoA. Co-expression of succinate coenzyme A transferase, CoA-dependent succinic semialdehyde dehydrogenase, NAD-dependent 4-hydroxybutyrate dehydrogenase and 4-hydroxybutyrate coenzyme A transferase in a host microbial organism resulted in significant production of 4-HB compared to host microbial organisms lacking a 4-HB biosynthetic pathway. In a further specific embodiment, 4-HB-producing microbial organisms were generated that utilized α-ketoglutarate as a substrate by introducing nucleic acids encoding α-ketoglutarate decarboxylase and NAD-dependent 4-hydroxybutyrate dehydrogenase.

[0027] In another specific embodiment, microbial organisms containing a 1,4-butanediol (BDO) biosynthetic pathway were constructed that biosynthesized BDO when cultured in the presence of 4-HB. The BDO biosynthetic pathway consisted of a nucleic acid encoding either a multifunctional aldehyde/alcohol dehydrogenase or nucleic acids encoding an aldehyde dehydrogenawse and an alcohol dehydrogenase. To support growth on 4-HB substrates, these BDO-producing microbial organisms also expressed 4-hydroxybutyrate CoA transferase or 4-butyrate kinase in conjunction with phosphotranshydroxybutyrlase. In yet a further specific embodiment, microbial organisms were generated that synthesized BDO through exogenous expression of nucleic acids encoding a functional 4-HB biosynthetic pathway and a functional BDO biosynthetic pathway. The 4-HB biosynthetic pathway consisted of succinate coenzyme A transferase, CoA-dependent succinic semialdehyde dehydrogenase, NAD-dependent 4-hydroxybutyrate dehydrogenase and 4-hydroxybutyrate coenzyme A transferase. The BDO pathway consisted of a multifunctional aldehyde/alcohol dehydrogenase.

[0028] As used herein, the term "non-naturally occurring" when used in reference to a microbial organism or microorganism of the invention is intended to mean that the microbial organism has at least one genetic alteration not normally found in a naturally occurring strain of the referenced species, including wild-type strains of the referenced species. Genetic alterations include, for example, modifications introducing expressible nucleic acids encoding metabolic polypeptides, other nucleic acid additions, nucleic acid deletions and/or other functional disruption of the microbial genetic material. Such modification include, for example, coding regions and functional fragments thereof, for heterologous, homologous or both heterologous and homologous polypeptides for the referenced species. Additional modifications include, for example, non-coding regulatory regions in which the modifications alter expression of a gene or operon. Exemplary metabolic polypeptides include enzymes within a 4-HB biosynthetic pathway and enzymes within a biosynthetic pathway for a BDO family of compounds.

[0029] A metabolic modification refers to a biochemical reaction that is altered from its naturally occurring state. Therefore, non-naturally occurring microorganisms having genetic modifications to nucleic acids encoding metabolic polypeptides or, functional fragments thereof. Exemplary metabolic modifications are described further below for both *E. coli* and yeast microbial organisms.

[0030] As used herein, the term "isolated" when used in reference to a microbial organism is intended to mean an organism that is substantially free of at least one component as the referenced microbial organism is found in nature. The term includes a microbial organism that is removed from some or all components as it is found in its natural environment. The term also includes a microbial organism that is removed from some or all components as the microbial organism is found in non-naturally occurring environments. Therefore, an isolated microbial organism is partly or completely separated from other substances as it is found in nature or as it is grown, stored or subsisted in non-naturally occurring environments. Specific examples of isolated microbial organisms include partially pure microbes, substantially pure microbes and microbes cultured in a medium that is non-naturally occurring.

[0031] As used herein, the terms "microbial," "microbial organism" or "microorganism" is intended to mean any organism that exists as a microscopic cell that is included within the domains of archaea, bacteria or eukarya. Therefore, the term is intended to encompass prokaryotic or eukaryotic cells or organisms having a microscopic size and includes bacteria, archaea and eubacteria of all species as well as eukaryotic microorganisms such as yeast and fungi. The term also includes cell cultures of any species that can be cultured for the production of a biochemical.

[0032] As used herein, the term "4-hydroxybutanoic acid" is intended to mean a 4-hydroxy derivative of butyric acid having the chemical formula $C_4H_8O_3$ and a molecular mass of 104.11 g/mol (126.09 g/mol for its sodium salt). The chemical compound 4-hydroxybutanoic acid also is known in the art as 4-HB, 4-hydroxybutyrate, gamma-hydroxybutyric acid or GHB. The term as it is used herein is intended to include any of the compound's various salt forms and include, for example, 4-hydroxybutanoate and 4-hydroxybutyrate. Specific examples of salt forms for 4-HB include sodium 4-HB and potassium 4-HB. Therefore, the terms 4-hydroxybutanoic acid, 4-HB, 4-hydroxybutyrate, 4-hydroxybutanoate, gamma-hydroxybutyric acid and GHB as well as other art recognized names are used synonymously herein.

[0033] As used herein, the term "monomeric" when used in reference to 4-HB is intended to mean 4-HB in a non-polymeric or underivatized form. Specific examples of polymeric 4-HB include poly-4-hydroxybutanoic acid and copolymers of, for example, 4-HB and 3-HB. A specific example of a derivatized form of 4-HB is 4-HB-CoA. Other polymeric 4-HB forms and other derivatized forms of 4-HB also are known in the art.

[0034] As used herein, the term "γ-butyrolactone" is intended to mean a lactone having the chemical formula $C_4H_6O_2$ and a molecular mass of 86.089 g/mol. The chemical compound γ-butyrolactone also is know in the art as GBL, butyrolactone, 1,4-lactone, 4-butyrolactone, 4-hydroxybutyric acid lactone, and gamma-hydroxybutyric acid lactone. The term as it is used herein is intended to include any of the compound's various salt forms.

[0035] As used herein, the term "1,4-butanediol" is intended to mean an alcohol derivative of the alkane butane, carrying two hydroxyl groups which has the chemical formula $C_4H_{10}O_2$ and a molecular mass of 90.12 g/mol. The chemical compound 1,4-butanediol also is known in the art as BDO and is a chemical intermediate or precursor for a family of compounds referred to herein as BDO family of compounds, some of which are exemplified in FIG. **1**.

[0036] As used herein, the term "tetrahydrofuran" is intended to mean a heterocyclic organic compound corresponding to the fully hydrogenated analog of the aromatic compound furan which has the chemical formula C_4H_8O and a molecular mass of 72.11 g/mol. The chemical compound tetrahydrofuran also is known in the art as THF, tetrahydrofuran, 1,4-epoxybutane, butylene oxide, cyclotetramethylene oxide, oxacyclopentane, diethylene oxide, oxolane, furanidine, hydrofuran, tetra-methylene oxide. The term as it is used herein is intended to include any of the compound's various salt forms.

[0037] As used herein, the term "CoA" or "coenzyme A" is intended to mean an organic cofactor or prosthetic group (nonprotein portion of an enzyme) whose presence is required for the activity of many enzymes (the apoenzyme) to form an active enzyme system. Coenzyme A functions in certain condensing enzymes, acts in acetyl or other acyl group transfer and in fatty acid synthesis and oxidation, pyruvate oxidation and in other acetylation.

[0038] As used herein, the term "substantially anaerobic" when used in reference to a culture or growth condition is intended to mean that the amount of oxygen is less than about 10% of saturation for dissolved oxygen in liquid media. The term also is intended to include sealed chambers of liquid or solid medium maintained with an atmosphere of less than about 1% oxygen.

[0039] The non-naturally occurring microbal organisms of the invention can contain stable genetic alterations, which refers to microorganisms that can be cultured for greater than five generations without loss of the alteration. Generally, stable genetic alterations include modifications that persist greater than 10 generations, particularly stable modifications will persist more than about 25 generations, and more particularly, stable genetic modifications will be greater than 50 generations, including indefinitely.

[0040] Those skilled in the art will understand that the genetic alterations, including metabolic modifications exemplified herein are described with reference to *E. coli* and yeast genes and their corresponding metabolic reactions. However, given the complete genome sequencing of a wide variety of organisms and the high level of skill in the area of genomics, those skilled in the art will readily be able to apply the teachings and guidance provided herein to essentially all other organisms. For example, the *E. coli* metabolic alterations exemplified herein can readily be applied to other species by incorporating the same or analogous encoding nucleic acid from species other than the referenced species. Such genetic alterations include, for example, genetic alterations of species homologs, in general, and in particular, orthologs, paralogs or nonorthologous gene displacements.

[0041] An ortholog is a gene or genes that are related by vertical descent and are responsible for substantially the same or identical functions in different organisms. For example, mouse epoxide hydrolase and human epoxide hydrolase can be considered orthologs for the biological function of hydrolysis of epoxides. Genes are related by vertical descent when, for example, they share sequence similarity of sufficient amount to indicate they are homologous, or related by evolution from a common ancestor. Genes can also be considered orthologs if they share three-dimensional structure but not necessarily sequence similarity, of a sufficient amount to indicate that they have evolved from a common ancestor to the extent that the primary sequence similarity is not identifiable. Genes that are orthologous can encode proteins with sequence similarity of about 25% to 100% amino acid sequence identity. Genes encoding proteins sharing an amino acid similarity less that 25% can also be considered to have arisen by vertical descent if their three-dimensional structure also shows similarities. Members of the serine protease family of enzymes, including tissue plasminogen activator and elastase, are considered to have arisen by vertical descent from a common ancestor.

[0042] Orthologs include genes or their encoded gene products that through, for example, evolution, have diverged in structure or overall activity. For example, where one species encodes a gene product exhibiting two functions and where such functions have been separated into distinct genes in a second species, the three genes and their corresponding products are considered to be orthologs. For the growth-coupled production of a biochemical product, those skilled in the art will understand that the orthologous gene harboring the metabolic activity to be disrupted is to be chosen for construction of the non-naturally occurring microorganism. An example of orthologs exhibiting separable activities is where distinct activities have been separated into distinct gene products between two or more species or within a single species. A specific example is the separation of elastase proteolysis and plasminogen proteolysis, two types of serine protease activity, into distinct molecules as plasminogen activator and elastase. A second example is the separation of mycoplasma 5'-3' exonuclease and *Drosophila* DNA polymerase III activity. The DNA polymerase from the first species can be considered an ortholog to either or both of the exonuclease or the polymerase from the second species and vice versa.

[0043] In contrast, paralogs are homologs related by, for example, duplication followed by evolutionary divergence and have similar or common, but not identical functions. Paralogs can originate or derive from, for example, the same species or from a different species. For example, microsomal epoxide hydrolase (epoxide hydrolase I) and soluble epoxide hydrolase (epoxide hydrolase II) can be considered paralogs because they represent two distinct enzymes, co-evolved from a common ancestor, that catalyze distinct reactions and have distinct functions in the same species. Paralogs are proteins from the same species with significant sequence similarity to each other suggesting that they are homologous, or related through co-evolution from a common ancestor. Groups of paralogous protein families include HipA homologs, luciferase genes, peptidases, and others.

[0044] A nonorthologous gene displacement is a nonorthologous gene from one species that can substitute for a referenced gene function in a different species. Substitution includes, for example, being able to perform substantially the same or a similar function in the species of origin compared to the referenced function in the different species. Although generally, a nonorthologous gene displacement will be identifiable as structurally related to a known gene encoding the referenced function, less structurally related but functionally similar genes and their corresponding gene products nevertheless will still fall within the meaning of the term as it is used herein. Functional similarity requires, for example, at least some structural similarity in the active site or binding region of a nonorthologous gene compared to a gene encoding the function sought to be substituted. Therefore, a nonorthologous gene includes, for example, a paralog or an unrelated gene.

[0045] Therefore, in identifying and constructing the non-naturally occurring microbial organisms of the invention having 4-HB, GBL and/or BDO biosynthetic capability, those skilled in the art will understand with applying the teaching and guidance provided herein to a particular species that the identification of metabolic modifications can include identification and inclusion or inactivation of orthologs. To the extent that paralogs and/or nonorthologous gene displacements are present in the referenced microorganism that encode an enzyme catalyzing a similar or substantially similar metabolic reaction, those skilled in the art also can utilize these evolutionally related genes.

[0046] Orthologs, paralogs and nonorthologous gene displacements can be determined by methods well known to those skilled in the art. For example, inspection of nucleic acid or amino acid sequences for two polypeptides will reveal sequence identity and similarities between the compared sequences. Based on such similarities, one skilled in the art can determine if the similarity is sufficiently high to indicate the proteins are related through evolution from a common ancestor. Algorithms well known to those skilled in the art, such as Align, BLAST, Clustal W and others compare and determine a raw sequence similarity or identity, and also determine the presence or significance of gaps in the sequence which can be assigned a weight or score. Such algorithms also are known in the art and are similarly applicable for determining nucleotide sequence similarity or identity. Parameters for sufficient similarity to determine relatedness are computed based on well known methods for calculating statistical similarity, or the chance of finding a similar match in a random polypeptide, and the significance of the match determined. A computer comparison of two or more sequences can, if desired, also be optimized visually by those skilled in the art. Related gene products or proteins can be expected to have a high similarity, for example, 25% to 100% sequence identity. Proteins that are unrelated can have an identity which is essentially the same as would be expected to occur by chance, if a database of sufficient size is scanned (about 5%). Sequences between 5% and 24% may or may not represent sufficient homology to conclude that the compared sequences are related. Additional statistical analysis to determine the significance of such matches given the size of the data set can be carried out to determine the relevance of these sequences.

[0047] Exemplary parameters for determining relatedness of two or more sequences using the BLAST algorithm, for example, can be as set forth below. Briefly, amino acid sequence alignments can be performed using BLASTP version 2.0.8 (Jan. 5, 1999) and the following parameters: Matrix: 0 BLOSUM62; gap open: 11; gap extension: 1; x_dropoff: 50; expect: 10.0; wordsize: 3; filter: on. Nucleic acid sequence alignments can be performed using BLASTN version 2.0.6 (Sep. 16, 1998) and the following parameters: Match: 1; mismatch: −2; gap open: 5; gap extension: 2; x_dropoff: 50; expect: 10.0; wordsize: 11; filter: off. Those skilled in the art will know what modifications can be made to the above parameters to either increase or decrease the stringency of the comparison, for example, and determine the relatedness of two or more sequences.

[0048] The invention provides a non-naturally occurring microbial biocatalyst including a microbial organism having a 4-hydroxybutanoic acid (4-HB) biosynthetic pathway that includes at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, CoA-independent succinic semialdehyde dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, glutamate:succinic semialdehyde transaminase, alpha-ketoglutarate decarboxylase, or glutamate decarboxylase, wherein the exogenous nucleic acid is expressed in sufficient amounts to produce monomeric 4-hydroxybutanoic acid (4-HB). 4-hydroxybutanoate dehydrogenase is also referred to as 4-hydroxybutyrate dehydrogenase or 4-HB dehydrogenase. Succinyl-CoA synthetase is also referred to as succinyl-CoA synthase or succinyl-CoA ligase.

[0049] Also provided is a non-naturally occurring microbial biocatalyst including a microbial organism having a 4-hydroxybutanoic acid (4-HB) biosynthetic pathway having at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, or α-ketoglutarate decarboxylase, wherein the exogenous nucleic acid is expressed in sufficient amounts to produce monomeric 4-hydroxybutanoic acid (4-HB).

[0050] The non-naturally occurring microbial biocatalysts of the invention include microbial organisms that employ combinations of metabolic reactions for biosynthetically producing the compounds of the invention. The biosynthesized compounds can be produced intracellularly and/or secreted into the culture medium. Exemplary compounds produced by the non-naturally occurring microorganisms include, for example, 4-hydroxybutanoic acid, 1,4-butanediol and γ-butyrolactone. The relationships of these exemplary compounds with respect to chemical synthesis or biosynthesis are exemplified in FIG. **1**.

[0051] In one embodiment, a non-naturally occurring microbial organism is engineered to produce 4-HB. This compound is one useful entry point into the 1,4-butanediol family of compounds. The biochemical reactions for formation of 4-HB from succinate, from succinate through succinyl-CoA or from α-ketoglutarate are shown in steps **1-8** of FIG. **2**.

[0052] The invention is described herein with general reference to the metabolic reaction, reactant or product thereof, or with specific reference to one or more nucleic acids or genes encoding an enzyme associated with or catalyzing the referenced metabolic reaction, reactant or product. Unless otherwise expressly stated herein, those skilled in the art will understand that reference to a reaction also constitutes reference to the reactants and products of the reaction. Similarly, unless otherwise expressly stated herein, reference to a reactant or product also references the reaction and that reference to any of these metabolic constitutes also references the gene or genes encoding the enzymes that catalyze the referenced reaction, reactant or product. Likewise, given the well known fields of metabolic biochemistry, enzymology and genomics, reference herein to a gene or encoding nucleic acid also constitutes a reference to the corresponding encoded enzyme and the reaction it catalyzes as well as the reactants and products of the reaction.

[0053] The production of 4-HB via biosynthetic modes using the microbial organisms of the invention is particularly useful because it can produce monomeric 4-HB. The non-naturally occurring microbial organisms of the invention and their biosynthesis of 4-HB and BDO family compounds also is particularly useful because the 4-HB product is (1) secreted; (2) can be devoid of any derivatizations such as Coenzyme A; (3) avoids thermodynamic changes during biosynthesis; (4) allows direct biosynthesis of BDO, and (5) allows for the spontaneous chemical conversion of 4-HB to γ-butyrolactone (GBL) in acidic pH medium. This latter characteristic also is particularly useful for efficient chemical synthesis or biosynthesis of BDO family compounds such as 1,4-butanediol and/or tetrahydrofuran (THF), for example.

[0054] Microbial organisms generally lack the capacity to synthesize 4-HB and therefore, any of the compounds shown in FIG. **1** are known to be within the 1,4-butanediol family of compounds or known by those in the art to be within the 1,4-butanediol family of compounds. Moreover, organisms having all of the requisite metabolic enzymatic capabilities are not known to produce 4-HB from the enzymes described and biochemical pathways exemplified herein. Rather, with the possible exception of a few anaerobic microorganisms described further below, the microorganisms having the enzymatic capability use 4-HB as a substrate to produce, for example, succinate. In contrast, the non-naturally occurring microbial organisms of the invention generate 4-HB as a product. As described above, the biosynthesis of 4-HB in its monomeric form is not only particularly useful in chemical synthesis of BDO family of compounds, it also allows for the further biosynthesis of BDO family compounds and avoids altogether chemical synthesis procedures.

[0055] The non-naturally occurring microbial organisms of the invention that can produce 4-HB are produced by ensuring that a host microbial organism includes functional capabilities for the complete biochemical synthesis of at least one 4-HB biosynthetic pathway of the invention. Ensuring at least one requisite 4-HB biosynthetic pathway confers 4-HB biosynthesis capability onto the host microbial organism.

[0056] Five requisite 4-HB biosynthetic pathways are exemplified herein and shown for purposes of illustration in FIG. **2**. One requisite 4-HB biosynthetic pathway includes the biosynthesis of 4-HB from succinate (the succinate pathway). The enzymes participating in this 4-HB pathway include CoA-independent succinic semialdehyde dehydrogenase and 4-hydroxybutanoate dehydrogenase. In this pathway, CoA-independent succinic semialdehyde dehydrogenase catalyzes the reverse reaction to the arrow shown in FIG. **2**. Another requisite 4-HB biosynthetic pathway includes the biosynthesis from succinate through succinyl-CoA (the succinyl-CoA pathway). The enzymes participating in this 4-HB pathway include succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase and 4-hydroxybutanoate dehydrogenase. Three other requisite 4-HB biosynthetic pathways include the biosynthesis of 4-HB from α-ketoglutarate (the α-ketoglutarate pathways). Hence, a third requisite 4-HB biosynthetic pathway is the biosynthesis of succinic semialdehyde through glutamate:succinic semialdehyde transaminase, glutamate decarboxylase and 4-hydroxybutanoate dehydrogenase. A fourth requisite 4-HB biosynthetic pathway also includes the biosynthesis of 4-HB from α-ketoglutarate, but utilizes α-ketoglutarate decarboxylase to catalyze succinic semialdehyde synthesis. 4-hydroxybutanoate dehydrogenase catalyzes the conversion of succinic semialdehyde to 4-HB. A fifth requisite 4-HB biosynthetic pathway includes the biosynthesis from α-ketoglutarate through succinyl-CoA and utilizes α-ketoglutarate dehydrogenase to produce succinyl-CoA, which funnels into the succinyl-CoA pathway described above. Each of these 4-HB biosynthetic pathways, their substrates, reactants and products are described further below in the Examples.

[0057] The non-naturally occurring microbial organisms of the invention can be produced by introducing expressible nucleic acids encoding one or more of the enzymes participating in one or more 4-HB biosynthetic pathways. Depending on the host microbial organism chosen for biosynthesis, nucleic acids for some or all of a particular 4-HB biosynthetic pathway can be expressed. For example, if a chosen host is deficient in both enzymes in the succinate to 4-HB pathway and this pathway is selected for 4-HB biosynthesis, then expressible nucleic acids for both CoA-independent succinic semialdehyde dehydrogenase and 4-hydroxybutanoate dehydrogenase are introduced into the host for subsequent exogenous expression. Alternatively, if the chosen host exhibits endogenous CoA-independent succinic semialdehyde dehydrogenase, but is deficient in 4-hydroxybutanoate dehydrogenase then an encoding nucleic acid is needed for this enzyme to achieve 4-HB biosynthesis.

[0058] In like fashion, where 4-HB biosynthesis is selected to occur through the succinate to succinyl-CoA pathway (the succinyl-CoA pathway), encoding nucleic acids for host deficiencies in the enzymes succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase and/or 4-hydroxybutanoate dehydrogenase are to be exogenously expressed in the recipient host. Selection of 4-HB biosynthesis through the α-ketoglutarate to succinic semialdehyde pathway (the α-ketoglutarate pathway) can utilize exogenous expression for host deficiencies in one or more of the enzymes for glutamate:succinic semialdehyde transaminase, glutamate decarboxylase and/or 4-hydroxybutanoate dehydrogenase, or α-ketoglutarate decarboxylase and 4-hydroxybutanoate dehydrogenase.

[0059] Depending on the 4-HB biosynthetic pathway constituents of a selected host microbial organism, the non-naturally occurring microbial 4-HB biocatalysts of the invention will include at least one exogenously expressed 4-HB pathway-encoding nucleic acid and up to all encoding nucleic acids for one or more 4-HB biosynthetic pathways. For example, 4-HB biosynthesis can be established from all five pathways in a host deficient in 4-hydroxybutanoate dehydrogenase through exogenous expression of a 4-hydroxybutanoate dehydrogenase encoding nucleic acid. In contrast, 4-HB biosynthesis can be established from all five pathways in a host deficient in all eight enzymes through exogenous expression of all eight of CoA-independent succinic semialdehyde dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, glutamate: succinic semialdehyde transaminase, glutamate decarboxylase, α-ketoglutarate decarboxylase, α-ketoglutarate dehydrogenase and 4-hydroxybutanoate dehydrogenase.

[0060] Given the teachings and guidance provided herein, those skilled in the art will understand that the number of encoding nucleic acids to introduce in an expressible form will, at least, parallel the 4-HB pathway deficiencies of the selected host microbial organism. Therefore, a non-naturally occurring microbial organism of the invention can have one, two, three, four, five, six, seven or eight nucleic acids encoding the above enzymes constituting one or more 4-HB biosynthetic pathways. In some embodiments, the non-naturally occurring microbial organisms also can include other genetic modifications that facilitate or optimize 4-HB biosynthesis or that confer other useful functions onto the host microbial organism. One such other functionality can include, for example, augmentation of the synthesis of one or more of the 4-HB pathway precursors such as succinate, succinyl-CoA and/or α-ketoglutarate.

[0061] In some embodiments, a non-naturally occurring microbial organism of the invention is generated from a host that contains the enzymatic capability to synthesize 4-HB. In this specific embodiment it can be useful to increase the synthesis or accumulation of a 4-HB pathway product to, for example, drive 4-HB pathway reactions toward 4-HB production. Increased synthesis or accumulation can be accomplished by, for example, overexpression of nucleic acids encoding one or more of the above-described 4-HB pathway enzymes. Over expression of the 4-HB pathway enzyme or enzymes can occur, for example, through exogenous expression of the endogenous gene or genes, or through exogenous expression of the heterologous gene or genes. Therefore, naturally occurring organisms can be readily generated to be non-naturally 4-HB producing microbial organisms of the invention through overexpression of one, two, three, four, five or all six nucleic acids encoding 4-HB biosynthetic pathway enzymes. In addition, a non-naturally occurring organism can be generated by mutagenesis of an endogenous gene that results in an increase in activity of an enzyme in the 4-HB biosynthetic pathway.

[0062] In particularly useful embodiments, exogenous expression of the encoding nucleic acids is employed. Exogenous expression confers the ability to custom tailor the expression and/or regulatory elements to the host and application to achieve a desired expression level that is controlled by the user. However, endogenous expression also can be utilized in other embodiments such as by removing a negative regulatory effector or induction of the gene's promoter when linked to an inducible promoter or other regulatory element. Thus, an endogenous gene having a naturally occurring inducible promoter can be up-regulated by providing the appropriate inducing agent, or the regulatory region of an endogenous gene can be engineered to incorporate an inducible regulatory element, thereby allowing the regulation of increased expression of an endogenous gene at a desired time. Similarly, an inducible promoter can be included as a regulatory element for an exogenous gene introduced into a non-naturally occurring microbial organism (see Examples II and IV, for example).

[0063] "Exogenous" as it is used herein is intended to mean that the referenced molecule or the referenced activity is introduced into the host microbial organism including, for example, introduction of an encoding nucleic acid into the host genetic material such as by integration into a host chromosome. Therefore, the term as it is used in reference to expression of an encoding nucleic acid refers to introduction of the encoding nucleic acid in an expressible form into the microbial organism. When used in reference to a biosynthetic activity, the term refers to an activity that is introduced into the host reference organism. The source can be, for example, a homologous or heterologous encoding nucleic acid that expresses the referenced activity following introduction into the host microbial organism. Therefore, the term "endogenous" refers to a referenced molecule or activity that is present in the host. Similarly, the term when used in reference to expression of an encoding nucleic acid refers to expression of an encoding nucleic acid contained within the microbial organism. The term "heterologous" refers to a molecule or activity derived from a source other than the referenced species whereas "homologous" refers to a molecule or activity derived from the host microbial organism. Accordingly, exogenous expression of an encoding nucleic acid of the invention can utilize either or both a heterologous or homologous encoding nucleic acid.

[0064] Sources of encoding nucleic acids for a 4-HB pathway enzyme can include, for example, any species where the encoded gene product is capable of catalyzing the referenced reaction. Such species include both prokaryotic and eukaryotic organisms including, but not limited to, bacteria, including archaea and eubacteria, and eukaryotes, including yeast, plant, insect, animal, and mammal, including human. Exemplary species for such sources include, for example, *E. coli, Saccharomyces cerevisiae, Clostridium kluyveri,*

Clostridium acetobutylicum, Clostridium beijerinckii, Clostridium saccharoperbutylacetonicum, Clostridium perfringens, Clostridium difficile, Ralstonia eutropha, Mycobacterium bovis, Mycobacterium tuberculosis and *Porphyromonas gingivalis*. For example, the microbial organisms having 4-HB biosynthetic production are exemplified herein with reference to *E. coli* and yeast hosts. However, with the complete genome sequence available for now more than 550 species (with more than half of these available on public databases such as the NCBI), including 395 microorganism genomes and a variety of yeast, fungi, plant, and mammalian genomes, the identification of genes encoding the requisite 4-HB biosynthetic activity for one or more genes in related or distant species, including for example, homologues, orthologs, paralogs and nonorthologous gene displacements of known genes, and the interchange of genetic alterations between organisms is routine and well known in the art. Accordingly, the metabolic alterations enabling biosynthesis of 4-HB and other compounds of the invention described herein with reference to a particular organism such as *E. coli* or yeast can be readily applied to other microorganisms, including prokaryotic and eukaryotic organisms alike. Given the teachings and guidance provided herein, those skilled in the art will know that a metabolic alteration exemplified in one organism can be applied equally to other organisms.

[0065] In some instances, such as when an alternative 4-HB biosynthetic pathway exists in an unrelated species, 4-HB biosynthesis can be conferred onto the host species by, for example, exogenous expression of a paralog or paralogs from the unrelated species that catalyzes a similar, yet non-identical metabolic reaction to replace the referenced reaction. Because certain differences among metabolic networks exist between different organisms, those skilled in the art will understand that the actual genes usage between different organisms may differ. However, given the teachings and guidance provided herein, those skilled in the art also will understand that the teachings and methods of the invention can be applied to all microbial organisms using the cognate metabolic alterations to those exemplified herein to construct a microbial organism in a species of interest that will synthesize monomeric 4-HB.

[0066] Host microbial organisms can be selected from, and the non-naturally occurring microbial organisms generated in, for example, bacteria, yeast, fungus or any of a variety of other microorganisms applicable to fermentation processes. Exemplary bacteria include species selected from *E. coli, Klebsiella oxytoca, Anaerobiospirillum succiniciproducens, Actinobacillus succinogenes, Mannheimia succiniciproducens, Rhizobium etli, Bacillus subtilis, Corynebacterium glutamicum, Gluconobacter oxydans, Zymomonas mobilis, Lactococcus lactis, Lactobacillus plantarum, Streptomyces coelicolor, Clostridium acetobutylicum, Pseudomonas fluorescens*, and *Pseudomonas putida*. Exemplary yeasts or fungi include species selected from *Saccharomyces cerevisiae, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces marxianus, Aspergillus terreus, Aspergillus niger* and *Pichia pastoris*.

[0067] Methods for constructing and testing the expression levels of a non-naturally occurring 4-HB-producing host can be performed, for example, by recombinant and detection methods well known in the art. Such methods can be found described in, for example, Sambrook et al., *Molecular Cloning: A Laboratory Manual, Third Ed.*, Cold Spring Harbor Laboratory, New York (2001); Ausubel et al., *Current Proto-*

cols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1999). 4-HB and GBL can be separated by, for example, HPLC using a Spherisorb 5 ODS1 column and a mobile phase of 70% 10 mM phosphate buffer (pH=7) and 30% methanol, and detected using a UV detector at 215 nm (Hennessy et al. 2004, J. Forensic Sci. 46(6):1-9). BDO is detected by gas chromatography or by HPLC and refractive index detector using an Aminex HPX-87H column and a mobile phase of 0.5 mM sulfuric acid (Gonzalez-Pajuelo et al., *Met. Eng.* 7:329-336 (2005)).

[0068] For example, an expression vector or vectors can be constructed to harbor one or more 4-HB biosynthetic pathway and/or one or more BDO biosynthetic encoding nucleic acids as exemplified herein operably linked to expression control sequences functional in the host organism. Expression vectors applicable for use in the microbial host organisms of the invention include, for example, plasmids, phage vectors, viral vectors, episomes and artificial chromosomes, including vectors and selection sequences or markers operable for stable integration into a host chromosome. Selectable marker genes also can be included that, for example, provide resistance to antibiotics or toxins, complement auxotrophic deficiencies, or supply critical nutrients not in the culture media. Expression control sequences can include constitutive and inducible promoters, transcription enhancers, transcription terminators, and the like which are well known in the art. When two or more exogenous encoding nucleic acids are to be co-expressed, both nucleic acids can be inserted, for example, into a single expression vector or in separate expression vectors. For single vector expression, the encoding nucleic acids can be operationally linked to one common expression control sequence or linked to different expression control sequences, such as one inducible promoter and one constitutive promoter. The transformation of exogenous nucleic acid sequences involved in a metabolic or synthetic pathway can be confirmed using methods well known in the art.

[0069] The non-naturally occurring microbial organisms of the invention are constructed using methods well known in the art as exemplified above to exogenously express at least one nucleic acid encoding a 4-HB pathway enzyme in sufficient amounts to produce monomeric 4-HB. Exemplary levels of expression for 4-HB enzymes in each pathway are described further below in the Examples. Following the teachings and guidance provided herein, the non-naturally occurring microbial organisms of the invention can achieve biosynthesis of monomeric 4-HB resulting in intracellular concentrations between about 0.1-25 mM or more. Generally, the intracellular concentration of monomeric 4-HB is between about 3-20 mM, particularly between about 5-15 mM and more particularly between about 8-12 mM, including about 10 mM or more. Intracellular concentrations between and above each of these exemplary ranges also can be achieved from the non-naturally occurring microbial organisms of the invention.

[0070] As described further below, one exemplary growth condition for achieving biosynthesis of 4-HB includes anaerobic culture or fermentation conditions. In certain embodiments, the non-naturally occurring microbial organisms of the invention can be sustained, cultured or fermented under anaerobic or substantially anaerobic conditions. Briefly, anaerobic conditions refers to an environment devoid of oxygen. Substantially anaerobic conditions include, for example, a culture, batch fermentation or continuous fermentation such that the dissolved oxygen concentration in the medium remains between 0 and 10% of saturation. Substantially anaerobic conditions also includes growing or resting cells in liquid medium or on solid agar inside a sealed chamber maintained with an atmosphere of less than 1% oxygen. The percent of oxygen can be maintained by, for example, sparging the culture with an N_2/CO_2 mixture or other suitable non-oxygen gas or gases.

[0071] The invention also provides a non-naturally occurring microbial biocatalyst including a microbial organism having 4-hydroxybutanoic acid (4-HB) and 1,4-butanediol (BDO) biosynthetic pathways that include at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, CoA-independent succinic semialdehyde dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, 4-hydroxybutyrate:CoA transferase, glutamate:succinic semialdehyde transaminase, glutamate decarboxylase, CoA-independent aldehyde dehydrogenase, CoA-dependent aldehyde dehydrogenase or alcohol dehydrogenase, wherein the exogenous nucleic acid is expressed in sufficient amounts to produce 1,4-butanediol (BDO). 4-Hydroxybutyrate:CoA transferase also is known as 4-hydroxybutyryl CoA:acetyl-CoA transferase.

[0072] The invention further provides non-naturally occurring microbial biocatalyst including a microbial organism having 4-hydroxybutanoic acid (4-HB) and 1,4-butanediol (BDO) biosynthetic pathways, the pathways include at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, 4-hydroxybutyrate:CoA transferase, 4-butyrate kinase, phosphotransbutyrylase, α-ketoglutarate decarboxylase, aldehyde dehydrogenase, alcohol dehydrogenase or an aldehyde/alcohol dehydrogenase, wherein the exogenous nucleic acid is expressed in sufficient amounts to produce 1,4-butanediol (BDO).

[0073] Non-naturally occurring microbial organisms also can be generated which biosynthesize BDO. As with the 4-HB producing microbial organisms of the invention, the BDO producing microbial organisms also can produce intracellularly or secret the BDO into the culture medium. Following the teachings and guidance provided previously for the construction of microbial organisms that synthesize 4-HB, additional BDO pathways can be incorporated into the 4-HB producing microbial organisms to generate organisms that also synthesize BDO and other BDO family compounds. The chemical synthesis of BDO and its downstream products are illustrated in FIG. **1**. The non-naturally occurring microbial organisms of the invention capable of BDO biosynthesis circumvent these chemical synthesis using 4-HB as an entry point as illustrated in FIG. **2**. As described further below, the 4-HB producers also can be used to chemically convert 4-HB to GBL and then to BDO or THF, for example. Alternatively, the 4-HB producers can be further modified to include biosynthetic capabilities for conversion of 4-HB and/or GBL to BDO.

[0074] The additional BDO pathways to introduce into 4-HB producers include, for example, the exogenous expression in a host deficient background or the overexpression of one or more of the enzymes exemplified in FIG. **2** as steps **9-13**. One such pathway includes, for example, the enzyme activities necessary to carryout the reactions shown as steps **9**, **12** and **13** in FIG. **2**, where the aldehyde and alcohol dehydrogenases can be separate enzymes or a multifunctional enzyme having both aldehyde and alcohol dehydrogenase activity. Another such pathway includes, for example, the enzyme activities necessary to carryout the reactions shown as steps **10**, **11**, **12** and **13** in FIG. **2**, also where the aldehyde and alcohol dehydrogenases can be separate enzymes or a multifunctional enzyme having both aldehyde and alcohol dehydrogenase activity. Accordingly, the additional BDO pathways to introduce into 4-HB producers include, for example, the exogenous expression in a host deficient background or the overexpression of one or more of a 4-hydroxybutyrate:CoA transferase, butyrate kinase, phosphotransbutyrylase, CoA-independent aldehyde dehydrogenase, CoA-dependent aldehyde dehydrogenase or an alcohol dehydrogenase. In the absence of endogenous acyl-CoA synthetase capable of modifying 4-HB, the non-naturally occurring BDO producing microbial organisms can further include an exogenous acyl-CoA synthetase selective for 4-HB, or the combination of multiple enzymes that have as a net reaction conversion of 4-HB into 4-HB-CoA. As exemplified further below in the Examples, butyrate kinase and phosphotransbutyrylase exhibit BDO pathway activity and catalyze the conversions illustrated in FIG. **2** with a 4-HB substrate. Therefore, these enzymes also can be referred to herein as 4-hydroxybutyrate kinase and phosphotranshydroxybutyrylase respectively.

[0075] Exemplary alcohol and aldehyde dehydrogenases that can be used for these in vivo conversions from 4-HB to BDO are listed below in Table 1.

TABLE 1

Alcohol and Aldehyde Dehydrogenases for Conversion of 4-HB to BDO.

	ALCOHOL DEHYDROGENASES
ec: 1.1.1.1	alcohol dehydrogenase
ec: 1.1.1.2	alcohol dehydrogenase (NADP+)
ec: 1.1.1.4	(R,R)-butanediol dehydrogenase
ec: 1.1.1.5	acetoin dehydrogenase
ec: 1.1.1.6	glycerol dehydrogenase
ec: 1.1.1.7	propanediol-phosphate dehydrogenase
ec: 1.1.1.8	glycerol-3-phosphate dehydrogenase (NAD+)
ec: 1.1.1.11	D-arabinitol 4-dehydrogenase
ec: 1.1.1.12	L-arabinitol 4-dehydrogenase
ec: 1.1.1.13	L-arabinitol 2-dehydrogenase
ec: 1.1.1.14	L-iditol 2-dehydrogenase
ec: 1.1.1.15	D-iditol 2-dehydrogenase
ec: 1.1.1.16	galactitol 2-dehydrogenase
ec: 1.1.1.17	mannitol-1-phosphate 5-dehydrogenase
ec: 1.1.1.18	inositol 2-dehydrogenase
ec: 1.1.1.21	aldehyde reductase
ec: 1.1.1.23	histidinol dehydrogenase
ec: 1.1.1.26	glyoxylate reductase
ec: 1.1.1.27	L-lactate dehydrogenase
ec: 1.1.1.28	D-lactate dehydrogenase
ec: 1.1.1.29	glycerate dehydrogenase
ec: 1.1.1.30	3-hydroxybutyrate dehydrogenase
ec: 1.1.1.31	3-hydroxyisobutyrate dehydrogenase
ec: 1.1.1.35	3-hydroxyacyl-CoA dehydrogenase
ec: 1.1.1.36	acetoacetyl-CoA reductase
ec: 1.1.1.37	malate dehydrogenase
ec: 1.1.1.38	malate dehydrogenase (oxaloacetate-decarboxylating)
ec: 1.1.1.39	malate dehydrogenase (decarboxylating)
ec: 1.1.1.40	malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+)
ec: 1.1.1.41	isocitrate dehydrogenase (NAD+)
ec: 1.1.1.42	isocitrate dehydrogenase (NADP+)
ec: 1.1.1.54	allyl-alcohol dehydrogenase
ec: 1.1.1.55	lactaldehyde reductase (NADPH)
ec: 1.1.1.56	ribitol 2-dehydrogenase
ec: 1.1.1.59	3-hydroxypropionate dehydrogenase
ec: 1.1.1.60	2-hydroxy-3-oxopropionate reductase
ec: 1.1.1.61	4-hydroxybutyrate dehydrogenase
ec: 1.1.1.66	omega-hydroxydecanoate dehydrogenase
ec: 1.1.1.67	mannitol 2-dehydrogenase
ec: 1.1.1.71	alcohol dehydrogenase [NAD(P)+]
ec: 1.1.1.72	glycerol dehydrogenase (NADP+)
ec: 1.1.1.73	octanol dehydrogenase
ec: 1.1.1.75	(R)-aminopropanol dehydrogenase
ec: 1.1.1.76	(S,S)-butanediol dehydrogenase
ec: 1.1.1.77	lactaldehyde reductase
ec: 1.1.1.78	methylglyoxal reductase (NADH-dependent)
ec: 1.1.1.79	glyoxylate reductase (NADP+)
ec: 1.1.1.80	isopropanol dehydrogenase (NADP+)
ec: 1.1.1.81	hydroxypyruvate reductase
ec: 1.1.1.82	malate dehydrogenase (NADP+)
ec: 1.1.1.83	D-malate dehydrogenase (decarboxylating)
ec: 1.1.1.84	dimethylmalate dehydrogenase
ec: 1.1.1.85	3-isopropylmalate dehydrogenase
ec: 1.1.1.86	ketol-acid reductoisomerase
ec: 1.1.1.87	homoisocitrate dehydrogenase
ec: 1.1.1.88	hydroxymethylglutaryl-CoA reductase
ec: 1.1.1.90	aryl-alcohol dehydrogenase
ec: 1.1.1.91	aryl-alcohol dehydrogenase (NADP+)
ec: 1.1.1.92	oxaloglycolate reductase (decarboxylating)
ec: 1.1.1.94	glycerol-3-phosphate dehydrogenase [NAD(P)+]
ec: 1.1.1.95	phosphoglycerate dehydrogenase
ec: 1.1.1.97	3-hydroxybenzyl-alcohol dehydrogenase
ec: 1.1.1.101	acylglycerone-phosphate reductase
ec: 1.1.1.103	L-threonine 3-dehydrogenase
ec: 1.1.1.104	4-oxoproline reductase
ec: 1.1.1.105	retinol dehydrogenase
ec: 1.1.1.110	indolelactate dehydrogenase
ec: 1.1.1.112	indanol dehydrogenase
ec: 1.1.1.113	L-xylose 1-dehydrogenase
ec: 1.1.1.129	L-threonate 3-dehydrogenase
ec: 1.1.1.137	ribitol-5-phosphate 2-dehydrogenase
ec: 1.1.1.138	mannitol 2-dehydrogenase (NADP+)
ec: 1.1.1.140	sorbitol-6-phosphate 2-dehydrogenase
ec: 1.1.1.142	D-pinitol dehydrogenase
ec: 1.1.1.143	sequoyitol dehydrogenase
ec: 1.1.1.144	perillyl-alcohol dehydrogenase
ec: 1.1.1.156	glycerol 2-dehydrogenase (NADP+)
ec: 1.1.1.157	3-hydroxybutyryl-CoA dehydrogenase
ec: 1.1.1.163	cyclopentanol dehydrogenase
ec: 1.1.1.164	hexadecanol dehydrogenase
ec: 1.1.1.165	2-alkyn-1-ol dehydrogenase
ec: 1.1.1.166	hydroxycyclohexanecarboxylate dehydrogenase
ec: 1.1.1.167	hydroxymalonate dehydrogenase
ec: 1.1.1.174	cyclohexane-1,2-diol dehydrogenase
ec: 1.1.1.177	glycerol-3-phosphate 1-dehydrogenase (NADP+)

TABLE 1-continued

Alcohol and Aldehyde Dehydrogenases for Conversion of 4-HB to BDO.	
ec: 1.1.1.178	3-hydroxy-2-methylbutyryl-CoA dehydrogenase
ec: 1.1.1.185	L-glycol dehydrogenase
ec: 1.1.1.190	indole-3-acetaldehyde reductase (NADH)
ec: 1.1.1.191	indole-3-acetaldehyde reductase (NADPH)
ec: 1.1.1.192	long-chain-alcohol dehydrogenase
ec: 1.1.1.194	coniferyl-alcohol dehydrogenase
ec: 1.1.1.195	cinnamyl-alcohol dehydrogenase
ec: 1.1.1.198	(+)-borneol dehydrogenase
ec: 1.1.1.202	1,3-propanediol dehydrogenase
ec: 1.1.1.207	(−)-menthol dehydrogenase
ec: 1.1.1.208	(+)-neomenthol dehydrogenase
ec: 1.1.1.216	farnesol dehydrogenase
ec: 1.1.1.217	benzyl-2-methyl-hydroxybutyrate dehydrogenase
ec: 1.1.1.222	(R)-4-hydroxyphenyllactate dehydrogenase
ec: 1.1.1.223	isopiperitenol dehydrogenase
ec: 1.1.1.226	4-hydroxycyclohexanecarboxylate dehydrogenase
ec: 1.1.1.229	diethyl 2-methyl-3-oxosuccinate reductase
ec: 1.1.1.237	hydroxyphenylpyruvate reductase
ec: 1.1.1.244	methanol dehydrogenase
ec: 1.1.1.245	cyclohexanol dehydrogenase
ec: 1.1.1.250	D-arabinitol 2-dehydrogenase
ec: 1.1.1.251	galactitol 1-phosphate 5-dehydrogenase
ec: 1.1.1.255	mannitol dehydrogenase
ec: 1.1.1.256	fluoren-9-ol dehydrogenase
ec: 1.1.1.257	4-(hydroxymethyl)benzenesulfonate dehydrogenase
ec: 1.1.1.258	6-hydroxyhexanoate dehydrogenase
ec: 1.1.1.259	3-hydroxypimeloyl-CoA dehydrogenase
ec: 1.1.1.261	glycerol-1-phosphate dehydrogenase [NAD(P)+]
ec: 1.1.1.265	3-methylbutanal reductase
ec: 1.1.1.283	methylglyoxal reductase (NADPH-dependent)
ec: 1.1.1.286	isocitrate-homoisocitrate dehydrogenase
ec: 1.1.1.287	D-arabinitol dehydrogenase (NADP+)
	butanol dehydrogenase
ALDEHYDE DEHYDROGENASES	
ec: 1.2.1.2	formate dehydrogenase
ec: 1.2.1.3	aldehyde dehydrogenase (NAD+)
ec: 1.2.1.4	aldehyde dehydrogenase (NADP+)
ec: 1.2.1.5	aldehyde dehydrogenase [NAD(P)+]
ec: 1.2.1.7	benzaldehyde dehydrogenase (NADP+)
ec: 1.2.1.8	betaine-aldehyde dehydrogenase
ec: 1.2.1.9	glyceraldehyde-3-phosphate dehydrogenase (NADP+)
ec: 1.2.1.10	acetaldehyde dehydrogenase (acetylating)
ec: 1.2.1.11	aspartate-semialdehyde dehydrogenase
ec: 1.2.1.12	glyceraldehyde-3-phosphate dehydrogenase (phosphorylating)
ec: 1.2.1.13	glyceraldehyde-3-phosphate dehydrogenase (NADP+) (phosphorylating)
ec: 1.2.1.15	malonate-semialdehyde dehydrogenase
ec: 1.2.1.16	succinate-semialdehyde dehydrogenase [NAD(P)+]
ec: 1.2.1.17	glyoxylate dehydrogenase (acylating)
ec: 1.2.1.18	malonate-semialdehyde dehydrogenase (acetylating)
ec: 1.2.1.19	aminobutyraldehyde dehydrogenase
ec: 1.2.1.20	glutarate-semialdehyde dehydrogenase
ec: 1.2.1.21	glycolaldehyde dehydrogenase
ec: 1.2.1.22	lactaldehyde dehydrogenase
ec: 1.2.1.23	2-oxoaldehyde dehydrogenase (NAD+)
ec: 1.2.1.24	succinate-semialdehyde dehydrogenase
ec: 1.2.1.25	2-oxoisovalerate dehydrogenase (acylating)
ec: 1.2.1.26	2,5-dioxovalerate dehydrogenase
ec: 1.2.1.27	methylmalonate-semialdehyde dehydrogenase (acylating)
ec: 1.2.1.28	benzaldehyde dehydrogenase (NAD+)
ec: 1.2.1.29	aryl-aldehyde dehydrogenase
ec: 1.2.1.30	aryl-aldehyde dehydrogenase (NADP+)
ec: 1.2.1.31	L-aminoadipate-semialdehyde dehydrogenase
ec: 1.2.1.32	aminomuconate-semialdehyde dehydrogenase
ec: 1.2.1.36	retinal dehydrogenase
ec: 1.2.1.39	phenylacetaldehyde dehydrogenase
ec: 1.2.1.41	glutamate-5-semialdehyde dehydrogenase
ec: 1.2.1.42	hexadecanal dehydrogenase (acylating)
ec: 1.2.1.43	formate dehydrogenase (NADP+)
ec: 1.2.1.45	4-carboxy-2-hydroxymuconate-6-semialdehyde dehydrogenase
ec: 1.2.1.46	formaldehyde dehydrogenase
ec: 1.2.1.47	4-trimethylammoniobutyraldehyde dehydrogenase
ec: 1.2.1.48	long-chain-aldehyde dehydrogenase
ec: 1.2.1.49	2-oxoaldehyde dehydrogenase (NADP+)
ec: 1.2.1.51	pyruvate dehydrogenase (NADP+)
ec: 1.2.1.52	oxoglutarate dehydrogenase (NADP+)
ec: 1.2.1.53	4-hydroxyphenylacetaldehyde dehydrogenase
ec: 1.2.1.57	butanal dehydrogenase
ec: 1.2.1.58	phenylglyoxylate dehydrogenase (acylating)
ec: 1.2.1.59	glyceraldehyde-3-phosphate dehydrogenase (NAD(P)+) (phosphorylating)
ec: 1.2.1.62	4-formylbenzenesulfonate dehydrogenase
ec: 1.2.1.63	6-oxohexanoate dehydrogenase
ec: 1.2.1.64	4-hydroxybenzaldehyde dehydrogenase
ec: 1.2.1.65	salicylaldehyde dehydrogenase
ec: 1.2.1.66	mycothiol-dependent formaldehyde dehydrogenase
ec: 1.2.1.67	vanillin dehydrogenase
ec: 1.2.1.68	coniferyl-aldehyde dehydrogenase
ec: 1.2.1.69	fluoroacetaldehyde dehydrogenase
ec: 1.2.1.71	succinylglutamate-semialdehyde dehydrogenase

[0076] Pathways other than those exemplified above also can be employed to generate the biosynthesis of BDO in non-naturally occurring microbial organisms. In one embodiment, biosynthesis can be achieved using a L-homoserine to BDO pathway. This pathway has a molar yield of 0.90 mol/mol glucose, which appears restricted by the availability of reducing equivalents. A second pathway synthesizes BDO from acetoacetate and is capable of achieving the maximum theoretical yield of 1.091 mol/mol glucose. Implementation of either pathway can be achieved by introduction of two exogenous enzymes, and both pathways can additionally complement BDO production via succinyl-CoA. Pathway enzymes, thermodynamics, theoretical yields and overall feasibility are described further below.

[0077] A homoserine pathway also can be engineered to generate BDO-producing microbial organisms. Homoserine is an intermediate in threonine and methionine metabolism, formed from oxaloacetate via aspartate. The conversion of oxaloacetate to homoserine requires one NADH, two NADPH, and one ATP (FIG. **3**). Once formed, homoserine feeds into biosynthetic pathways for both threonine and methionine. In most organisms, high levels of threonine or methionine feedback to repress the homoserine biosynthesis pathway (Caspi et al., *Nucleic Acids Res.* 34:D511-D516 (1990)).

[0078] The transformation of homoserine to 4-hydroxybutyrate (4-HB) can be accomplished in two enzymatic steps as shown in FIG. **4**. The first step of this pathway is deamination of homoserine by a putative ammonia lyase. This reaction has an estimated thermodynamic barrier of 12 kJ/mol, but can likely be driven in the forward direction by a concentration gradient. In step **2**, the product alkene, 4-hydroxybut-2-enoate is reduced to 4-HB by a putative reductase at the cost of one NADH. This reaction step is highly thermodynamically favorable in the direction of 4-HB synthesis, with an estimated $\Delta_{rxn}G$ of −59 kJ/mol. 4-HB can then be converted to BDO as in FIG. **2** above.

[0079] Enzymes available for catalyzing the above transformations are shown in FIG. **5**. For example, the ammonia lyase in step **1** of the pathway closely resembles the chemistry of aspartate ammonia-lyase (aspartase). Aspartase is a widespread enzyme in microorganisms, and has been characterized extensively (Viola, R. E., *Mol. Biol.* 74:295-341 (2008)). The crystal structure of the *E. coli* aspartase has been solved (Shi et al., *Biochemistry* 36:9136-9144 (1997)), so it is therefore possible to directly engineer mutations in the enzyme's active site that would alter its substrate specificity to include homoserine. The oxidoreductase in step **2** has chemistry similar to several well-characterized enzymes including fumarate reductase in the *E. coli* TCA cycle. Since the thermodynamics of this reaction are highly favorable, an endogenous reductase with broad substrate specificity will likely be able to reduce 4-hydroxybut-2-enoate. The yield of this pathway under anaerobic conditions is 0.9 mol BDO per mol glucose although, when compared to the pathway in FIG. **2** (1.09 mol/mol glucose), both pathways appear to have similar energetic and reductive requirements from the metabolic precursor oxaloacetate (FIG. **6**).

[0080] The succinyl-CoA pathway was found to have a higher yield due to the fact that it is more energetically efficient. The conversion of one oxaloacetate molecule to BDO via the homoserine pathway will require the expenditure of 2 ATP equivalents. Because the conversion of glucose to two oxaloacetate molecules can generate a maximum of 3 ATP molecules assuming PEP carboxykinase to be reversible, the overall conversion of glucose to BDO via homoserine has a negative energetic yield. As expected, if we assume that energy can be generated via respiration, the maximum yield of the homoserine pathway increases to 1.05 mol/mol glucose which is 96% of the succinyl-CoA pathway yield. The succinyl-CoA pathway can channel some of the carbon flux through pyruvate dehydrogenase and the oxidative branch of the TCA cycle to generate both reducing equivalents and succinyl-CoA without an energetic expenditure. Thus, it does not encounter the same energetic difficulties as the homoserine pathway because not all of the flux is channeled through oxaloacetate to succinyl-CoA to BDO. Overall, the homoserine pathway demonstrates a moderately high-yielding route to BDO. One particularly useful characteristic is that it involves minimal engineering, with only two non-native steps. The pathway is likely to be thermodynamically favorable in the direction of BDO synthesis.

[0081] An acetoacetate pathway also can be engineered to generate BDO-producing microbial organisms. In *E. coli* acetoacetate is produced from acetone and leucine degradation. Acetoacetate also can be formed from acetyl-CoA by enzymes involved in fatty acid metabolism, including acetyl-CoA acetyltransferase and acetoacetyl-CoA transferase (FIG. **7**). Biosynthetic routes through acetoacetate are also particularly useful in microbial organisms that can metabolize single carbon compounds to form acetyl-CoA.

[0082] A three step route from acetoacetate to succinic semialdehyde (FIG. **8**) can be used to synthesize BDO through acetoacetate. Succinic semialdehyde, which is one reduction step removed from succinyl-CoA or one decarboxylation step removed from α-ketoglutarate, can be converted to BDO following three reductions steps (FIG. **2**). Briefly, step **1** of the acetoacetate biopathway entails conversion of acetoacetate to 3-aminobutanoate by an ω-aminotransferase. The ω-amino acid:pyruvate aminotransferase (ω-APT) from *Alcaligens denitrificans* was overexpressed in *E. coli* and shown to have a high activity toward 3-aminobutanoate in vitro (Yun et al., *Appl. Environ. Microbiol.* 70:2529-2534 (2004)). The activity of ω-APT in the direction required here was not measured in this study, due to spontaneous decomposition of acetoacetate to acetone in the reaction mixture. However, the thermodynamics indicate that it is feasible.

[0083] In step **2**, a putative aminomutase shifts the amine group from the 3- to the 4-position of the carbon backbone. An aminomutase performing this function on 3-aminobutanoate has not been characterized, but an enzyme from *Clostridium sticklandii* has a very similar mechanism (FIG. **9**). The enzyme, D-lysine-5,6-aminomutase, is involved in lysine biosynthesis.

[0084] The synthetic route to BDO from acetoacetate passes through 4-aminobutanoate, a metabolite in *E. coli* that's normally formed from decarboxylation of glutamate. Once formed, 4-aminobutanoate can be converted to succinic semialdehyde by 4-aminobutanoate transaminase (2.6.1.19), an enzyme which has been biochemically characterized. The thermodynamics of this enzyme and other steps of the pathway are close to equilibrium, so the operation of enzymes in the direction of interest is likely to be driven by substrate and product concentrations

[0085] One consideration for selecting candidate enzymes in this pathway is the stereoselectivity of the enzymes involved in the first two steps. The ω-ABT in *Alcaligens denitrificans* is specific to the L-stereoisomer of 3-aminobutanoate, while D-lysine-5,6-aminomutase likely requires the D-stereoisomer. If enzymes with complementary stereoselectivity can't be found or engineered, it would be necessary to add a third enzyme to the pathway with racemase activity that can convert L-3-aminobutanoate to D-3-aminobutanoate.

While amino acid racemases are widespread, whether these enzymes can function on ω-amino acids is not known.

[0086] The maximum theoretical molar yield of this pathway under anaerobic conditions is 1.091 mol/mol glucose. In order to generate flux from acetoacetate to BDO it was necessary to assume that acetyl-CoA:acetoacetyl-CoA transferase (enzyme 3 in FIG. **10**) is reversible. The function of this enzyme in *E. coli* is to metabolize short-chain fatty acids by first converting them into thioesters.

[0087] While the operation of acetyl-CoA:acetoacetyl-CoA transferase in the acetate-consuming direction has not been demonstrated experimentally in *E. coli*, studies on similar enzymes in other organisms support the assumption that this reaction is reversible. The enzyme butyryl-CoA:acetate:CoA transferase in gut microbes *Roseburia* sp. and *F. prasnitzii* operates in the acetate utilizing direction to produce butyrate (Duncan et al., *Appl. Environ. Microbiol.* 68:5186-5190 (2002)). Another very similar enzyme, acetyl:succinate CoA-transferase in *Trypanosoma brucei*, also operates in the acetate utilizing direction. This reaction has a $\Delta_{rxn}G$ close to equilibrium, so high concentrations of acetate can likely drive the reaction in the direction of interest. At the maximum theoretical BDO production rate of 1.09 mol/mol glucose simulations predict that *E. coli* can generate 1.098 mol ATP per mol glucose with no fermentation byproducts. This ATP yield should be sufficient for cell growth, maintenance, and production. The acetoacetate biopathway is a high-yielding route to BDO from acetyl-CoA. Like the homoserine pathway, this pathway requires minimal strain engineering, with only two non-native steps in addition to the BDO pathway.

[0088] Therefore, in addition to any of the various modifications exemplified previously for establishing 4-HB biosynthesis in a selected host, the BDO producing microbial organisms can include any of the previous combinations and permutations of 4-HB pathway metabolic modifications as well as any combination of expression for CoA-independent aldehyde dehydrogenase, CoA-dependent aldehyde dehydrogenase or an alcohol dehydrogenase to generate biosynthetic pathways for GBL and/or BDO. Therefore, the BDO producers of the invention can have exogenous expression of, for example, one, two, three, four, five, six, seven, eight, nine or all 10 enzymes corresponding to any of the six 4-HB pathway and/or any of the 4 BDO pathway enzymes.

[0089] Design and construction of the genetically modified microbial organisms is carried out using methods well known in the art to achieve sufficient amounts of expression to produce BDO. In particular, the non-naturally occurring microbial organisms of the invention can achieve biosynthesis of BDO resulting in intracellular concentrations between about 0.1-25 mM or more. Generally, the intracellular concentration of BDO is between about 3-20 mM, particularly between about 5-15 mM and more particularly between about 8-12 mM, including about 10 mM or more. Intracellular concentrations between and above each of these exemplary ranges also can be achieved from the non-naturally occurring microbial organisms of the invention. As with the 4-HB producers, the BDO producers also can be sustained, cultured or fermented under anaerobic conditions.

[0090] The invention further provides a method for the production of 4-HB. The method includes culturing a non-naturally occurring microbial organism having a 4-hydroxybutanoic acid (4-HB) biosynthetic pathway comprising at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, CoA-independent succinic semialdehyde dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, glutamate:succinic semialdehyde transaminase, α-ketoglutarate decarboxylase, or glutamate decarboxylase under substantially anaerobic conditions for a sufficient period of time to produce monomeric 4-hydroxybutanoic acid (4-HB). The method can additionally include chemical conversion of 4-HB to GBL and to BDO or THF, for example.

[0091] Additionally provided is a method for the production of 4-HB. The method includes culturing a non-naturally occurring microbial organism having a 4-hydroxybutanoic acid (4-HB) biosynthetic pathway including at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase or α-ketoglutarate decarboxylase under substantially anaerobic conditions for a sufficient period of time to produce monomeric 4-hydroxybutanoic acid (4-HB). The 4-HB product can be secreted into the culture medium.

[0092] Further provided is a method for the production of BDO. The method includes culturing a non-naturally occurring microbial biocatalyst, comprising a microbial organism having 4-hydroxybutanoic acid (4-HB) and 1,4-butanediol (BDO) biosynthetic pathways, the pathways including at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, 4-hydroxybutyrate:CoA transferase, 4-hydroxybutyrate kinase, phosphotranshydroxybutyrylase, α-ketoglutarate decarboxylase, aldehyde dehydrogenase, alcohol dehydrogenase or an aldehyde/alcohol dehydrogenase for a sufficient period of time to produce 1,4-butanediol (BDO). The BDO product can be secreted into the culture medium.

[0093] It is understood that, in methods of the invention, any of the one or more exogenous nucleic acids can be introduced into a microbial organism to produce a non-naturally occurring microbial organism of the invention. The nucleic acids can be introduced so as to confer, for example, a 4-HB, BDO, THF or GBL biosynthetic pathway onto the microbial organism. Alternatively, encoding nucleic acids can be introduced to produce an intermediate microbial organism having the biosynthetic capability to catalyze some of the required reactions to confer 4-HB, BDO, THF or GBL biosynthetic capability. For example, a non-naturally occurring microbial organism having a 4-HB biosynthetic pathway can comprise at least two exogenous nucleic acids encoding desired enzymes, such as the combination of 4-hydroxybutanoate dehydrogenase and α-ketoglutarate decarboxylase; 4-hydroxybutanoate dehydrogenase and CoA-independent succinic semialdehyde dehydrogenase; 4-hydroxybutanoate dehydrogenase and CoA-dependent succinic semialdehyde dehydrogenase; CoA-dependent succinic semialdehyde dehydrogenase and succinyl-CoA synthetase; succinyl-CoA synthetase and glutamate decarboxylase, and the like. Thus, it is understood that any combination of two or more enzymes of a biosynthetic pathway can be included in a non-naturally occurring microbial organism of the invention. Similarly, it is understood that any combination of three or more enzymes of a biosynthetic pathway can be included in a non-naturally occurring microbial organism of the invention, for example, 4-hydroxybutanoate dehydrogenase, α-ketoglutarate decarboxylase and CoA-dependent succinic semialdehyde dehydrogenase; CoA-independent succinic semialdehyde dehydrogenase and succinyl-CoA synthetase;

4-hydroxybutanoate dehydrogenase, CoA-dependent succinic semialdehyde dehydrogenase and glutamate:succinic semialdehyde transaminase, and so forth, as desired, so long as the combination of enzymes of the desired biosynthetic pathway results in production of the corresponding desired product.

[0094] Similarly, for example, with respect to any one or more exogenous nucleic acids introduced to confer BDO production, a non-naturally occurring microbial organism having a BDO biosynthetic pathway can comprise at least two exogenous nucleic acids encoding desired enzymes, such as the combination of 4-hydroxybutanoate dehydrogenase and α-ketoglutarate decarboxylase; 4-hydroxybutanoate dehydrogenase and 4-hydroxybutyryl CoA:acetyl-CoA transferase; 4-hydroxybutanoate dehydrogenase and butyrate kinase; 4-hydroxybutanoate dehydrogenase and phosphotransbutyrylase; 4-hydroxybutyryl CoA:acetyl-CoA transferase and aldehyde dehydrogenase; 4-hydroxybutyryl CoA:acetyl-CoA transferase and alcohol dehydrogenase; 4-hydroxybutyryl CoA:acetyl-CoA transferase and an aldehyde/alcohol dehydrogenase, and the like. Thus, it is understood that any combination of two or more enzymes of a biosynthetic pathway can be included in a non-naturally occurring microbial organism of the invention. Similarly, it is understood that any combination of three or more enzymes of a biosynthetic pathway can be included in a non-naturally occurring microbial organism of the invention, for example, 4-hydroxybutanoate dehydrogenase, α-ketoglutarate decarboxylase and 4-hydroxybutyryl CoA:acetyl-CoA transferase; 4-hydroxybutanoate dehydrogenase, butyrate kinase and phosphotransbutyrylase; 4-hydroxybutanoate dehydrogenase, 4-hydroxybutyryl CoA:acetyl-CoA transferase and aldehyde dehydrogenase; 4-hydroxybutyryl CoA:acetyl-CoA transferase, aldehyde dehydrogenase and alcohol dehydrogenase; butyrate kinase, phosphotransbutyrylase and an aldehyde/alcohol dehydrogenase, and the like. Similarly, any combination of four, five or more enzymes of a biosynthetic pathway as disclosed herein can be included in a non-naturally occurring microbial organism of the invention, as desired, so long as the combination of enzymes of the desired biosynthetic pathway results in production of the corresponding desired product.

[0095] Any of the non-naturally occurring microbial organisms described previously can be cultured to produce and/or secrete the biosynthetic products of the invention. For example, the 4-HB producers can be cultured for the biosynthetic production of 4-HB. The 4-HB can be isolated or be treated as described below to generate GBL, THF and/or BDO. Similarly, the BDO producers can be cultured for the biosynthetic production of BDO. The BDO can be isolated or subjected to further treatments for the chemical synthesis of BDO family compounds such as those downstream compounds exemplified in FIG. **1**.

[0096] The growth medium can be, for example, any carbohydrate source which can supply a source of carbon to the non-naturally occurring microorganism. Such sources include, for example, sugars such as glucose, xylose, arabinose, galactose, mannose, fructose and starch. Other sources of carbohydrate include, for example, renewable feedstocks and biomass. Exemplary types of biomasses that can be used as feedstocks in the methods of the invention include cellulosic biomass, hemicellulosic biomass and lignin feedstocks or portions of feedstocks. Such biomass feedstocks contain, for example, carbohydrate substrates useful as carbon sources such as glucose, xylose, arabinose, galactose, mannose, fructose and starch. Given the teachings and guidance provided herein, those skilled in the art will understand that renewable feedstocks and biomass other than those exemplified above also can be used for culturing the microbial organisms of the invention for the production of 4-HB and other compounds of the invention.

[0097] Accordingly, given the teachings and guidance provided herein, those skilled in the art will understand that a non-naturally occurring microbial organism can be produced that secretes the biosynthesized compounds of the invention when grown on a carbon source such as a carbohydrate. Such compounds include, for example, 4-HB, BDO and any of the intermediates metabolites in the 4-HB pathway, the BDO pathway and/or the combined 4-HB and BDO pathways. All that is required is to engineer in one or more of the enzyme activities shown in FIG. **2** to achieve biosynthesis of the desired compound or intermediate including, for example, inclusion of some or all of the 4-HB and/or BDO biosynthetic pathways. Accordingly, the invention provides a non-naturally occurring microbial organism that secretes 4-HB when grown on a carbohydrate, secretes BDO when grown on a carbohydrate and/or secretes any of the intermediate metabolites shown in FIG. **2** when grown on a carbohydrate. The BDO producing microbial organisms of the invention can initiate synthesis from, for example, succinate, succinyl-CoA, α-ketogluterate, succinic semialdehyde, 4-HB, 4-hydroxybutyrylphosphate, 4-hydroxybutyryl-CoA (4-HB-CoA) and/or 4-hydroxybutyraldehyde.

[0098] In some embodiments, culture conditions include anaerobic or substantially anaerobic growth or maintenance conditions. Exemplary anaerobic conditions have been described previously and are well known in the art. Exemplary anaerobic conditions for fermentation processes are described below in the Examples. Any of these conditions can be employed with the non-naturally occurring microbial organisms as well as other anaerobic conditions well known in the art. Under such anaerobic conditions, the 4-HB and BDO producers can synthesize monomeric 4-HB and BDO, respectively, at intracellular concentrations of 5-10 mM or more as well as all other concentrations exemplified previously.

[0099] A number of downstream compounds also can be generated for the 4-HB and BDO producing non-naturally occurring microbial organisms of the invention. With respect to the 4-HB producing microbial organisms of the invention, monomeric 4-HB and GBL exist in equilibrium in the culture medium. The conversion of 4-HB to GBL can be efficiently accomplished by, for example, culturing the microbial organisms in acid pH medium. A pH less than or equal to 7.5, in particular at or below pH 5.5, spontaneously converts 4-HB to GBL as illustrated in FIG. **1**.

[0100] The resultant GBL can be separated from 4-HB and other components in the culture using a variety of methods well known in the art. Such separation methods include, for example, the extraction procedures exemplified in the Examples as well as methods which include continuous liquid-liquid extraction, pervaporation, membrane filtration, membrane separation, reverse osmosis, electrodialysis, distillation, crystallization, centrifugation, extractive filtration, ion exchange chromatography, size exclusion chromatography, adsorption chromatography, and ultrafiltration. All of the above methods are well known in the art. Separated GBL can be further purified by, for example, distillation.

[0101] Another down stream compound that can be produced from the 4-HB producing non-naturally occurring microbial organisms of the invention includes, for example, BDO. This compound can be synthesized by, for example, chemical hydrogenation of GBL. Chemical hydrogenation reactions are well known in the art. One exemplary procedure includes the chemical reduction of 4-HB and/or GBL or a mixture of these two components deriving from the culture using a heterogeneous or homogeneous hydrogenation catalyst together with hydrogen, or a hydride-based reducing agent used stoichiometrically or catalytically, to produce 1,4-butanediol.

[0102] Other procedures well known in the art are equally applicable for the above chemical reaction and include, for example, WO No. 82/03854 (Bradley, et al.), which describes the hydrogenolysis of gamma-butyrolactone in the vapor phase over a copper oxide and zinc oxide catalyst. British Pat. No. 1,230,276, which describes the hydrogenation of gamma-butyrolactone using a copper oxide-chromium oxide catalyst. The hydrogenation is carried out in the liquid phase. Batch reactions also are exemplified having high total reactor pressures. Reactant and product partial pressures in the reactors are well above the respective dew points. British Pat. No. 1,314,126, which describes the hydrogenation of gamma-butyrolactone in the liquid phase over a nickel-cobalt-thorium oxide catalyst. Batch reactions are exemplified as having high total pressures and component partial pressures well above respective component dew points. British Pat. No. 1,344,557, which describes the hydrogenation of gamma-butyrolactone in the liquid phase over a copper oxide-chromium oxide catalyst. A vapor phase or vapor-containing mixed phase is indicated as suitable in some instances. A continuous flow tubular reactor is exemplified using high total reactor pressures. British Pat. No. 1,512,751, which describes the hydrogenation of gamma-butyrolactone to 1,4-butanediol in the liquid phase over a copper oxide-chromium oxide catalyst. Batch reactions are exemplified with high total reactor pressures and, where determinable, reactant and product partial pressures well above the respective dew points. U.S. Pat. No. 4,301,077, which describes the hydrogenation to 1,4-butanediol of gamma-butyrolactone over a Ru—Ni—Co—Zn catalyst. The reaction can be conducted in the liquid or gas phase or in a mixed liquid-gas phase. Exemplified are continuous flow liquid phase reactions at high total reactor pressures and relatively low reactor productivities. U.S. Pat. No. 4,048,196, which describes the production of 1,4-butanediol by the liquid phase hydrogenation of gamma-butyrolactone over a copper oxide-zinc oxide catalyst. Further exemplified is a continuous flow tubular reactor operating at high total reactor pressures and high reactant and product partial pressures. And U.S. Pat. No. 4,652,685, which describes the hydrogenation of lactones to glycols.

[0103] A further downstream compound that can be produced form the 4-HB producing microbial organisms of the invention includes, for example, THF. This compound can be synthesized by, for example, chemical hydrogenation of GBL. One exemplary procedure well known in the art applicable for the conversion of GBL to THF includes, for example, chemical reduction of 4-HB and/or GBL or a mixture of these two components deriving from the culture using a heterogeneous or homogeneous hydrogenation catalyst together with hydrogen, or a hydride-based reducing agent used stoichiometrically or catalytically, to produce tetrahydrofuran. Other procedures well know in the art are equally applicable for the above chemical reaction and include, for example, U.S. Pat. No. 6,686,310, which describes high surface area sol-gel route prepared hydrogenation catalysts. Processes for the reduction of maleic acid to tetrahydrofuran (THF) and 1,4-butanediol (BDO) and for the reduction of gamma butyrolactone to tetrahydrofuran and 1,4-butanediol also are described.

[0104] The culture conditions can include, for example, liquid culture procedures as well as fermentation and other large scale culture procedures. As described further below in the Examples, particularly useful yields of the biosynthetic products of the invention can be obtained under anaerobic or substantially anaerobic culture conditions.

[0105] The invention further provides a method of manufacturing 4-HB. The method includes fermenting a non-naturally occurring microbial organism having a 4-hydroxybutanoic acid (4-HB) biosynthetic pathway comprising at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, CoA-independent succinic semialdehyde dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, glutamate:succinic semialdehyde transaminase, a-ketoglutarate decarboxylase, or glutamate decarboxylase under substantially anaerobic conditions for a sufficient period of time to produce monomeric 4-hydroxybutanoic acid (4-HB), the process comprising fed-batch fermentation and batch separation; fed-batch fermentation and continuous separation, or continuous fermentation and continuous separation.

[0106] The culture and chemical hydrogenations described above also can be scaled up and grown continuously for manufacturing of 4-HB, GBL, BDO and/or THF. Exemplary growth procedures include, for example, fed-batch fermentation and batch separation; fed-batch fermentation and continuous separation, or continuous fermentation and continuous separation. All of these processes are well known in the art. Employing the 4-HB producers allows for simultaneous 4-HB biosynthesis and chemical conversion to GBL, BDO and/or THF by employing the above hydrogenation procedures simultaneous with continuous cultures methods such as fermentation. Other hydrogenation procedures also are well known in the art and can be equally applied to the methods of the invention.

[0107] Fermentation procedures are particularly useful for the biosynthetic production of commercial quantities of 4-HB and/or BDO. Generally, and as with non-continuous culture procedures, the continuous and/or near-continuous production of 4-HB or BDO will include culturing a non-naturally occurring 4-HB or BDO producing organism of the invention in sufficient nutrients and medium to sustain and/or nearly sustain growth in an exponential phase. Continuous culture under such conditions can be include, for example, 1 day, 2, 3, 4, 5, 6 or 7 days or more. Additionally, continuous culture can include 1 week, 2, 3, 4 or 5 or more weeks and up to several months. Alternatively, organisms of the invention can be cultured for hours, if suitable for a particular application. It is to be understood that the continuous and/or near-continuous culture conditions also can include all time intervals in between these exemplary periods.

[0108] Fermentation procedures are well known in the art. Briefly, fermentation for the biosynthetic production of 4-HB, BDO or other 4-HB derived products of the invention can be utilized in, for example, fed-batch fermentation and batch separation; fed-batch fermentation and continuous separation, or continuous fermentation and continuous separation. Examples of batch and continuous fermentation procedures well known in the art are exemplified further below in the Examples.

[0109] In addition, to the above fermentation procedures using the 4-HB or BDO producers of the invention for continuous production of substantial quantities of monomeric 4-HB and BDO, respectively, the 4-HB producers also can be, for example, simultaneously subjected to chemical synthesis procedures as described previously for the chemical conversion of monomeric 4-HB to, for example, GBL, BDO and/or THF. The BDO producers can similarly be, for example, simultaneously subjected to chemical synthesis procedures as described previously for the chemical conversion of BDO to, for example, THF, GBL, pyrrolidones and/or other BDO family compounds. In addition, the products of the 4-HB and BDO producers can be separated from the fermentation culture and sequentially subjected to chemical conversion, as disclosed herein.

[0110] Briefly, hydrogenation of GBL in the fermentation broth can be performed as described by Frost et al., *Biotechnology Progress* 18: 201-211 (2002). Another procedure for hydrogenation during fermentation include, for example, the methods described in, for example, U.S. Pat. No. 5,478,952. This method is further exemplified in the Examples below.

[0111] Therefore, the invention additionally provides a method of manufacturing γ-butyrolactone (GBL), tetrahydrofuran (THF) or 1,4-butanediol (BDO). The method includes fermenting a non-naturally occurring microbial organism having 4-hydroxybutanoic acid (4-HB) and/or 1,4-butanediol (BDO) biosynthetic pathways, the pathways comprise at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, CoA-independent succinic semialdehyde dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, 4-hydroxybutyrate: CoA transferase, glutamate:succinic semialdehyde transaminase, α-ketoglutarate decarboxylase, glutamate decarboxylase, 4-hydroxybutanoate kinase, phosphotransbutyrylase, CoA-independent 1,4-butanediol semialdehyde dehydrogenase, CoA-dependent 1,4-butanediol semialdehyde dehydrogenase, CoA-independent 1,4-butanediol alcohol dehydrogenase or CoA-dependent 1,4-butanediol alcohol dehydrogenase, under substantially anaerobic conditions for a sufficient period of time to produce 1,4-butanediol (BDO), GBL or THF, the fermenting comprising fed-batch fermentation and batch separation; fed-batch fermentation and continuous separation, or continuous fermentation and continuous separation.

[0112] In addition to the biosynthesis of 4-HB, BDO and other products of the invention as described herein, the non-naturally occurring microbial organisms and methods of the invention also can be utilized in various combinations with each other and with other microbial organisms and methods well known in the art to achieve product biosynthesis by other routes. For example, one alternative to produce BDO other than use of the 4-HB producers and chemical steps or other than use of the BDO producer directly is through addition of another microbial organism capable of converting 4-HB or a 4-HB product exemplified herein to BDO.

[0113] One such procedure includes, for example, the fermentation of a 4-HB producing microbial organism of the invention to produce 4-HB, as described above and below. The 4-HB can then be used as a substrate for a second microbial organism that converts 4-HB to, for example, BDO, GBL and/or THF. The 4-HB can be added directly to another culture of the second organism or the original culture of 4-HB producers can be depleted of these microbial organisms by, for example, cell separation, and then subsequent addition of the second organism to the fermentation broth can utilized to produce the final product without intermediate purification steps. One exemplary second organism having the capacity to biochemically utilize 4-HB as a substrate for conversion to BDO, for example, is *Clostridium acetobutylicum* (see, for example, Jewell et al., *Current Microbiology,* 13:215-19 (1986)).

[0114] In other embodiments, the non-naturally occurring microbial organisms and methods of the invention can be assembled in a wide variety of subpathways to achieve biosynthesis of, for example, 4-HB and/or BDO as described. In these embodiments, biosynthetic pathways for a desired product of the invention can be segregated into different microbial organisms and the different microbial organisms can be co-cultured to produce the final product. In such a biosynthetic scheme, the product of one microbial organism is the substrate for a second microbial organism until the final product is synthesized. For example, the biosynthesis of BDO can be accomplished as described previously by constructing a microbial organism that contains biosynthetic pathways for conversion of a substrate such as endogenous succinate through 4-HB to the final product BDO. Alternatively, BDO also can be bio synthetically produced from microbial organisms through co-culture or co-fermentation using two organisms in the same vessel. A first microbial organism being a 4-HB producer with genes to produce 4-HB from succinic acid, and a second microbial organism being a BDO producer with genes to convert 4-HB to BDO.

[0115] Given the teachings and guidance provided herein, those skilled in the art will understand that a wide variety of combinations and permutations exist for the non-naturally occurring microbial organisms and methods of the invention together with other microbial organisms, with the co-culture of other non-naturally occurring microbial organisms having subpathways and with combinations of other chemical and/or biochemical procedures well known in the art to produce 4-HB, BDO, GBL and THF products of the invention.

[0116] One computational method for identifying and designing metabolic alterations favoring biosynthesis of a product is the OptKnock computational framework, Burgard et al., *Biotechnol Bioeng,* 84: 647-57 (2003). OptKnock is a metabolic modeling and simulation program that suggests gene deletion strategies that result in genetically stable microorganisms which overproduce the target product. Specifically, the framework examines the complete metabolic and/or biochemical network of a microorganism in order to suggest genetic manipulations that force the desired biochemical to become an obligatory byproduct of cell growth. By coupling biochemical production with cell growth through strategically placed gene deletions or other functional gene disruption, the growth selection pressures imposed on the engineered strains after long periods of time in a bioreactor lead to improvements in performance as a result of the compulsory growth-coupled biochemical production. Lastly, when gene deletions are constructed there is a negligible possibility of the designed strains reverting to their wild-type states because the genes selected by OptKnock are to be completely removed from the genome. Therefore, this computational methodology can be used to either identify alternative pathways that lead to biosynthesis of 4-HB and/or BDO or used in connection with the non-naturally occurring microbial organisms for further optimization of 4-HB and/or BDO biosynthesis.

[0117] Briefly, OptKnock is a term used herein to refer to a computational method and system for modeling cellular metabolism. The OptKnock program relates to a framework of models and methods that incorporate particular constraints into flux balance analysis (FBA) models. These constraints include, for example, qualitative kinetic information, qualitative regulatory information, and/or DNA microarray experimental data. OptKnock also computes solutions to various metabolic problems by, for example, tightening the flux boundaries derived through flux balance models and subsequently probing the performance limits of metabolic networks in the presence of gene additions or deletions. OptKnock computational framework allows the construction of model formulations that enable an effective query of the performance limits of metabolic networks and provides methods for solving the resulting mixed-integer linear programming problems. The metabolic modeling and simulation methods referred to herein as OptKnock are described in, for example, U.S. patent application Ser. No. 10/043,440, filed Jan. 10, 2002, and in International Patent No. PCT/US02/00660, filed Jan. 10, 2002.

[0118] Another computational method for identifying and designing metabolic alterations favoring biosynthetic production of a product is metabolic modeling and simulation system termed SimPheny®. This computational method and system is described in, for example, U.S. patent application Ser. No. 10/173,547, filed Jun. 14, 2002, and in International Patent Application No. PCT/US03/18838, filed Jun. 13, 2003.

[0119] SimPheny® is a computational system that can be used to produce a network model in silico and to simulate the flux of mass, energy or charge through the chemical reactions of a biological system to define a solution space that contains any and all possible functionalities of the chemical reactions in the system, thereby determining a range of allowed activities for the biological system. This approach is referred to as constraints-based modeling because the solution space is defined by constraints such as the known stoichiometry of the included reactions as well as reaction thermodynamic and capacity constraints associated with maximum fluxes through reactions. The space defined by these constraints can be interrogated to determine the phenotypic capabilities and behavior of the biological system or of its biochemical components. Analysis methods such as convex analysis, linear programming and the calculation of extreme pathways as described, for example, in Schilling et al., *J. Theor. Biol.* 203:229-248 (2000); Schilling et al., *Biotech. Bioeng.* 71:286-306 (2000) and Schilling et al., *Biotech. Prog.* 15:288-295 (1999), can be used to determine such phenotypic capabilities. As described in the Examples below, this computation methodology was used to identify and analyze the feasible as well as the optimal 4-HB biosynthetic pathways in 4-HB non-producing microbial organisms.

[0120] As described above, one constraints-based method used in the computational programs applicable to the invention is flux balance analysis. Flux balance analysis is based on flux balancing in a steady state condition and can be performed as described in, for example, Varma and Palsson, *Biotech. Bioeng.* 12:994-998 (1994). Flux balance approaches have been applied to reaction networks to simulate or predict systemic properties of, for example, adipocyte metabolism as described in Fell and Small, *J. Biochem.* 138: 781-786 (1986), acetate secretion from *E. coli* under ATP maximization conditions as described in Majewski and Domach, *Biotech. Bioeng.* 35:732-738 (1990) or ethanol secretion by yeast as described in Vanrolleghem et al., *Biotech. Prog.* 12:434-448 (1996). Additionally, this approach can be used to predict or simulate the growth of *E. coli* on a variety of single-carbon sources as well as the metabolism of *H. influenzae* as described in Edwards and Palsson, *Proc. Natl. Acad. Sci.* 97:5528-5533 (2000), Edwards and Palsson, *J. Bio. Chem.* 274:17410-17416 (1999) and Edwards et al., *Nature Biotech.* 19:125-130 (2001).

[0121] Once the solution space has been defined, it can be analyzed to determine possible solutions under various conditions. This computational approach is consistent with biological realities because biological systems are flexible and can reach the same result in many different ways. Biological systems are designed through evolutionary mechanisms that have been restricted by fundamental constraints that all living systems must face. Therefore, constraints-based modeling strategy embraces these general realities. Further, the ability to continuously impose further restrictions on a network model via the tightening of constraints results in a reduction in the size of the solution space, thereby enhancing the precision with which physiological performance or phenotype can be predicted.

[0122] Given the teachings and guidance provided herein, those skilled in the art will be able to apply various computational frameworks for metabolic modeling and simulation to design and implement biosynthesis of 4-HB, BDO, GBL, THF and other BDO family compounds in host microbial organisms other than *E. coli* and yeast. Such metabolic modeling and simulation methods include, for example, the computational systems exemplified above as SimPheny® and OptKnock. For illustration of the invention, some methods are described herein with reference to the OptKnock computation framework for modeling and simulation. Those skilled in the art will know how to apply the identification, design and implementation of the metabolic alterations using OptKnock to any of such other metabolic modeling and simulation computational frameworks and methods well known in the art.

[0123] The ability of a cell or organism to biosynthetically produce a biochemical product can be illustrated in the context of the biochemical production limits of a typical metabolic network calculated using an in silico model. These limits are obtained by fixing the uptake rate(s) of the limiting substrate(s) to their experimentally measured value(s) and calculating the maximum and minimum rates of biochemical production at each attainable level of growth. The production of a desired biochemical generally is in direct competition with biomass formation for intracellular resources. Under these circumstances, enhanced rates of biochemical production will necessarily result in sub-maximal growth rates. The knockouts suggested by the above metabolic modeling and simulation programs such as OptKnock are designed to restrict the allowable solution boundaries forcing a change in metabolic behavior from the wild-type strain. Although the actual solution boundaries for a given strain will expand or contract as the substrate uptake rate(s) increase or decrease, each experimental point will lie within its calculated solution boundary. Plots such as these enable accurate predictions of how close the designed strains are to their performance limits which also indicates how much room is available for improvement.

[0124] The OptKnock mathematical framework is exemplified herein for pinpointing gene deletions leading to product biosynthesis and, particularly, growth-coupled product biosynthesis. The procedure builds upon constraint-based metabolic modeling which narrows the range of possible phenotypes that a cellular system can display through the successive imposition of governing physico-chemical constraints, Price et al., *Nat Rev Microbiol,* 2: 886-97 (2004). As described above, constraint-based models and simulations are well known in the art and generally invoke the optimization of a particular cellular objective, subject to network stoichiometry, to suggest a likely flux distribution.

[0125] Briefly, the maximization of a cellular objective quantified as an aggregate reaction flux for a steady state metabolic network comprising a set N={1, . . . , N} of metabolites and a set M={1, . . . , M} of metabolic reactions is expressed mathematically as follows: maximize $\nu_{cellular\ objective}$ subject to

$$\sum_{j=1}^{M} S_{ij} v_j = 0, \quad \forall\ i \in N$$

$\nu_{substrate}=\nu_{substrate\_uptake}$ mmol/gDW·hr $\forall i \in$ {limiting substrate(s)}

$\nu_{atp} \geqq \nu_{atp\_main}$ mmol/gDW·hr

$\nu_j \geqq 0$, $\forall j \in$ {irrev. reactions}

[0126] where S_{ij} is the stoichiometric coefficient of metabolite i in reaction j, ν_j is the flux of reaction j, $\nu_{substrate\_uptake}$ represents the assumed or measured uptake rate(s) of the limiting substrate(s), and ν_{atp_main} is the non-growth associated ATP maintenance requirement. The vector ν includes both internal and external fluxes. In this study, the cellular objective is often assumed to be a drain of biosynthetic precursors in the ratios required for biomass formation, Neidhardt, F. C. et al., *Escherichia coli and Salmonella: Cellular and Molecular Biology,* 2nd ed. 1996, Washington, D.C.: ASM Press. 2 v. (xx, 2822, lxxvi). The fluxes are generally reported per 1 gDW·hr (gram of dry weight times hour) such that biomass formation is expressed as g biomass produced/gDW·hr or l/hr.

[0127] The modeling of gene deletions, and thus reaction elimination, first employs the incorporation of binary variables into the constraint-based approach framework, Burgard et al., *Biotechnol Bioeng,* 74: 364-375 (2001), Burgard et al., *Biotechnol Prog,* 17: 791-797 (2001). These binary variables,

$$y_j = \begin{cases} 1, & \text{if reaction flux } v_j \text{ is active} \\ 0, & \text{if reaction flux } v_j \text{ is not active} \end{cases}, \forall\ j \in M$$

assume a value of 1 if reaction j is active and a value of 0 if it is inactive. The following constraint,

$$v_j^{min} \cdot y_j \leqq \nu_j \leqq v_j^{max} \cdot y_j,\ \forall j \in M$$

ensures that reaction flux ν_j is set to zero only if variable y_j is equal to zero. Alternatively, when y_j is equal to one, ν_j is free to assume any value between a lower ν_j^{min} and an upper ν_j^{max} bound. Here, ν_j^{min} and ν_j^{max} are identified by minimizing and maximizing, respectively, every reaction flux subject to the network constraints described above, Mahadevan et al., *Metab Eng,* 5: 264-76 (2003).

[0128] Optimal gene/reaction knockouts are identified by solving a bilevel optimization problem that chooses the set of active reactions (y_j=1) such that an optimal growth solution for the resulting network overproduces the chemical of interest. Mathematically, this bilevel optimization problem is expressed as the following bilevel mixed-integer optimization problem:

$$\begin{aligned} &\underset{y_j}{\text{maximize}} \quad v_{chemical} \quad (OptKnock) \\ &\left(\begin{array}{llll} \underset{v_j}{\text{subject to}} & \text{maximize} & v_{biomass} & \\ & \text{subject to} & \sum_{j=1}^{M} S_{ij} v_j = 0, & \forall\ i \in N \\ & & v_{substrate} = v_{substrate\_uptake} & \forall\ i \in \{\text{limiting substrate}(s)\} \\ & & v_{atp} \geq v_{atp\_main} & \end{array} \right) \\ &v_{biomass} \geq v_{biomass}^{target} \\ &v_j^{min} \cdot y_j \leq v_j \leq v_j^{max} \cdot y_j,\ \forall\ j \in M \\ &\sum_{j \in M^{forward}} (1 - y_j) = K \\ &y_j \in \{0, 1\},\ \forall\ j \in M \end{aligned}$$

where $\nu_{chemical}$ is the production of the desired target product, for example succinate or other biochemical product, and K is the number of allowable knockouts. Note that setting K equal to zero returns the maximum biomass solution of the complete network, while setting K equal to one identifies the single gene/reaction knockout (y_j=0) such that the resulting network involves the maximum overproduction given its maximum biomass yield. The final constraint ensures that the resulting network meets a minimum biomass yield. Burgard et al., *Biotechnol Bioeng,* 84: 647-57 (2003), provide a more detailed description of the model formulation and solution procedure. Problems containing hundreds of binary variables can be solved in the order of minutes to hours using CPLEX 8.0, *GAMS: The Solver Manuals*. 2003: GAMS Development Corporation, accessed via the GAMS, Brooke et al., *GAMS Development Corporation* (1998), modeling environment on an IBM RS6000-270 workstation. The OptKnock framework has already been able to identify promising gene deletion strategies for biochemical overproduction, Burgard et al., *Biotechnol Bioeng,* 84: 647-57 (2003), Pharkya et al., *Biotechnol Bioeng,* 84: 887-899 (2003), and establishes a systematic framework that will naturally encompass future improvements in metabolic and regulatory modeling frameworks.

[0129] Any solution of the above described bilevel OptKnock problem will provide one set of metabolic reactions to disrupt. Elimination of each reaction within the set or metabolic modification can result in 4-HB or BDO as an obligatory product during the growth phase of the organism. Because the reactions are known, a solution to the bilevel OptKnock problem also will provide the associated gene or genes encoding one or more enzymes that catalyze each reaction within the set of reactions. Identification of a set of reactions and their corresponding genes encoding the enzymes participating in each reaction is generally an automated process, accomplished through correlation of the reactions with a reaction database having a relationship between enzymes and encoding genes.

[0130] Once identified, the set of reactions that are to be disrupted in order to achieve 4-HB or BDO production are implemented in the target cell or organism by functional disruption of at least one gene encoding each metabolic reaction within the set. One particularly useful means to achieve functional disruption of the reaction set is by deletion of each encoding gene. However, in some instances, it can be beneficial to disrupt the reaction by other genetic aberrations including, for example, mutation, deletion of regulatory regions such as promoters or cis binding sites for regulatory factors, or by truncation of the coding sequence at any of a number of locations. These latter aberrations, resulting in less than total deletion of the gene set can be useful, for example, when rapid assessments of the succinate coupling are desired or when genetic reversion is less likely to occur.

[0131] To identify additional productive solutions to the above described bilevel OptKnock problem which lead to further sets of reactions to disrupt or metabolic modifications that can result in the biosynthesis, including growth-coupled biosynthesis of 4-HB or other biochemical product, an optimization method, termed integer cuts, can be implemented. This method proceeds by iteratively solving the OptKnock problem exemplified above with the incorporation of an additional constraint referred to as an integer cut at each iteration. Integer cut constraints effectively prevent the solution procedure from choosing the exact same set of reactions identified in any previous iteration that obligatory couples product biosynthesis to growth. For example, if a previously identified growth-coupled metabolic modification specifies reactions 1, 2, and 3 for disruption, then the following constraint prevents the same reactions from being simultaneously considered in subsequent solutions: $y_1+y_2+y_3 \geqq 1$. The integer cut method is well known in the art and can be found described in, for example, reference, Burgard et al., *Biotechnol Prog,* 17: 791-797 (2001). As with all methods described herein with reference to their use in combination with the OptKnock computational framework for metabolic modeling and simulation, the integer cut method of reducing redundancy in iterative computational analysis also can be applied with other computational frameworks well known in the art including, for example, SimPheny®.

[0132] Constraints of the above form preclude identification of larger reaction sets that include previously identified sets. For example, employing the integer cut optimization method above in a further iteration would preclude identifying a quadruple reaction set that specified reactions 1, 2, and 3 for disruption since these reactions had been previously identified. To ensure identification of all possible reaction sets leading to biosynthetic production of a product, a modification of the integer cut method can be employed.

[0133] Briefly, the modified integer cut procedure begins with iteration 'zero' which calculates the maximum production of the desired biochemical at optimal growth for a wild-type network. This calculation corresponds to an OptKnock solution with K equaling 0. Next, single knockouts are considered and the two parameter sets, $objstore_{iter}$ and $ystore_{iter,j}$, are introduced to store the objective function ($v_{chemical}$) and reaction on-off information (y_j), respectively, at each iteration, iter. The following constraints are then successively added to the OptKnock formulation at each iteration.

$$v_{chemical} \geqq objstore_{iter} + \epsilon - M \cdot \Sigma_{j \epsilon ystore_{iter,j}=0} y_j$$

[0134] In the above equation, ϵ and M are a small and a large numbers, respectively. In general, ϵ can be set at about 0.01 and M can be set at about 1000. However, numbers smaller and/or larger then these numbers also can be used. M ensures that the constraint can be binding only for previously identified knockout strategies, while ϵ ensures that adding knockouts to a previously identified strategy must lead to an increase of at least ϵ in biochemical production at optimal growth. The approach moves onto double deletions whenever a single deletion strategy fails to improve upon the wild-type strain. Triple deletions are then considered when no double deletion strategy improves upon the wild-type strain, and so on. The end result is a ranked list, represented as desired biochemical production at optimal growth, of distinct deletion strategies that differ from each other by at least one knockout. This optimization procedure as well as the identification of a wide variety of reaction sets that, when disrupted, lead to the biosynthesis, including growth-coupled production, of a biochemical product. Given the teachings and guidance provided herein, those skilled in the art will understand that the methods and metabolic engineering designs exemplified herein are equally applicable to identify new biosynthetic pathways and/or to the obligatory coupling of cell or microorganism growth to any biochemical product.

[0135] The methods exemplified above and further illustrated in the Examples below enable the construction of cells and organisms that biosynthetically produce, including obligatory couple production of a target biochemical product to growth of the cell or organism engineered to harbor the identified genetic alterations. In this regard, metabolic alterations have been identified that result in the biosynthesis of 4-HB and 1,4-butanediol. Microorganism strains constructed with the identified metabolic alterations produce elevated levels of 4-HB or BDO compared to unmodified microbial organisms. These strains can be beneficially used for the commercial production of 4-HB, BDO, THF and GBL, for example, in continuous fermentation process without being subjected to the negative selective pressures.

[0136] Therefore, the computational methods described herein enable the identification and implementation of metabolic modifications that are identified by an in silico method selected from OptKnock or SimPheny. The set of metabolic modifications can include, for example, addition of one or more biosynthetic pathway enzymes and/or functional disruption of one or more metabolic reactions including, for example, disruption by gene deletion.

[0137] It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also included within the definition of the invention provided herein. Accordingly, the following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

Biosynthesis of 4-Hydroxybutanoic Acid

[0138] This Example describes the biochemical pathways for 4-HB production.

[0139] Previous reports of 4-HB synthesis in microbes have focused on this compound as an intermediate in production of the biodegradable plastic poly-hydroxyalkanoate (PHA) (U.S. Pat. No. 6,117,658). The use of 4-HB/3-HB copolymers over poly-3-hydroxybutyrate polymer (PHB) can result in plastic that is less brittle (Saito and Doi, *Intl. J. Biol. Macromol.* 16:99-104 (1994)). The production of monomeric 4-HB described herein is a fundamentally distinct process for several reasons: (1) the product is secreted, as opposed to PHA which is produced intracellularly and remains in the cell; (2) for organisms that produce hydroxybutanoate polymers, free 4-HB is not produced, but rather the Coenzyme A derivative is used by the polyhydroxyalkanoate synthase; (3) in the case of the polymer, formation of the granular product changes thermodynamics; and (4) extracellular pH is not an issue for production of the polymer, whereas it will affect whether 4-HB is present in the free acid or conjugate base state, and also the equilibrium between 4-HB and GBL.

[0140] 4-HB can be produced in two enzymatic reduction steps from succinate, a central metabolite of the TCA cycle, with succinic semialdehyde as the intermediate (FIG. **2**). The first of these enzymes, succinic semialdehyde dehydrogenase, is native to many organisms including *E. coli*, in which both NADH- and NADPH-dependent enzymes have been found (Donnelly and Cooper, *Eur. J. Biochem.* 113:555-561 (1981); Donnelly and Cooper, *J. Bacteriol.* 145:1425-1427 (1981); Marek and Henson, *J. Bacteriol.* 170:991-994 (1988)). There is also evidence supporting succinic semialdehyde dehydrogenase activity in *S. cerevisiae* (Ramos et al., *Eur. J. Biochem.* 149:401-404 (1985)), and a putative gene has been identified by sequence homology. However, most reports indicate that this enzyme proceeds in the direction of succinate synthesis, as shown in FIG. **2** (Donnelly and Cooper, supra; Lutke-Eversloh and Steinbuchel, *FEMS Microbiol. Lett.* 181:63-71 (1999)), participating in the degradation pathway of 4-HB and gamma-aminobutyrate. Succinic semialdehyde also is natively produced by certain microbial organisms such as *E. coli* through the TCA cycle intermediate α-ketoglutarate via the action of two enzymes: glutamate:succinic semialdehyde transaminase and glutamate decarboxylase. An alternative pathway, used by the obligate anaerobe *Clostridium kluyveri* to degrade succinate, activates succinate to succinyl-CoA, then converts succinyl-CoA to succinic semialdehyde using an alternative succinic semialdehyde dehydrogenase which is known to function in this direction (Sohling and Gottschalk, *Eur. J. Biochem.* 212:121-127 (1993)). However, this route has the energetic cost of ATP required to convert succinate to succinyl-CoA.

[0141] The second enzyme of the pathway, 4-hydroxybutanoate dehydrogenase, is not native to *E. coli* or yeast but is found in various bacteria such as *C. kluyveri* and *Ralstonia eutropha* (Lutke-Eversloh and Steinbuchel, supra; Sohling and Gottschalk, *J. Bacteriol.* 178:871-880 (1996); Valentin et al., *Eur. J. Biochem.* 227:43-60 (1995); Wolff and Kenealy, *Protein Expr. Purif.* 6:206-212 (1995)). These enzymes are known to be NADH-dependent, though NADPH-dependent forms also exist. An additional pathway to 4-HB from alpha-ketoglutarate was demonstrated in *E. coli* resulting in the accumulation of poly(4-hydroxybutyric acid) (Song et al., *Wei Sheng Wu Xue. Bao.* 45:382-386 (2005)). The recombinant strain required the overexpression of three heterologous genes, PHA synthase (*R. eutropha*), 4-hydroxybutyrate dehydrogenase (*R. eutropha*) and 4-hydroxybutyrate:CoA transferase (*C. kluyveri*), along with two native *E. coli* genes: glutamate:succinic semialdehyde transaminase and glutamate decarboxylase. Steps **4** and **5** in FIG. **2** can alternatively be carried out by an alpha-ketoglutarate decarboxylase such as the one identified in *Euglena gracilis* (Shigeoka et al., *Biochem. J.* 282(Pt2):319-323 (1992); Shigeoka and Nakano, *Arch. Biochem. Biophys.* 288:22-28 (1991); Shigeoka and Nakano, *Biochem J.* 292(Pt 2):463-467 (1993)). However, this enzyme has not previously been applied to impact the production of 4-HB or related polymers in any organism.

[0142] The reported directionality of succinic semialdehyde dehydrogenase led to the investigation of the thermodynamics of 4-HB metabolism. Specifically, this study investigated whether or not the reactions involved in the conversion of succinate or succinyl-CoA to 4-HB are thermodynamically favorable (i.e., $\Delta G_r < 0$) under the typical physiological conditions present in *E. coli* and *S. cerevisiae*. All oxidation/reduction reactions were assumed to utilize NADH, although the results for assuming NADPH utilization would be similar. Standard Gibbs free energies of formation (ΔG_f^o) were calculated for each compound in the succinate and succinyl-CoA pathways shown in FIG. **2** based on the group contribution method (Mavrovouniotis, M. L., *J. Biol. Chem.* 266: 14440-14445 (1991)). Each standard Gibbs energy of formation was then transformed in order to obtain a criterion of spontaneous change at specified pressure, temperature, pH, and ionic strength (Alberty, R. A., *Biochem. Biophys. Acta* 1207:1-11 (1994)) (equation 1).

$$\Delta G'_f(I, \mathrm{pH}) = \Delta G^o_f(I=0) + N_H RT \ln(10^{pH}) - 2.915\sqrt{I}\,\frac{z^2 - N_H}{1 + B\sqrt{I}} \tag{1}$$

[0143] Where ΔG_f^o is the standard Gibbs energy of formation, N_H is the number of hydrogen atoms in the compound, R is the universal gas constant, T is constant at 298K, z is the charge of the molecule at the pH of interest, I is the ionic strength in M, and B is a constant equal to 1.6 $L^{0.5}/mol^{0.5}$.

[0144] Equation 1 reveals that both intracellular pH and ionic strength play a role in determining thermodynamic feasibility. Normally, intracellular pH of cells is very well regulated, even when there are large variations in the culture pH. The intracellular pH of *E. coli* and *S. cerevisiae* have both been reported in the literature. *E. coli* maintains an intracellular pH of 7.4-7.7 during typical growth conditions in neutral buffers, but can drop to 7.2 in pH 6 medium, and even go as low as 6.9 for external pH of 5 (Riondet et al., *Biotechnology Tech.* 11:735-738 (1997)). However, growth of *E. coli* is severely inhibited at external pH below 6. Yeast pH exhibits more variation. During exponential growth phase, *S. cerevisiae* internal pH has been measured to be in the range of 6.7-7.0 with external pH controlled at 5.0 (Dombek and Ingram, *Appl. Environ. Microbiol.* 53:1286-1291 (1987)). On the other hand, in resting cells the internal pH drops to below 6 when the external pH is 6 or less (Imai and Ohno, *J. Biotechnol.* 38:165-172 (1995)). This analysis assumes an intracellular pH of 7.4 for *E. coli* and 6.8 for *S. cerevisiae*. An ionic strength of 0.15 also was assumed (Valenti et al., supra).

[0145] Transformed Gibbs energies of formation were calculated at the standard state (pH=7.0, I=0) and at physiological states of *E. coli* (pH=7.4, 10.15) and *S. cerevisiae* (pH=6.8, I=0.15). Transformed Gibbs energies of reaction ($\Delta G_r'$) were then calculated by taking the difference in $\Delta G_f'$ between the products and reactants. The transformed Gibbs energies of the reactions necessary to convert succinate or succinyl-CoA to 4-HB are provided in Table 2. Although some of the steps have calculated positive delta G values, the standard errors for these calculations and concentration gradients indicate that any of the steps are feasible. Note that the standard error, $U_{f,est}$, on ΔG_f calculated by the group contribution theory is 4 kcal/mol. The uncertainty in ΔG_r, $U_{r,est}$, can be calculated as the Euclidean norm of the uncertainty for ΔG_f of each compound (Equation).

$$U_{r,est} = \sqrt{\sum_{i=1}^{m} n_i^2 * U_{f,est}^2} = \sqrt{\sum_{i=1}^{m} 16 n_i^2} \tag{2}$$

[0146] Where n is the stoichiometric coefficient and i is the compound. For the examined reactions, this uncertainty is on the order of 8 kcal/mol.

TABLE 2

Gibbs free energy of reaction (kcal/mole) at different pH and ionic strength values.

Reaction	$\Delta G_r^{o'}$ pH = 7.0 IS = 0	$\Delta G_r'$ pH = 7.4 IS = 0.15 M	$\Delta G_r'$ pH = 6.8 IS = 0.15 M
succ + NADH + 2 H+ → sucsa + NAD + h2o	12.0	14.4	12.8
succ + coa + ATP → succoa + ADP + Pi	0.30	−0.03	−0.03
succoa + NADH + H+ → sucsa + NAD + coa	4.4	7.0	6.2
sucsa + NADH + H+ → 4-HB + NAD	−5.0	−3.8	−4.6

The first column is under standard conditions, while the others are adjusted according to equation 1.
Temperature is constant at 298 K.
Error bars for these values are on the order of 8 kcal/mol, as calculated by equation 2.
Abbreviations:
suc, succinate;
sucsa, succinic semialdehyde;
succoa, succinyl-CoA;
Pi, inorganic phosphate.

[0147] Table 2 reveals that the reaction most likely to encounter a thermodynamic barrier after considering potential uncertainty in our calculations is succinic semialdehyde dehydrogenase (step **1** in FIG. **2**). Whether this reaction can be driven closer to thermodynamic feasibility by varying the assumed concentrations of the participating metabolites also was studied. For example, the standard Gibbs energies assume concentrations of 1 M for all participating compounds (except water). In an anaerobic environment, NADH will be present at a several-fold higher concentration than NAD. Assuming [NADH]=5×[NAD], we calculated the effect on $\Delta G_r'$ using the equation

$$\Delta G_f' = \Delta G_f^{0'} + RT\ln\frac{\prod[prod]}{\prod[react]} \tag{3}$$

[0148] This change results in a difference of about 1 kcal/mol in the delta G values for succinic semialdehyde dehydrogenase. Equation 3 was also used to calculate other effects on ΔG_r, such as high succinate concentration to drive the reactions. A 1000-fold difference in the concentrations of succinate and succinic semialdehyde will contribute about 5 kcal/mol to delta G. Taken together with an assumed uncertainty of 8 kcal/mol, the possibility that succinic semialdehyde dehydrogenase will operate in the direction towards succinic semialdehyde under some set of physiological conditions cannot be eliminated. Thus, the direct route from succinate to 4-HB remains a consideration in subsequent analysis.

[0149] The microbial production capabilities of 4-hydroxybutyrate were explored in two microbes, *Escherichia coli* and *Saccharomyces cerevisiae*, using in silico metabolic models of each organism. Potential pathways to 4-HB proceed via a succinate, succinyl-CoA, or alpha-ketoglutarate intermediate as shown in FIG. **2**.

[0150] A first step in the 4-HB production pathway from succinate involves the conversion of succinate to succinic semialdehyde via an NADH- or NADPH-dependant succinic semialdehyde dehydrogenase. In *E. coli*, gabD is an NADP-dependant succinic semialdehyde dehydrogenase and is part of a gene cluster involved in 4-aminobutyrate uptake and degradation (Niegemann et al., *Arch. Microbiol.* 160:454-460 (1993); Schneider et al., *J. Bacteriol.* 184:6976-6986 (2002)). sad is believed to encode the enzyme for NAD-dependant succinic semialdehyde dehydrogenase activity (Marek and Henson, supra). *S. cerevisiae* contains only the NADPH-dependant succinic semialdehyde dehydrogenase, putatively assigned to UGA2, which localizes to the cytosol (Huh et al., *Nature* 425:686-691 (2003)). The maximum yield calculations assuming the succinate pathway to 4-HB in both *E. coli* and *S. cerevisiae* require only the assumption that a non-native 4-HB dehydrogenase has been added to their metabolic networks.

[0151] The pathway from succinyl-CoA to 4-hydroxybutyrate was described in U.S. Pat. No. 6,117,658 as part of a process for making polyhydroxyalkanoates comprising 4-hydroxybutyrate monomer units. *Clostridium kluyveri* is one example organism known to possess CoA-dependant succinic semialdehyde dehydrogenase activity (Sohling and Gottschalk, supra; Sohling and Gottschalk, supra). In this study, it is assumed that this enzyme, from *C. kluyveri* or another organism, is expressed in *E. coli* or *S. cerevisiae* along with a non-native or heterologous 4-HB dehydrogenase to complete the pathway from succinyl-CoA to 4-HB. The pathway from alpha-ketoglutarate to 4-HB was demonstrated in *E. coli* resulting in the accumulation of poly(4-hydroxybutyric acid) to 30% of dry cell weight (Song et al., supra). As *E. coli* and *S. cerevisiae* natively or endogenously possess both glutamate:succinic semialdehyde transaminase and glutamate decarboxylase (Coleman et al., *J. Biol. Chem.* 276: 244-250 (2001)), the pathway from AKG to 4-HB can be completed in both organisms by assuming only that a non-native 4-HB dehydrogenase is present.

EXAMPLE II

Production of 4-Hydroxybutanoic Acid in *E. coli*

[0152] This Example describes the biosynthetic yields for 4-hydroxybutanoic acid resulting from each biochemical pathway.

[0153] In this section, the maximum theoretical yields of 4-HB from glucose are calculated assuming that each of the three metabolic pathways depicted in FIG. **2** are functional in *E. coli*. A genome-scale metabolic model of *E. coli*, similar to the one described in Reed et al., *Genome Biol.* 4:R54 (2003), was used as the basis for the analysis. The energetic gain, in terms of ATP molecules produced, of each maximum yielding pathway is calculated assuming anaerobic conditions, unless otherwise stated. 4-Hydroxybutyrate is assumed to exit in *E. coli* via proton symport, as is the case with most organic acids. It is also possible that GBL is secreted by simple diffusion, and in this case the energetics would be more favorable than in the case considered here. The impact of cofactor specificity (i.e., NADH or NADPH-dependence) of the participating enzymes on the maximum yield and energetics of each pathway also was investigated.

[0154] The results from the analysis are shown in Tables 3 A-C. From an energetic and yield standpoint, the succinate to 4-HB pathway is the most promising. Specifically, the calculations reveal that the maximum theoretical yield of 4-HB from glucose is 1.33 mol/mol (0.77 g/g; 0.89 Cmol/Cmol) assuming the succinate to 4-HB pathway is functional. In addition, the anaerobic production of 4-HB via succinate would result in the net production of either 1.8, 1.5, or 1.1 mol of ATP per glucose depending upon the assumed cofactor specificity of the participating enzymes. These energetic yields are comparable to the 2.0 ATP per glucose that can be obtained via substrate level phosphorylation by the production of ethanol or lactate suggesting the potential for anaerobic homo-4-HB production in *E. coli*.

[0155] The succinyl-CoA route to 4-HB is another promising pathway when considering maximum yield and energetics. A 1.33 mol/mol yield of 4-HB is achievable in *E. coli* if at least one of the pathway steps is assumed NADH-dependant. However, because this pathway requires the formation of succinyl-CoA, its energetic yield is lower than that of the succinate pathway. An oxygen requirement is anticipated at high 4-HB yields if both the CoA-dependant succinic semialdehyde dehydrogenase and 4-HB dehydrogenase steps are assumed NADPH-dependant. In this case, the production of 4-HB at the maximum yield would result in no net ATP gain and possibly not support the energetic maintenance demands needed for *E. coli* survival. Thus, some energy would have to originate from oxidative phosphorylation to enable homo-fermentative 4-HB production. The alpha-ketoglutarate pathway utilizing glutamate:succinate semialdehyde transaminase and glutamate decarboxylase toward 4-HB is the least favorable of the three potential routes with a maximum achievable yield of 1.0 mol 4-HB per mol of glucose. In addition to the lower maximum yield, this pathway requires the utilization of 1.5 moles of oxygen per mol of glucose converted to 4-HB. The energetics of this pathway are unaffected by the assumed cofactor specificity of 4-HB dehydrogenase.

TABLE 3

The overall substrate conversion stoichiometry to 4-HB assuming the A) succinate, B) succinyl-CoA, or C) alpha-ketoglutarate production routes are functional in *E. coli*.
Glucose and oxygen are taken up while all other molecules are produced.

A) Succinate Pathway

Cofactor Specificity	2 NADH steps	1 NADH step 1 NADPH step	2 NADPH steps
Glucose	−1.000	−1.000	−1.000
Oxygen	0.000	0.000	0.000
Protons	1.333	1.333	1.333
4HB	1.333	1.333	1.333
CO2	0.667	0.667	0.667
H2O	0.667	0.667	0.667
ATP	1.800	1.510	1.097

B) Succinyl-CoA Pathway

Cofactor Specificity	2 NADH steps	1 NADH step 1 NADPH step	2 NADPH steps	2 NADPH steps
Glucose	−1.000	−1.000	−1.000	−1.000
Oxygen	0.000	0.000	−0.036	0.000
Protons	1.333	1.333	1.325	1.294
4HB	1.333	1.333	1.325	1.294
CO2	0.667	0.667	0.698	0.082
H2O	0.667	0.667	0.698	0.470
ATP	0.467	0.177	0.000	0.000

C) Alpha-ketoglutarate Pathway

Cofactor Specificity	1 NADH step	1 NADPH step
Glucose	−1.000	−1.000
Oxygen	−1.500	−1.500
Protons	1.000	1.000
4HB	1.000	1.000
CO2	2.000	2.000
H2O	2.000	2.000
ATP	5.500	5.500

[0156] In order to corroborate the computational predictions proposed in this report, the strains expressing a complete pathway to 4-HB can be constructed and tested. Corroboration is performed with both *E. coli* (Examples II and IV) and *S. cerevisiae* (Example III). In *E. coli*, the relevant genes are expressed in a synthetic operon behind an inducible promoter on a medium- or high-copy plasmid; for example the P_{BAD} promoter which is induced by arabinose, on a plasmid of the pBAD series (Guzman et al., *J. Bacteriol.* 177: 4121-4130 (1995)). In *S. cerevisiae*, genes are integrated into the chromosome behind the PDC1 promoter, replacing the native pyruvate carboxylase gene. It has been reported that this results in higher expression of foreign genes than from a plasmid (Ishida et al., *Appl. Environ. Microbiol.* 71:1964-1970 (2005)), and will also ensure expression during anaerobic conditions.

[0157] Cells containing the relevant constructs are grown in minimal media containing glucose, with addition of arabinose in the case of *E. coli* containing genes expressed under the P_{BAD} promoter. Periodic samples are taken for both gene expression and enzyme activity analysis. Enzyme activity assays are performed on crude cell extracts using procedures well known in the art. Alternatively, assays based on the oxidation of NAD(P)H, which is produced in all dehydrogenase reaction steps and detectable by spectrophotometry can be utilized. In addition, antibodies can be used to detect the level of particular enzymes. In lieu of or in addition to enzyme activity measurements, RNA can be isolated from parallel samples and transcript of the gene of interest measured by reverse transcriptase PCR. Any constructs lacking detectable transcript expression are reanalyzed to ensure the encoding nucleic acids are harbored in an expressible form. Where transcripts are detected, this result indicates either a lack of translation or production of inactive enzyme. A variety of methods well known in the art can additionally be employed, such as codon optimization, engineering a strong ribosome binding site, use of a gene from a different species, and prevention of N-glycosylation (for expression of bacterial enzymes in yeast) by conversion of Asn residues to Asp. Once all required enzyme activities are detected, the next step is to measure the production of 4-HP in vivo. Triplicate shake flask cultures are grown either anaerobically or microaerobically, depending on the conditions required (see above), and periodic samples taken. Organic acids present in the culture supernatants are analyzed by HPLC using the Aminex AH-87× column. The elution time of 4-HB will be determined using a standard purchased from a chemical supplier.

[0158] The CoA-independent pathway can be implemented and tested for corroboration. In this case, the genes overexpressed are the native succinic semialdehyde dehydrogenase from each organism, and the 4-hydroxybutanoate dehydrogenase from *Ralstonia eutropha*. Once both enzyme activities are detected as discussed above, the strains are tested for 4-HB production. Corroboration also can be obtained from implementing the CoA-dependent pathway. The CoA-dependent succinic semialdehyde dehydrogenase and the 4-hydroxybutanoate dehydrogenase from *Clostridium kluyveri* are expressed as described above. In addition, overexpression of the native succinyl-CoA synthetase also can be performed, to funnel more succinate into the heterologous pathway. Finally, if 4-HB production is unfavorable, different culture conditions can be tested, such as a change in oxygenation status which can manipulate the NAD(P)H/NAD(P) ratio.

EXAMPLE III

Production of 4-Hydroxybutanoic Acid in Yeast

[0159] This Example describes the biosynthetic yields for 4-hydroxybutanoic acid resulting from each biochemical pathway in *S. cerevisiae*.

[0160] In this section, the maximum theoretical yields of 4-HB from glucose are calculated assuming that each of the three metabolic pathways depicted in FIG. **2** are functional in *S. cerevisiae*. A genome-scale metabolic model of *S. cerevisiae*, similar to the one described in Forster et al. *Genome Res.* 13:244-253 (2003) was used as the basis for the analysis. The energetic gain of each maximum yielding pathway is calculated assuming anaerobic conditions unless otherwise stated. 4-hydroxybutyrate is assumed to exit *S. cerevisiae* via proton symport, as is the case with most organic acids. The impact of cofactor specificity (i.e., NADH or NADPH-dependence) of the participating enzymes on the maximum yield and energetics of each pathway was also investigated.

[0161] The results from the analysis are shown in Tables 4 A-C. As with *E. coli*, the succinate to 4-HB pathway is the most promising provided that the thermodynamic concerns raised in Example I can be overcome. The calculations reveal that the maximum theoretical yield of 4-HB from glucose is 1.33 mol/mol (0.77 g/g; 0.89 Cmol/Cmol) in *S. cerevisiae*. In addition, the anaerobic production of 4-HB via succinate would result in the net production of either 1.4, 1.1, or 0.5 mol of ATP per glucose depending upon the assumed cofactor specificity of the participating enzymes.

[0162] The succinyl-CoA route to 4-HB is the second most favorable pathway. A maximum yield of 1.33 mol 4-HB/mol glucose is achievable in *S. cerevisiae* regardless of cofactor specificity. However, net energy generation at the maximum theoretical yield is possible only if both the CoA-dependant succinic semialdehyde dehydrogenase and 4-HB dehydrogenase steps are assumed to be NADH-dependant. If either step is NADPH-dependant, no net ATP will be gained from anaerobic 4-HB production and an alternate energy source (e.g., oxidative phosphorylation) would be required to support cell growth and maintenance. The alpha-ketoglutarate route toward 4-HB is the least favorable of the three potential pathways in *S. cerevisiae* although the maximum yield of 1.1-1.2 mol 4-HB per mol glucose is slightly higher than was found in *E. coli*. Nevertheless, this pathway requires an oxygen uptake of 0.8-0.9 mol oxygen per mol glucose to become energetically neutral.

TABLE 4

The overall substrate conversion stoichiometry to 4-HB in *S. cerevisiae*., assuming the A) succinate, B) succinyl-CoA, or C) alpha-ketoglutarate production routes are functional in *S. cerevisiae*. Glucose and oxygen are taken up while all other molecules are produced.

Cofactor Specificity	2 NADH steps	1 NADH step 1 NADPH step	2 NADPH steps
	A) Succinate Pathway		
Glucose	−1.000	−1.000	−1.000
Oxygen	0.000	0.000	0.000
Protons	1.333	1.333	1.333
4HB	1.333	1.333	1.333
CO2	0.667	0.667	0.667
H2O	0.667	0.667	0.667
ATP	1.444	1.067	0.533
	B) Succinyl-CoA Pathway		
Glucose	−1.000	−1.000	−1.000
Oxygen	0.000	0.000	0.000
Protons	1.333	1.333	1.333
4HB	1.333	1.333	1.333
CO2	0.667	0.667	0.667
H2O	0.667	0.667	0.667
ATP	0.533	0.000	0.000

C) Alpha-ketoglutarate Pathway

Cofactor Specificity	1 NADH step	1 NADPH step
Glucose	−1.000	−1.000
Oxygen	−0.785	−0.879
Protons	1.159	1.138
4HB	1.159	1.138
CO2	1.364	1.448
H2O	1.364	1.448
ATP	0.000	0.000

EXAMPLE IV

Biosynthesis of 1,4-Butanediol from Succinate and α-Ketoglutarate

[0163] This Example illustrates the construction and biosynthetic production of 4-HB and BDO from microbial organisms.

[0164] As described previously in Examples I-III, the thermodynamic characteristics of the biotransformation steps from 4-HB to BDO shown in FIG. **1** also were calculated based on standard Gibbs free energy of formation determined by group contribution. The results are provide in Table 5. Similarly, although some of the steps have calculated positive delta G values, the standard errors for these calculations and concentration gradients indicate that any of the steps are feasible.

TABLE 5

Gibbs free energy of reaction (kcal/mole) under standard conditions (pH and ionic strength values).

Reaction	$\Delta G_r^{o\prime}$ pH = 7.0 IS = 0
4-HB + NADH + H+ → 4-HBald + NAD	2.4
4-HB + acetyl-CoA → 4-HB-CoA + acetate	−5.0
4-HB-CoA + NADH + H+ → 4-HBald + NAD + CoA	9.5
4-HBald + NADH + H+ → bdo + NAD	−5.0

Temperature was constant at 298 K.
Error bars for these values are on the order of 8 kcal/mol, as calculated by equation 2.
Abbreviations:
4-HBald, 4-hydroxybutyraldehyde.

[0165] Theoretical yields were calculated assuming all the pathways in FIG. **2** are incorporated into *E. coli*. A genome-scale metabolic model of *E. coli*, similar to the one described in Reed et al., *Genome Biol* 4:R54 (2003), was used as the basis for the analysis. The maximum theoretical yield assuming energetic neutrality and no cell growth or maintenance was 1.09 mol BDO/mol glucose under microaerobic conditions. Simulations performed under anaerobic conditions, which can be utilized to drive the pathway toward BDO production, either acetate or ethanol is produced as a co-product. Under these conditions, the maximum yields were 1.04 and 1.00 mol/mol, respectively. One alternative is to add limiting amounts of nitrate as an electron acceptor, thus controlling the amount of respiration that can occur. Under this condition, the maximum yield returns to 1.09 mol/mol. Another alternative is to replace the native *E. coli* phosphoenolpyruvate (PEP) carboxylase with a heterologous or engineered phosphoenolpyruvate carboxykinase that is capable of functioning in the direction of PEP carboxylation. This enzyme produces ATP, whereas the PEP carboxylase does not. Under this assumption, the maximum yield returns to 1.09 mol/mol.

[0166] In addition, there are several alternative enzymes that can be utilized in the pathway described above. The native or endogenous enzyme for conversion of succinate to succinyl-CoA (Step **1** in FIG. **2**) can be replaced by a CoA transferase such as that encoded by the cat1 gene *C. kluyveri* (Sohling, B. and G. Gottschalk, *Eur. J. Biochem.* 212:121-127 (1993)), which functions in a similar manner to Step **9**. However, the production of acetate by this enzyme may not be optimal, as it might be secreted rather than being converted back to acetyl-CoA. In this respect, it also can be beneficial to eliminate acetate formation in Step **9**. As one alternative to this CoA transferase, a mechanism can be employed in which the 4-HB is first phosphorylated by ATP and then converted to the CoA derivative, similar to the acetate kinase/phosphotransacetylase pathway in *E. coli* for the conversion of acetate to acetyl-CoA. The net cost of this route is one ATP, which is the same as is required to regenerate acetyl-CoA from acetate. The enzymes phosphotransbutyrylase (ptb) and butyrate kinase (bk) are known to carry out these steps on the non-hydroxylated molecules for butyrate production in *C. acetobutylicum* (Cary et al., *Appl Environ Microbiol* 56:1576-1583 (1990); Valentine, R. C. and R. S. Wolfe, *J Biol Chem.* 235:1948-1952 (1960)). These enzymes are reversible, allowing synthesis to proceed in the direction of 4-HB.

[0167] BDO also can be produced via α-ketoglutarate in addition to or instead of through succinate. A described previously, and exemplified further below, one pathway to accomplish product biosynthesis is with the production of succinic semialdehyde via α-ketoglutarate using the endogenous enzymes (FIG. **2**, Steps **4-5**). An alternative is to use an α-ketoglutarate decarboxylase that can perform this conversion in one step (FIG. **2**, Step **8**; Tian et al., *Proc Natl Acad Sci USA* 102:10670-10675 (2005)).

[0168] For the construction of different strains of BDO-producing microbial organisms, a list of applicable genes was assembled for corroboration. Briefly, one or more genes within the 4-HB and/or BDO biosynthetic pathways were identified for each step of the complete BDO-producing pathway shown in FIG. **2**, using available literature resources, the NCBI genetic database, and homology searches. The genes cloned and assessed in this study are presented below in Table 6, along with the appropriate references and URL citations to the polypeptide sequence. As discussed further below, some genes were synthesized for codon optimization while others were cloned via PCR from the genomic DNA of the native or wild-type organism. For some genes both approaches were used, and in this case the native genes are indicated by an "n" suffix to the gene identification number when used in an experiment. Note that only the DNA sequences differ; the proteins are identical.

TABLE 6

Genes expressed in host BDO-producting microbial organisms.

Gene ID number	Reaction number (FIG. 1)	Gene name	Source organism	Enzyme name	Link to protein sequence	Reference
0001	9	Cat2	*Clostridium kluyveri* DSM 555	4-hydroxybutyrate coenzyme A transferase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=1228100	(29)d}

TABLE 6-continued

Genes expressed in host BDO-producting microbial organisms.

Gene ID number	Reaction number (FIG. 1)	Gene name	Source organism	Enzyme name	Link to protein sequence	Reference
0002	12/13	adhE	*Clostridium acetobutylicum* ATCC 824	Aldehyde/alcohol dehydrogenase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&val=15004739	(22)d}
0003	12/13	adhE2	*Clostridium acetobutylicum* ATCC 824	Aldehyde/alcohol dehydrogenase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=NP__149325.1	(12)d}
0004	1	Cat1	*Clostridium kluyveri* DSM 555	Succinate coenzyme A transferase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=1228100	(29)d}
0008	6	sucD	*Clostridium kluyveri* DSM 555	Succinic semialdehyde dehydrogenase (CoA-dependent)	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=1228100	(29)d}
0009	7	4-HBd	*Ralstonia eutropha H16*	4-hydroxybutyrate dehydrogenase (NAD-dependent)	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=YP__726053.1	(32)d}
0010	7	4-HBd	*Clostridium kluyveri* DSM 555	4-hydroxybutyrate dehydrogenase (NAD-dependent)	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=1228100	(29)d}
0011	12/13	adhE	*E. coli*	Aldehyde/alcohol dehydrogenase	www.shigen.nig.ac.jp/*ecoli*/pec/genes.List.DetailAction.do?fromListFlag=true&featureType=1&orfId=1219	
0012	12/13	yqhD	*E. coli*	Aldehyde/alcohol dehydrogenase	www.shigen.nig.ac.jp/*ecoli*/pec/genes.List.DetailAction.do	
0013	13	bdhB	*Clostridium acetobutylicum* ATCC 824	Butanol dehydrogenase II	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=NP__349891.1	(35)d}
0020	11	ptb	*Clostridium acetobutylicum* ATCC 824	Phospho-transbutyrylase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&id=15896327	(4)d}
0021	10	buk1	*Clostridium acetobutylicum* ATCC 824	Butyrate kinase I	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&id=20137334	(4)d}
0022	10	buk2	*Clostridium acetobutylicum* ATCC 824	Butyrate kinase II	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&id=20137415	(4)d}
0023	13	adhEm	isolated from metalibrary of anaerobic sewage digester microbial consortia	Alcohol dehydrogenase		(37)d}
0024	13	adhE	*Clostridium thermocellum*	Alcohol dehydrogenase	www.genome.jp/dbget-bin/www__bget?cth:Cthe__0423	
0025	13	ald	*Clostridium beijerinckii*	Coenzyme A-acylating aldehyde dehydrogenase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&id=49036681	(31)d}
0026	13	bdhA	*Clostridium acetobutylicum* ATCC 824	Butanol dehydrogenase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=NP__349892.1	(35)d}
0027	12	bld	*Clostridium saccharoperbutylacetonicum*	Butyraldehyde dehydrogenase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&id=31075383	(18)d}
0028	13	bdh	*Clostridium saccharoperbutylacetonicum*	Butanol dehydrogenase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&id=124221917	(18)d}
0029	12/13	adhE	*Clostridium tetani*	Aldehyde/alcohol dehydrogenase	www.genome.jp/dbget-bin/www__bget?ctc:CTC01366	
0030	12/13	adhE	*Clostridium perfringens*	Aldehyde/alcohol dehydrogenase	www.genome.jp/dbget-bin/www__bget?cpe:CPE2531	
0031	12/13	adhE	*Clostridium difficile*	Aldehyde/alcohol dehydrogenase	www.genome.jp/dbget-bin/www__bget?cdf:CD2966	
0032	8	sucA	*Mycobacterium bovis* BCG, Pasteur	α-ketoglutarate decarboxylase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=YP__977400.1	(30)d}
0033	9	cat2	*Clostridium aminobutyricum*	4-hydroxybutyrate coenzyme A transferase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&val=6249316	
0034	9	cat2	*Porphyromonas gingivalis* W83	4-hydroxybutyrate coenzyme A transferase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&val=34541558	

TABLE 6-continued

Genes expressed in host BDO-producting microbial organisms.

Gene ID number	Reaction number (FIG. 1)	Gene name	Source organism	Enzyme name	Link to protein sequence	Reference
0035	6	sucD	*Porphyromonas gingivalis* W83	Succinic semialdehyde dehydrogenase (CoA-dependent)	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=NP__904963.1	
0036	7	4-HBd	*Porphyromonas gingivalis* W83	NAD-dependent 4-hydroxybutyrate dehydrogenase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?val=NP__904964.1	
0037	7	gbd	Uncultured bacterium	4-hydroxybutyrate dehydrogenase	www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=5916168	(16)d}
0038	1	sucCD	*E. coli*	Succinyl-CoA synthetase	www.shigen.nig.ac.jp/*ecoli*/pec/genes.List.DetailAction.do	

[0169] Expression Vector Construction for BDO pathway. Vector backbones and some strains were obtained from Dr. Rolf Lutz of Expressys (www.expressys.de/). The vectors and strains are based on the pZ Expression System developed by Dr. Rolf Lutz and Prof. Hermann Bujard (Lutz, R. and H. Bujard, *Nucleic Acids Res* 25:1203-1210 (1997)). Vectors obtained were pZE13luc, pZA33luc, pZS*13luc and pZE22luc and contained the luciferase gene as a stuffer fragment. To replace the luciferase stuffer fragment with a lacZ-alpha fragment flanked by appropriate restriction enzyme sites, the luciferase stuffer fragment was first removed from each vector by digestion with EcoRI and XbaI. The lacZ-alpha fragment was PCR amplified from pUC19 with the following primers:

```
lacZalpha-RI
5'GACGAATTCGCTAGCAAGAGGAGAAGTCGACATGTCCAATTCACTGGC
CGTCGTTTTAC3'

lacZalpha 3'BB
5'-GACCCTAGGAAGCTTTCTAGAGTCGACCTATGCGGCATCAGAGCAG
A-3'.
```

[0170] This generated a fragment with a 5' end of EcoRI site, NheI site, a Ribosomal Binding Site, a SalI site and the start codon. On the 3' end of the fragment contained the stop codon, XbaI, HindIII, and AvrII sites. The PCR product was digested with EcoRI and AvrII and ligated into the base vectors digested with EcoRI and XbaI (XbaI and AvrII have compatible ends and generate a non-site). Because NheI and XbaI restriction enzyme sites generate compatible ends that can be ligated together (but generate a NheI/XbaI non-site that is not digested by either enzyme), the genes cloned into the vectors could be "Biobricked" together (http://openwetware.org/wiki/Synthetic_Biology:BioBricks). Briefly, this method enables joining an unlimited number of genes into the vector using the same 2 restriction sites (as long as the sites do not appear internal to the genes), because the sites between the genes are destroyed after each addition.

[0171] All vectors have the pZ designation followed by letters and numbers indication the origin of replication, antibiotic resistance marker and promoter/regulatory unit. The origin of replication is the second letter and is denoted by E for ColE1, A for p15A and S for pSC101-based origins. The first number represents the antibiotic resistance marker (1 for Ampicillin, 2 for Kanamycin, 3 for Chloramphenicol, 4 for Spectinomycin and 5 for Tetracycline). The final number defines the promoter that regulated the gene of interest (1 for $P_{LtetO\text{-}1}$, 2 for $P_{LlacO\text{-}1}$, 3 for $P_{AllacO\text{-}1}$, and 4 for $P_{lac/ara\text{-}1}$). The MCS and the gene of interest follows immediately after. For the work discussed here we employed two base vectors, pZA33 and pZE13, modified for the biobricks insertions as discussed above. Once the gene(s) of interest have been cloned into them, resulting plasmids are indicated using the four digit gene codes given in Table 6; e.g., pZA33-XXXX-YYYY- . . . .

[0172] Host Strain Construction. The parent strain in all studies described here is *E. coli* K-12 strain MG1655. Markerless deletion strains in adhE, gabD, and aldA were constructed under service contract by a third party using the redET method (Datsenko, K. A. and B. L. Wanner, *Proc Natl Acad Sci USA* 97:6640-6645 (2000)). Subsequent strains were constructed via bacteriophage P1 mediated transduction (Miller, J. 1973. Experiments in Molecular Genetics. Cold Spring Harbor Laboratories, New York). Strain C600Z1 (laci^q, PN25-tetR, Sp^R, lacY1, leuB6,mcrB+, supE44, thi-1, thr-1, tonA21) was obtained from Expressys and was used as a source of a lacI^q allele for P1 transduction. Bacteriophage P1vir was grown on the C600Z1 *E. coli* strain, which has the spectinomycin resistance gene linked to the lacI^q. The P1 lysate grown on C600Z1 was used to infect MG1655 with selection for spectinomycin resistance. The spectinomycin resistant colonies were then screened for the linked lacIq by determining the ability of the transductants to repress expression of a gene linked to a $P_{AllacO\text{-}1}$ promoter. The resulting strain was designated MG1655 lacI^q. A similar procedure was used to introduce lacI^Q into the deletion strains.

[0173] Production of 4-HB From Succinate. For construction of a 4-HB producer from succinate, genes encoding steps from succinate to 4-HB and 4-HB-CoA (**1**, **6**, **7**, and **9** in FIG. **2**) were assembled onto the pZA33 and pZE13 vectors as described below. Various combinations of genes were assessed, as well as constructs bearing incomplete pathways as controls (Tables 7 and 8). The plasmids were then transformed into host strains containing lacI^Q, which allow inducible expression by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG). Both wild-type and hosts with deletions in genes encoding the native succinic semialdehyde dehydrogenase (step **2** in FIG. **1**) were tested.

[0174] Activity of the heterologous enzymes were first tested in in vitro assays, using strain MG1655 lacI^Q as the host for the plasmid constructs containing the pathway genes. Cells were grown aerobically in LB media (Difco) containing the appropriate antibiotics for each construct, and induced by addition of IPTG at 1 mM when the optical density (OD600) reached approximately 0.5. Cells were harvested after 6 hours, and enzyme assays conducted as discussed below.

[0175] In Vitro Enzyme Assays. To obtain crude extracts for activity assays, cells were harvested by centrifugation at 4,500 rpm (Beckman-Coulter, Allegera X-15R) for 10 min. The pellets were resuspended in 0.3 mL BugBuster (Novagen) reagent with benzonase and lysozyme, and lysis proceeded for 15 minutes at room temperature with gentle shaking. Cell-free lysate was obtained by centrifugation at 14,000 rpm (Eppendorf centrifuge 5402) for 30 min at 4° C. Cell protein in the sample was determined using the method of Bradford et al., *Anal. Biochem.* 72:248-254 (1976), and specific enzyme assays conducted as described below. Activities are reported in Units/mg protein, where a unit of activity is defined as the amount of enzyme required to convert 1 μmol of substrate in 1 min. at room temperature. In general, reported values are averages of at least 3 replicate assays.

[0176] Succinyl-CoA transferase (Cat1) activity was determined by monitoring the formation of acetyl-CoA from succinyl-CoA and acetate, following a previously described procedure Sohling and Gottschalk, *J. Bacteriol.* 178:871-880 (1996). Succinyl-CoA synthetase (SucCD) activity was determined by following the formation of succinyl-CoA from succinate and CoA in the presence of ATP. The experiment followed a procedure described by Cha and Parks, *J. Biol. Chem.* 239:1961-1967 (1964). CoA-dependent succinate semialdehyde dehydrogenase (SucD) activity was determined by following the conversion of NAD to NADH at 340 nm in the presence of succinate semialdehyde and CoA (Sohling and Gottschalk, *Eur. J. Biochem.* 212:121-127 (1993)). 4-HB dehydrogenase (4-HBd) enzyme activity was determined by monitoring the oxidation of NADH to NAD at 340 nm in the presence of succinate semialdehyde. The experiment followed a published procedure Gerhardt et al. *Arch. Microbiol.* 174:189-199 (2000). 4-HB CoA transferase (Cat2) activity was determined using a modified procedure from Scherf and Buckel, *Appl. Environ. Microbiol.* 57:2699-2702 (1991). The formation of 4-HB-CoA or butyryl-CoA formation from acetyl-CoA and 4-HB or butyrate was determined using HPLC.

[0177] Alcohol (ADH) and aldehyde (ALD) dehydrogenase was assayed in the reductive direction using a procedure adapted from several literature sources (Durre et al., *FEMS Microbiol. Rev.* 17:251-262 (1995); Palosaari and Rogers, *J. Bacteriol.* 170:2971-2976 (1988) and Welch et al., *Arch. Biochem. Biophys.* 273:309-318 (1989). The oxidation of NADH is followed by reading absorbance at 340 nM every four seconds for a total of 240 seconds at room temperature. The reductive assays were performed in 100 mM MOPS (adjusted to pH 7.5 with KOH), 0.4 mM NADH, and from 1 to 50 μl of cell extract. The reaction is started by adding the following reagents: 100 μl of 100 mM acetaldehyde or butyraldehyde for ADH, or 100 μl of 1 mM acetyl-CoA or butyryl-CoA for ALD. The Spectrophotometer is quickly blanked and then the kinetic read is started. The resulting slope of the reduction in absorbance at 340 nM per minute, along with the molar extinction coefficient of NAD(P)H at 340 nM (6000) and the protein concentration of the extract, can be used to determine the specific activity.

[0178] The enzyme activity of PTB is measured in the direction of butyryl-CoA to butyryl-phosphate as described in Cary et al. *J. Bacteriol.* 170:4613-4618 (1988). It provides inorganic phosphate for the conversion, and follows the increase in free CoA with the reagent 5,5'-dithiobis-(2-nitrobenzoic acid), or DTNB. DTNB rapidly reacts with thiol groups such as free CoA to release the yellow-colored 2-nitro-5-mercaptobenzoic acid (TNB), which absorbs at 412 nm with a molar extinction coefficient of 14,140 M cm^{-1}. The assay buffer contained 150 mM potassium phosphate at pH 7.4, 0.1 mM DTNB, and 0.2 mM butyryl-CoA, and the reaction was started by addition of 2 to 50 μL cell extract. The enzyme activity of BK is measured in the direction of butyrate to butyryl-phosphate formation at the expense of ATP. The procedure is similar to the assay for acetate kinase previously described Rose et al., *J. Biol. Chem.* 211:737-756 (1954). However we have found another acetate kinase enzyme assay protocol provided by Sigma to be more useful and sensitive. This assay links conversion of ATP to ADP by acetate kinase to the linked conversion of ADP and phosphoenol pyruvate (PEP) to ATP and pyruvate by pyruvate kinase, followed by the conversion of pyruvate and NADH to lactate and NAD+ by lactate dehydrogenase. Substituting butyrate for acetate is the only major modification to enable the assay to follow BK enzyme activity. The assay mixture contained 80 mM triethanolamine buffer at pH 7.6, 200 mM sodium butyrate, 10 mM MgCl2, 0.1 mM NADH, 6.6 mM ATP, 1.8 mM phosphoenolpyruvate. Pyruvate kinase, lactate dehydrogenase, and myokinase were added according to the manufacturer's instructions. The reaction was started by adding 2 to 50 μL cell extract, and the reaction was monitored based on the decrease in absorbance at 340 nm indicating NADH oxidation.

[0179] Analysis of CoA Derivatives by HPLC. An HPLC based assay was developed to monitor enzymatic reactions involving coenzyme A (CoA) transfer. The developed method enabled enzyme activity characterization by quantitative determination of CoA, acetyl CoA (AcCoA), butyryl CoA (BuCoA) and 4-hydroxybutyrate CoA (4-HBCoA) present in in-vitro reaction mixtures. Sensitivity down to low μM was achieved, as well as excellent resolution of all the CoA derivatives of interest.

[0180] Chemical and sample preparation was performed as follows. Briefly, CoA, AcCoA, BuCoA and all other chemicals, were obtained from Sigma-Aldrich. The solvents, methanol and acetonitrile, were of HPLC grade. Standard calibration curves exhibited excellent linearity in the 0.01-1 mg/mL concentration range. Enzymatic reaction mixtures contained 100 mM Tris HCl buffer (pH 7), aliquots were taken at different time points, quenched with formic acid (0.04% final concentration) and directly analyzed by HPLC.

[0181] HPLC analysis was performed using an Agilent 1100 HPLC system equipped with a binary pump, degasser, thermostated autosampler and column compartment, and diode array detector (DAD), was used for the analysis. A reversed phase column, Kromasil 100 5 um C18, 4.6×150 mm (Peeke Scientific), was employed. 25 mM potassium phosphate (pH 7) and methanol or acetonitrile, were used as aqueous and organic solvents at 1 mL/min flow rate. Two methods were developed: a short one with a faster gradient for the analysis of well-resolved CoA, AcCoA and BuCoA, and a longer method for distinguishing between closely eluting AcCoA and 4-HBCoA. Short method employed acetonitrile gradient (0 min-5%, 6 min-30%, 6.5 min-5%, 10 min-5%) and resulted in the retention times 2.7, 4.1 and 5.5 min for CoA, AcCoA and BuCoA, respectively. In the long method methanol was used with the following linear gradient: 0 min-5%, 20 min-35%, 20.5 min-5%, 25 min-5%. The retention times for CoA, AcCoA, 4-HBCoA and BuCoA were 5.8, 8.4, 9.2 and 16.0 min, respectively. The injection volume was 5 μL, column temperature 30° C., and UV absorbance was monitored at 260 nm.

[0182] The results demonstrated activity of each of the four pathway steps (Table 6), though activity is clearly dependent on the gene source, position of the gene in the vector, and the context of other genes with which it is expressed. For example, gene 0035 encodes a succinic semialdehyde dehydrogenase that is more active than that encoded by 0008, and 0036 and 0010n are more active 4-HB dehydrogenase genes than 0009. There also seems to be better 4-HB dehydrogenase activity when there is another gene preceding it on the same operon.

TABLE 7

In vitro enzyme activities in cell extracts from MG1655 $lacI^{Q}$ containing the plasmids expressing genes in the 4-HB-CoA pathway. Activities are reported in Units/mg protein, where a unit of activity is defined as the amount of enzyme required to convert 1 μmol of substrate in 1 min. at room temperature.

Sample #	pZE13 (a)	pZA33 (b)	OD600	Cell Prot (c)	Cat1	SucD	4HBd	Cat2
1	cat1 (0004)		2.71	6.43	1.232	0.00		
2	cat1 (0004)-sucD (0035)		2.03	5.00	0.761	2.57		
3	cat1 (0004)-sucD (0008)		1.04	3.01	0.783	0.01		
4	sucD (0035)		2.31	6.94		2.32		
5	sucD (0008)		1.10	4.16		0.05		
6		4hbd (0009)	2.81	7.94	0.003		0.25	
7		4hbd (0036)	2.63	7.84			3.31	
8		4hbd (0010n)	2.00	5.08			2.57	
9	cat1 (0004)-sucD (0035)	4hbd (0009)	2.07	5.04	0.600	1.85	0.01	
10	cat1 (0004)-sucD (0035)	4hbd (0036)	2.08	5.40	0.694	1.73	0.41	
11	cat1 (0004)-sucD (0035)	4hbd (0010n)	2.44	4.73	0.679	2.28	0.37	
12	cat1 (0004)-sucD (0008)	4hbd (0009)	1.08	3.99	0.572	−0.01	0.02	
13	cat1 (0004)-sucD (0008)	4hbd (0036)	0.77	2.60	0.898	−0.01	0.04	
14	cat1 (0004)-sucD (0008)	4hbd (0010n)	0.63	2.47	0.776	0.00	0.00	
15		cat2 (0034)	2.56	7.86				1.283
16		cat2 (0034)-4hbd (0036)	3.13	8.04			24.86	0.993
17		cat2 (0034)-4hbd (0010n)	2.38	7.03			7.45	0.675
18		4hbd (0036)-cat2 (0034)	2.69	8.26			2.15	7.490
19		4hbd (0010n)-cat2 (0034)	2.44	6.59			0.59	4.101

(a) Genes expressed from Plac on pZE13, a high-copy plasmid with colE1 origin and ampicillin resistance. Gene identification numbers are as given in Table 2

(b) Genes expressed from Plac on pZA33, a medium-copy plasmid with pACYC origin and chloramphenicol resistance.

(c) Cell protein given as mg protein per mL extract.

[0183] Recombinant strains containing genes in the 4-HB pathway were then evaluated for the ability to produce 4-HB in vivo from central metabolic intermediates. Cells were grown anaerobically in LB medium to OD600 of approximately 0.4, then induced with 1 mM IPTG. One hour later, sodium succinate was added to 10 mM, and samples taken for analysis following an additional 24 and 48 hours. 4-HB in the culture broth was analyzed by GC-MS as described below. The results indicate that the recombinant strain can produce over 2 mM 4-HB after 24 hours, compared to essentially zero in the control strain (Table 8).

TABLE 8

Production of 4-HB from succinate in *E. coli* strains harboring plasmids expressing various combinations of 4-HB pathway genes.

Sample				24 Hours			48 Hours		
#	Host Strain	pZE13	pZA33	OD600	4HB, μM	4HB norm. (a)	OD600	4HB, μM	4HB norm. (a)
1	MG1655 lacIq	cat1 (0004)-sucD (0035)	4hbd (0009)	0.47	487	1036	1.04	1780	1711
2	MG1655 lacIq	cat1 (0004)-sucD (0035)	4hbd (0027)	0.41	111	270	0.99	214	217
3	MG1655 lacIq	cat1 (0004)-sucD (0035)	4hbd (0036)	0.47	863	1835	0.48	2152	4484
4	MG1655 lacIq	cat1 (0004)-sucD (0035)	4hbd (0010n)	0.46	956	2078	0.49	2221	4533
5	MG1655 lacIq	cat1 (0004)-sucD (0008)	4hbd (0009)	0.38	493	1296	0.37	1338	3616
6	MG1655 lacIq	cat1 (0004)-sucD (0008)	4hbd (0027)	0.32	26	81	0.27	87	323
7	MG1655 lacIq	cat1 (0004)-sucD (0008)	4hbd (0036)	0.24	506	2108	0.31	1448	4672
8	MG1655 lacIq	cat1 (0004)-sucD (0008)	4hbd (0010n)	0.24	78	324	0.56	233	416

TABLE 8-continued

Production of 4-HB from succinate in *E. coli* strains harboring plasmids expressing various combinations of 4-HB pathway genes.

Sample				24 Hours			48 Hours		
#	Host Strain	pZE13	pZA33	OD600	4HB, μM	4HB norm. (a)	OD600	4HB, μM	4HB norm. (a)
9	MG1655 lacIq gabD	cat1 (0004)-sucD (0035)	4hbd (0009)	0.53	656	1237	1.03	1643	1595
10	MG1655 lacIq gabD	cat1 (0004)-sucD (0035)	4hbd (0027)	0.44	92	209	0.98	214	218
11	MG1655 lacIq gabD	cat1 (0004)-sucD (0035)	4hbd (0036)	0.51	1072	2102	0.97	2358	2431
12	MG1655 lacIq gabD	cat1 (0004)-sucD (0035)	4hbd (0010n)	0.51	981	1924	0.97	2121	2186
13	MG1655 lacIq gabD	cat1 (0004)-sucD (0008)	4hbd (0009)	0.35	407	1162	0.77	1178	1530
14	MG1655 lacIq gabD	cat1 (0004)-sucD (0008)	4hbd (0027)	0.51	19	36	1.07	50	47
15	MG1655 lacIq gabD	cat1 (0004)-sucD (0008)	4hbd (0036)	0.35	584	1669	0.78	1350	1731
16	MG1655 lacIq gabD	cat1 (0004)-sucD (0008)	4hbd (0010n)	0.32	74	232	0.82	232	283
17	MG1655 lacIq	vector only	vector only	0.8	1	2	1.44	3	2
18	MG1655 lacIq gabD	vector only	vector only	0.89	1	2	1.41	7	5

(a) Normalized 4-HB concentration, μM/OD600 units

[0184] An alternate to using a CoA transferase (cat1) to produce succinyl-CoA from succinate is to use the native *E. coli* sucCD genes, encoding succinyl-CoA synthetase. This gene cluster was cloned onto pZE13 along with candidate genes for the remaining steps to 4-HB to create pZE13-0038-0035-0036.

[0185] Production of 4-HB from Glucose. Although the above experiments demonstrate a functional pathway to 4-HB from a central metabolic intermediate (succinate), an industrial process would require the production of chemicals from low-cost carbohydrate feedstocks such as glucose or sucrose. Thus, the next set of experiments was aimed to determine whether endogenous succinate produced by the cells during growth on glucose could fuel the 4-HB pathway. Cells were grown anaerobically in M9 minimal medium (6.78 g/L Na_2HPO_4, 3.0 g/L KH_2PO_4, 0.5 g/L NaCl, 1.0 g/L NH_4Cl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$) supplemented with 20 g/L glucose, 100 mM 3-(N-morpholino)propanesulfonic acid (MOPS) to improve the buffering capacity, 10 μg/mL thiamine, and the appropriate antibiotics. 0.25 mM IPTG was added when OD600 reached approximately 0.2, and samples taken for 4-HB analysis every 24 hours following induction. In all cases 4-HB plateaued after 24 hours, with a maximum of about 1 mM in the best strains (FIG. **11***a*), while the succinate concentration continued to rise (FIG. **11***b*). This indicates that the supply of succinate to the pathway is likely not limiting, and that the bottleneck may be in the activity of the enzymes themselves or in NADH availability. 0035 and 0036 are clearly the best gene candidates for CoA-dependent succinic semialdehyde dehydrogenase and 4-HB dehydrogenase, respectively. The elimination of one or both of the genes encoding known (gabD) or putative (aldA) native succinic semialdehyde dehydrogenases had little effect on performance. Finally, it should be noted that the cells grew to a much lower OD in the 4-HB-producing strains than in the controls (FIG. **11***c*).

[0186] An alternate pathway for the production of 4-HB from glucose is via a-ketoglutarate. We explored the use of an α-ketoglutarate decarboxylase from *Mycobacterium tuberculosis* Tian et al., *Proc. Natl. Acad. Sci. USA* 102:10670-10675 (2005) to produce succinic semialdehyde directly from α-ketoglutarate (step **8** in FIG. **2**). To demonstrate that this gene (0032) was functional in vivo, we expressed it on pZE13 in the same host as 4-HB dehydrogenase (gene 0036) on pZA33. This strain was capable of producing over 1.0 mM 4-HB within 24 hours following induction with 1 mM IPTG (FIG. **12**). Since this strain does not express a CoA-dependent succinic semialdehyde dehydrogenase, the possibility of succinic semialdehyde production via succinyl-CoA is eliminated. It is also possible that the native genes responsible for producing succinic semialdehyde could function in this pathway (steps **4** and **5** in FIG. **2**); however, the amount of 4-HB produced when the pZE13-0032 plasmid was left out of the host is the negligible.

[0187] Production of BDO from 4-HB. The production of BDO from 4-HB required two reduction steps, catalyzed by dehydrogenases. Alcohol and aldehyde dehydrogenases (ADH and ALD, respectively) are NAD+/H and/or NADP+/H-dependent enzymes that together can reduce a carboxylic acid group on a molecule to an alcohol group, or in reverse, can perform the oxidation of an alcohol to a carboxylic acid. This biotransformation has been demonstrated in wild-type *Clostridium acetobutylicum* (Jewell et al., *Current Microbiology,* 13:215-19 (1986)), but neither the enzymes responsible nor the genes responsible were identified. In addition, it is not known whether activation to 4-HB-CoA is first required (step **9** in FIG. **2**), or if the aldehyde dehydrogenase (step **12**) can act directly on 4-HB. We developed a list of candidate enzymes from *C. acetobutylicum* and related organisms based on known activity with the non-hydroxylated analogues to 4-HB and pathway intermediates, or by similarity to these characterized genes (Table 6). Since some of the candidates are multifunctional dehydrogenases, they could potentially catalyze both the NAD(P)H-dependent reduction of the acid (or CoA-derivative) to the aldehyde, and of the aldehyde to the alcohol. Before beginning work with these genes in *E. coli*, we first validated the result referenced above using *C. acetobutylicum* ATCC 824. Cells were grown in Schaedler broth (Accumedia, Lansing, Mich.) supplemented with 10 mM 4-HB, in an anaerobic atmosphere of 10% CO_2, 10% H_2, and 80% N_2 at 30° C. Periodic culture samples were taken, centrifuged, and the broth analyzed for BDO by GC-MS as described below. BDO concentrations of 0.1 mM, 0.9 mM, and 1.5 mM were detected after 1 day, 2 days, and 7 days incubation, respectively. No BDO was detected in culture grown without 4-HB addition. To demonstrate that the BDO produced was derived from glucose, we grew the best BDO producing strain MG1655 lacI^Q pZE13-0004-0035-0002 pZA33-0034-0036 in M9 minimal medium supplemented with 4 g/L uniformly labeled ^{13}C-glucose. Cells were induced at OD of 0.67 with 1 mM IPTG, and a sample taken after 24 hours. Analysis of the culture supernatant was performed by mass spectrometry.

[0188] Gene candidates for the 4-HB to BDO conversion pathway were next tested for activity when expressed in the *E. coli* host MG1655 lacI^Q. Recombinant strains containing each gene candidate expressed on pZA33 were grown in the presence of 0.25 mM IPTG for four hours at 37° C. to fully induce expression of the enzyme. Four hours after induction, cells were harvested and assayed for ADH and ALD activity as described above. Since 4-HB-CoA and 4-hydroxybutyraldehyde are not available commercially, assays were performed using the non-hydroxylated substrates (Table 9). The ratio in activity between 4-carbon and 2-carbon substrates for *C. acetobutylicum* adhE2 (0002) and *E. coli* adhE (0011) were similar to those previously reported in the literature a Atsumi et al., *Biochim. Biophys. Acta.* 1207:1-11 (1994).

TABLE 9

In vitro enzyme activities in cell extracts from MG1655 lacI^Q containing pZA33 expressing gene candidates for aldehyde and alcohol dehydrogenases. Activities are expressed in μmol min^{-1} mg cell $protein^{-1}$.

		Aldehyde dehydrogenase			
		Butyryl-	Acetyl-	Alcohol dehydrogenase	
Gene	Substrate	CoA	CoA	Butyraldehyde	Acetaldehyde
0002		0.0076	0.0046	0.0264	0.0247
0003n		0.0060	0.0072	0.0080	0.0075
0011		0.0069	0.0095	0.0265	0.0093
0013		N.D.	N.D.	0.0130	0.0142
0023		0.0089	0.0137	0.0178	0.0235
0025		0	0.0001	N.D.	N.D.
0026		0	0.0005	0.0024	0.0008

N.D., not determined.

[0189] For the BDO production experiments, cat2 from *Porphyromonas gingivalis* W83 (gene 0034) was included on pZA33 for the conversion of 4-HB to 4-HB-CoA, while the candidate dehydrogenase genes were expressed on pZE13. The host strain was MG1655 lacI^Q. Along with the alcohol and aldehyde dehydrogenase candidates, we also tested the ability of CoA-dependent succinic semialdehyde dehydrogenases (sucD) to function in this step, due to the similarity of the substrates. Cells were grown to an OD of about 0.5 in LB medium supplemented with 10 mM 4-HB, induced with 1 mM IPTG, and culture broth samples taken after 24 hours and analyzed for BDO as described below. The best BDO production occurred using adhE2 from *C. acetobutylicum*, sucD from *C. kluyveri*, or sucD from *P. gingivalis* (FIG. **13**). Interestingly, the absolute amount of BDO produced was higher under aerobic conditions; however, this is primarily due to the lower cell density achieved in anaerobic cultures. When normalized to cell OD, the BDO production per unit biomass is higher in anaerobic conditions (Table 10).

TABLE 10

Absolute and normalized BDO concentrations from cultures of cells expressing adhE2 from *C. acetobutylicum*, sucD from *C. kluyveri*, or sucD from *P. gingivalis* (data from experiments 2, 9, and 10 in FIG. 11), as well as the negative control (experiment 1).

Gene expressed	Conditions	BDO (μM)	OD (600 nm)	BDO/OD
none	Aerobic	0	13.4	0
none	Microaerobic	0.5	6.7	0.09
none	Anaerobic	2.2	1.26	1.75
0002	Aerobic	138.3	9.12	15.2
0002	Microaerobic	48.2	5.52	8.73
0002	Anaerobic	54.7	1.35	40.5
0008n	Aerobic	255.8	5.37	47.6
0008n	Microaerobic	127.9	3.05	41.9
0008n	Anaerobic	60.8	0.62	98.1
0035	Aerobic	21.3	14.0	1.52
0035	Microaerobic	13.1	4.14	3.16
0035	Anaerobic	21.3	1.06	20.1

[0190] As discussed in Section 2, it may be advantageous to use a route for converting 4-HB to 4-HB-CoA that does not generate acetate as a byproduct. To this aim, we tested the use of phosphotransbutyrylase (ptb) and butyrate kinase (bk) from *C. acetobutylicum* to carry out this conversion via steps **10** and **11** in FIG. **2**. The native ptb/bk operon from *C. acetobutylicum* (genes 0020 and 0021) was cloned and expressed in pZA33. Extracts from cells containing the resulting construct were taken and assayed for the two enzyme activities as described herein. The specific activity of BK was approximately 65 U/mg, while the specific activity of PTB was approximately 5 U/mg. One unit (U) of activity is defined as conversion of 1 μM substrate in 1 minute at room temperature. Finally, the construct was tested for participation in the conversion of 4-HB to BDO. Host strains were transformed with the pZA33-0020-0021 construct described and pZE13-0002, and compared to use of cat2 in BDO production using the aerobic procedure used above in FIG. **13**. The BK/PTB strain produced 1 mM BDO, compared to 2 mM when using cat2 (Table 11). Interestingly, the results were dependent on whether the host strain contained a deletion in the native adhE gene.

TABLE 11

Absolute and normalized BDO concentrations from cultures of cells expressing adhE2 from *C. acetobutylicum* in pZE13 along with either cat2 from *P. gingivalis* (0034) or the PTB/BK genes from *C. acetobutylicum* on pZA33. Host strains were either MG1655 lacI^Q or MG1655 ΔadhE lacI^Q.

Genes	Host Strain	BDO (μM)	OD (600 nm)	BDO/OD
0034	MG1655 lacI^Q	0.827	19.9	0.042
0020 + 0021	MG1655 lacI^Q	0.007	9.8	0.0007
0034	MG1655 ΔadhE lacI^Q	2.084	12.5	0.166
0020 + 0021	MG1655 ΔadhE lacI^Q	0.975	18.8	0.052

[0191] Production of BDO from Glucose. The final step of pathway corroboration is to express both the 4-HB and BDO segments of the pathway in *E. coli* and demonstrate production of BDO in glucose minimal medium. New plasmids were constructed so that all the required genes fit on two plasmids. In general, cat1, adhE, and sucD genes were expressed from pZE13, and cat2 and 4-HBd were expressed from pZA33. Various combinations of gene source and gene order were tested in the MG1655 lacI^Q background. Cells were grown anaerobically in M9 minimal medium (6.78 g/L Na_2HPO_4, 3.0 g/L KH_2PO_4, 0.5 g/L NaCl, 1.0 g/L NH_4Cl, 1 mM $MgSO_4$, 0.1 mM $CaCl_2$) supplemented with 20 g/L glucose, 100 mM 3-(N-morpholino)propanesulfonic acid (MOPS) to improve the buffering capacity, 10 μg/mL thiamine, and the appropriate antibiotics. 0.25 mM IPTG was added approximately 15 hours following inoculation, and culture supernatant samples taken for BDO, 4-HB, and succinate analysis 24 and 48 hours following induction. The production of BDO appeared to show a dependency on gene order (Table 12). The highest BDO production, over 0.5 mM, was obtained with cat2 expressed first, followed by 4-HBd on pZA33, and cat1 followed by *P. gingivalis* sucD on pZE13. The addition of *C. acetobutylicum* adhE2 in the last position on pZE13 resulted in slight improvement. 4-HB and succinate were also produced at higher concentrations.

TABLE 12

Production of BDO, 4-HB, and succinate in recombinant *E. coli* strains expressing combinations of BDO pathway genes, grown in minimal medium supplemented with 20 g/L glucose. Concentrations are given in mM.

				24 Hours				48 Hours			
Sample	pZE13	pZA33	Induction OD	OD600 nm	Su	4HB	BDO	OD600 nm	Su	4HB	BDO
1	cat1 (0004)-sucD (0035)	4hbd (0036)-cat2 (0034)	0.92	1.29	5.44	1.37	0.240	1.24	6.42	1.49	0.280
2	cat1 (0004)-sucD (0008N)	4hbd (0036)-cat2 (0034)	0.36	1.11	6.90	1.24	0.011	1.06	7.63	1.33	0.011
3	adhE (0002)-cat1 (0004)-sucD (0035)	4hbd (0036)-cat2 (0034)	0.20	0.44	0.34	1.84	0.050	0.60	1.93	2.67	0.119
4	cat1 (0004)-sucD (0035)-adhE (0002)	4hbd (0036)-cat2 (0034)	1.31	1.90	9.02	0.73	0.073	1.95	9.73	0.82	0.077
5	adhE (0002)-cat1 (0004)-sucD (0008N)	4hbd (0036)-cat2 (0034)	0.17	0.45	1.04	1.04	0.008	0.94	7.13	1.02	0.017
6	cat1 (0004)-sucD (0008N)-adhE (0002)	4hbd (0036)-cat2 (0034)	1.30	1.77	10.47	0.25	0.004	1.80	11.49	0.28	0.003
7	cat1 (0004)-sucD (0035)	cat2 (0034)-4hbd (0036)	1.09	1.29	5.63	2.15	0.461	1.38	6.66	2.30	0.520
8	cat1 (0004)-sucD (0008N)	cat2 (0034)-4hbd (0036)	1.81	2.01	11.28	0.02	0.000	2.24	11.13	0.02	0.000
9	adhE (0002)-cat1 (0004)-sucD (0035)	cat2 (0034)-4hbd (0036)	0.24	1.99	2.02	2.32	0.106	0.89	4.85	2.41	0.186
10	cat1 (0004)-sucD (0035)-adhE (0002)	cat2 (0034)-4hbd (0036)	0.98	1.17	5.30	2.08	0.569	1.33	6.15	2.14	0.640
11	adhE (0002)-cat1 (0004)-sucD (0008N)	cat2 (0034)-4hbd (0036)	0.20	0.53	1.38	2.30	0.019	0.91	8.10	1.49	0.034
12	cat1 (0004)-sucD (0008N)-adhE (0002)	cat2 (0034)-4hbd (0036)	2.14	2.73	12.07	0.16	0.000	3.10	11.79	0.17	0.002
13	vector only	vector only	2.11	2.62	9.03	0.01	0.000	3.00	12.05	0.01	0.000

[0192] Analysis of BDO, 4-HB and succinate by GCMS. BDO, 4-HB and succinate in fermentation and cell culture samples were derivatized by silylation and quantitatively analyzed by GCMS using methods adapted from literature reports ((Simonov et al., *J. Anal Chem.* 59:965-971 (2004)). The developed method demonstrated good sensitivity down to 1 μM, linearity up to at least 25 mM, as well as excellent selectivity and reproducibility.

[0193] Sample preparation was performed as follows: 100 μL filtered (0.2 μm or 0.45 μm syringe filters) samples, e.g. fermentation broth, cell culture or standard solutions, were dried down in a Speed Vac Concentrator (Savant SVC-100H) for approximately 1 hour at ambient temperature, followed by the addition of 20 μL 10 mM cyclohexanol solution, as an internal standard, in dimethylformamide. The mixtures were vortexed and sonicated in a water bath (Branson 3510) for 15 min to ensure homogeneity. 100 μL silylation derivatization reagent, N,O-bis(trimethylsilyl)trifluoro-acetimide (BSTFA) with 1% trimethylchlorosilane, was added, and the mixture was incubated at 70° C. for 30 min. The derivatized samples were centrifuged for 5 min, and the clear solutions were directly injected into GCMS. All the chemicals and reagents were from Sigma-Aldrich, with the exception of BDO which was purchased from J. T. Baker.

[0194] GCMS was performed on an Agilent gas chromatograph 6890N, interfaced to a mass-selective detector (MSD) 5973N operated in electron impact ionization (EI) mode has been used for the analysis. A DB-5MS capillary column (J&W Scientific, Agilent Technologies), 30 m×0.25 mm i.d.×0.25 μm film thickness, was used. The GC was operated in a split injection mode introducing 1 μL of sample at 20:1 split ratio. The injection port temperature was 250° C. Helium was used as a carrier gas, and the flow rate was maintained at 1.0 mL/min. A temperature gradient program was optimized to ensure good resolution of the analytes of interest and minimum matrix interference. The oven was initially held at 80° C. for 1 min, then ramped to 120° C. at 2° C./min, followed by fast ramping to 320° C. at 100° C./min and final hold for 6 min at 320° C. The MS interface transfer line was maintained at 280° C. The data were acquired using 'lowmass' MS tune settings and 30-400 m/z mass-range scan. The total analysis time was 29 min including 3 min solvent delay. The retention times corresponded to 5.2, 10.5, 14.0 and 18.2 min for BSTFA-derivatized cyclohexanol, BDO, 4-HB and succinate, respectively. For quantitative analysis, the following specific mass fragments were selected (extracted ion chromatograms): m/z 157 for internal standard cyclohexanol, 116 for BDO, and 147 for both 4-HB and succinate. Standard calibration curves were constructed using analyte solutions in the corresponding cell culture or fermentation medium to match sample matrix as close as possible. GCMS data were processed using Environmental Data Analysis ChemStation software (Agilent Technologies).

[0195] The results indicated that most of the 4-HB and BDO produced were labeled with ^{13}C (FIG. **14**, right-hand sides). Mass spectra from a parallel culture grown in unlabeled glucose are shown for comparison (FIG. **14**, left-hand sides). Note that the peaks seen are for fragments of the derivatized molecule containing different numbers of carbon atoms from the metabolite. The derivatization reagent also contributes some carbon and silicon atoms that naturally-occurring label distribution, so the results are not strictly quantitative.

[0196] Production of BDO from 4-HB using alternate pathways. The various alternate pathways were also tested for BDO production. This includes use of the native *E. coli* SucCD enzyme to convert succinate to succinyl-CoA (Table 13, rows 2-3), use of α-ketoglutarate decarboxylase in the α-ketoglutarate pathway (Table 13, row 4), and use of PTB/BK as an alternate means to generate the CoA-derivative of 4HB (Table 13, row 1). Strains were constructed containing plasmids expressing the genes indicated in Table 13, which encompass these variants. The results show that in all cases, production of 4-HB and BDO occurred (Table 13).

TABLE 13

Production of BDO, 4-HB, and succinate in recombinant *E. coli* strains genes for different BDO pathway variants, grown anaerobically in minimal medium supplemented with 20 g/L glucose, and harvested 24 hours after induction with 0.1 mM IPTG. Concentrations are given in mM.

Genes on pZE13	Genes on pZA33	Succinate	4-HB	BDO
0002 + 0004 + 0035	0020n – 0021n – 0036	0.336	2.91	0.230
0038 + 0035	0034 – 0036	0.814	2.81	0.126
0038 + 0035	0036 – 0034	0.741	2.57	0.114
0035 + 0032	0034 – 0036	5.01	0.538	0.154

EXAMPLE V

Biosynthesis of 4-Hydroxybutanoic Acid, γ-Butyrolactone and 1,4-Butanediol

[0197] This Example describes the biosynthetic production of 4-hydroxybutanoic acid, γ-butyrolactone and 1,4-butanediol using fermentation and other bioprocesses.

[0198] Methods for the integration of the 4-HB fermentation step into a complete process for the production of purified GBL, 1,4-butanediol (BDO) and tetrahydrofuran (THF) are described below. Since 4-HB and GBL are in equilibrium, the fermentation broth will contain both compounds. At low pH this equilibrium is shifted to favor GBL. Therefore, the fermentation can operate at pH 7.5 or less, generally pH 5.5 or less. After removal of biomass, the product stream enters into a separation step in which GBL is removed and the remaining stream enriched in 4-HB is recycled. Finally, GBL is distilled to remove any impurities. The process operates in one of three ways: 1) fed-batch fermentation and batch separation; 2) fed-batch fermentation and continuous separation; 3) continuous fermentation and continuous separation. The first two of these modes are shown schematically in FIG. **15**. The integrated fermentation procedures described below also are used for the BDO producing cells of the invention for biosynthesis of BDO and subsequent BDO family products.

[0199] Fermentation protocol to produce 4-HB/GBL (batch): The production organism is grown in a 10 L bioreactor sparged with an N_2/CO_2 mixture, using 5 L broth containing 5 g/L potassium phosphate, 2.5 g/L ammonium chloride, 0.5 g/L magnesium sulfate, and 30 g/L corn steep liquor, and an initial glucose concentration of 20 g/L. As the cells grow and utilize the glucose, additional 70% glucose is fed into the bioreactor at a rate approximately balancing glucose consumption. The temperature of the bioreactor is maintained at 30 degrees C. Growth continues for approximately 24 hours, until 4-HB reaches a concentration of between 20-200 g/L, with the cell density being between 5 and 10 g/L. The pH is not controlled, and will typically decrease to pH 3-6 by the end of the run. Upon completion of the cultivation period, the fermenter contents are passed through a cell separation unit (e.g., centrifuge) to remove cells and cell debris, and the fermentation broth is transferred to a product separations unit. Isolation of 4-HB and/or GBL would take place by standard separations procedures employed in the art to separate organic products from dilute aqueous solutions, such as liquid-liquid extraction using a water immiscible organic solvent (e.g., toluene) to provide an organic solution of 4-HB/GBL. The resulting solution is then subjected to standard distillation methods to remove and recycle the organic solvent and to provide GBL (boiling point 204-205° C.) which is isolated as a purified liquid.

[0200] Fermentation protocol to produce 4-HB/GBL (fully continuous): The production organism is first grown up in batch mode using the apparatus and medium composition described above, except that the initial glucose concentration is 30-50 g/L. When glucose is exhausted, feed medium of the same composition is supplied continuously at a rate between 0.5 L/hr and 1 L/hr, and liquid is withdrawn at the same rate. The 4-HB concentration in the bioreactor remains constant at 30-40 g/L, and the cell density remains constant between 3-5 g/L. Temperature is maintained at 30 degrees C., and the pH is maintained at 4.5 using concentrated NaOH and HCl, as required. The bioreactor is operated continuously for one month, with samples taken every day to assure consistency of 4-HB concentration. In continuous mode, fermenter contents are constantly removed as new feed medium is supplied. The exit stream, containing cells, medium, and products 4-HB and/or GBL, is then subjected to a continuous product separations procedure, with or without removing cells and cell debris, and would take place by standard continuous separations methods employed in the art to separate organic products from dilute aqueous solutions, such as continuous liquid-liquid extraction using a water immiscible organic solvent (e.g., toluene) to provide an organic solution of 4-HB/GBL. The resulting solution is subsequently subjected to standard continuous distillation methods to remove and recycle the organic solvent and to provide GBL (boiling point 204-205° C.) which is isolated as a purified liquid.

[0201] GBL Reduction Protocol: Once GBL is isolated and purified as described above, it will then be subjected to reduction protocols such as those well known in the art (references cited) to produce 1,4-butanediol or tetrahydrofuran (THF) or a mixture thereof. Heterogeneous or homogeneous hydrogenation catalysts combined with GBL under hydrogen pressure are well known to provide the products 1,4-butanediol or tetrahydrofuran (THF) or a mixture thereof. It is important to note that the 4-HB/GBL product mixture that is separated from the fermentation broth, as described above, may be subjected directly, prior to GBL isolation and purification, to these same reduction protocols to provide the products 1,4-butanediol or tetrahydrofuran or a mixture thereof. The resulting products, 1,4-butanediol and THF are then isolated and purified by procedures well known in the art.

[0202] Fermentation and hydrogenation protocol to produce BDO or THF directly (batch): Cells are grown in a 10 L bioreactor sparged with an N_2/CO_2 mixture, using 5 L broth containing 5 g/L potassium phosphate, 2.5 g/L ammonium chloride, 0.5 g/L magnesium sulfate, and 30 g/L corn steep liquor, and an initial glucose concentration of 20 g/L. As the cells grow and utilize the glucose, additional 70% glucose is fed into the bioreactor at a rate approximately balancing glucose consumption. The temperature of the bioreactor is maintained at 30 degrees C. Growth continues for approximately 24 hours, until 4-HB reaches a concentration of between 20-200 g/L, with the cell density being between 5 and 10 g/L. The pH is not controlled, and will typically decrease to pH 3-6 by the end of the run. Upon completion of the cultivation period, the fermenter contents are passed through a cell separation unit (e.g., centrifuge) to remove cells and cell debris, and the fermentation broth is transferred to a reduction unit (e.g., hydrogenation vessel), where the mixture 4-HB/GBL is directly reduced to either 1,4-butanediol or THF or a mixture thereof. Following completion of the reduction procedure, the reactor contents are transferred to a product separations unit. Isolation of 1,4-butanediol and/or THF would take place by standard separations procedures employed in the art to separate organic products from dilute aqueous solutions, such as liquid-liquid extraction using a water immiscible organic solvent (e.g., toluene) to provide an organic solution of 1,4-butanediol and/or THF. The resulting solution is then subjected to standard distillation methods to remove and recycle the organic solvent and to provide 1,4-butanediol and/or THF which are isolated as a purified liquids.

[0203] Fermentation and hydrogenation protocol to produce BDO or THF directly (fully continuous): The cells are first grown up in batch mode using the apparatus and medium composition described above, except that the initial glucose concentration is 30-50 g/L. When glucose is exhausted, feed medium of the same composition is supplied continuously at a rate between 0.5 L/hr and 1 L/hr, and liquid is withdrawn at the same rate. The 4-HB concentration in the bioreactor remains constant at 30-40 g/L, and the cell density remains constant between 3-5 g/L. Temperature is maintained at 30 degrees C., and the pH is maintained at 4.5 using concentrated NaOH and HCl, as required. The bioreactor is operated continuously for one month, with samples taken every day to assure consistency of 4-HB concentration. In continuous mode, fermenter contents are constantly removed as new feed medium is supplied. The exit stream, containing cells, medium, and products 4-HB and/or GBL, is then passed through a cell separation unit (e.g., centrifuge) to remove cells and cell debris, and the fermentation broth is transferred to a continuous reduction unit (e.g., hydrogenation vessel), where the mixture 4-HB/GBL is directly reduced to either 1,4-butanediol or THF or a mixture thereof. Following completion of the reduction procedure, the reactor contents are transferred to a continuous product separations unit. Isolation of 1,4-butanediol and/or THF would take place by standard continuous separations procedures employed in the art to separate organic products from dilute aqueous solutions, such as liquid-liquid extraction using a water immiscible organic solvent (e.g., toluene) to provide an organic solution of 1,4-butanediol and/or THF. The resulting solution is then subjected to standard continuous distillation methods to remove and recycle the organic solvent and to provide 1,4-butanediol and/or THF which are isolated as a purified liquids.

[0204] Fermentation protocol to produce BDO directly (batch): The production organism is grown in a 10 L bioreactor sparged with an N_2/CO_2 mixture, using 5 L broth containing 5 g/L potassium phosphate, 2.5 g/L ammonium chloride, 0.5 g/L magnesium sulfate, and 30 g/L corn steep liquor, and an initial glucose concentration of 20 g/L. As the cells grow and utilize the glucose, additional 70% glucose is fed into the bioreactor at a rate approximately balancing glucose consumption. The temperature of the bioreactor is maintained at 30 degrees C. Growth continues for approximately 24 hours, until BDO reaches a concentration of between 20-200 g/L, with the cell density generally being between 5 and 10 g/L. Upon completion of the cultivation period, the fermenter contents are passed through a cell separation unit (e.g., centrifuge) to remove cells and cell debris, and the fermentation broth is transferred to a product separations unit. Isolation of BDO would take place by standard separations procedures employed in the art to separate organic products from dilute aqueous solutions, such as liquid-liquid extraction using a water immiscible organic solvent (e.g., toluene) to provide an organic solution of BDO. The resulting solution is then subjected to standard distillation methods to remove and recycle the organic solvent and to provide BDO (boiling point 228-229° C.) which is isolated as a purified liquid.

[0205] Fermentation protocol to produce BDO directly (fully continuous): The production organism is first grown up in batch mode using the apparatus and medium composition described above, except that the initial glucose concentration is 30-50 g/L. When glucose is exhausted, feed medium of the same composition is supplied continuously at a rate between 0.5 L/hr and 1 L/hr, and liquid is withdrawn at the same rate. The BDO concentration in the bioreactor remains constant at 30-40 g/L, and the cell density remains constant between 3-5 g/L. Temperature is maintained at 30 degrees C., and the pH is maintained at 4.5 using concentrated NaOH and HCl, as required. The bioreactor is operated continuously for one month, with samples taken every day to assure consistency of BDO concentration. In continuous mode, fermenter contents are constantly removed as new feed medium is supplied. The exit stream, containing cells, medium, and the product BDO, is then subjected to a continuous product separations procedure, with or without removing cells and cell debris, and would take place by standard continuous separations methods employed in the art to separate organic products from dilute aqueous solutions, such as continuous liquid-liquid extraction using a water immiscible organic solvent (e.g., toluene) to provide an organic solution of BDO. The resulting solution is subsequently subjected to standard continuous distillation methods to remove and recycle the organic solvent and to provide BDO (boiling point 228-229° C.) which is isolated as a purified liquid (mpt 20° C.).

[0206] Throughout this application various publications have been referenced within parentheses. The disclosures of these publications in their entireties are hereby incorporated by reference in this application in order to more fully describe the state of the art to which this invention pertains.

[0207] Although the invention has been described with reference to the disclosed embodiments, those skilled in the art will readily appreciate that the specific examples and studies detailed above are only illustrative of the invention. It should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

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```

What is claimed is:

1. A non-naturally occurring microbial biocatalyst, comprising a microbial organism having a 4-hydroxybutanoic acid (4-HB) biosynthetic pathway comprising at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, or α-ketoglutarate decarboxylase, wherein said exogenous nucleic acid is expressed in sufficient amounts to secrete monomeric 4-hydroxybutanoic acid (4-HB).

2. The non-naturally occurring microbial biocatalyst of claim **1**, wherein said 4-HB biosynthetic pathway comprises 4-hydroxybutanoate dehydrogenase and succinyl-CoA synthetase and CoA-dependent succinic semialdehyde dehydrogenase, or α-ketoglutarate decarboxylase.

3. The non-naturally occurring microbial biocatalyst of claim **1**, wherein said exogenous nucleic acid encodes 4-hydroxybutanoate dehydrogenase.

4. The non-naturally occurring microbial biocatalyst of claim **3**, further comprising a nucleic acid encoding an exogenous α-ketoglutarate decarboxylase.

5. The non-naturally occurring microbial biocatalyst of claim **1**, wherein said at least one exogenous nucleic acid further comprises two or more exogenous nucleic acids.

6. The non-naturally occurring microbial biocatalyst of claim **3**, further comprising a nucleic acid encoding an exogenous succinyl-CoA synthetase, exogenous CoA-dependent succinic semialdehyde dehydrogenase or exogenous succinyl-CoA synthetase and exogenous CoA-dependent succinic semialdehyde dehydrogenase.

7. The non-naturally occurring microbial biocatalyst of claim **1**, wherein said microbial organism lacks an endogenous 4-HB biosynthetic activity selected from 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, and α-ketoglutarate decarboxylase.

8. The non-naturally occurring microbial biocatalyst of claim **1**, wherein said at least one exogenous nucleic acid further comprises a heterologous encoding nucleic acid.

9. The non-naturally occurring microbial biocatalyst of claim **1**, wherein said monomeric 4-HB is expressed at an intracellular concentration of at least about 5 mM.

10. The non-naturally occurring microbial biocatalyst of claim **9**, further comprising an intracellular concentration of said monomeric 4-HB of about 10 mM or more.

11. The non-naturally occurring microbial biocatalyst of claim **1**, further comprising a substantially anaerobic culture medium.

12. A non-naturally occurring microbial biocatalyst, comprising a microbial organism having 4-hydroxybutanoic acid (4-HB) and 1,4-butanediol (BDO) biosynthetic pathways, said pathways comprise at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, 4-hydroxybutyrate:CoA transferase, 4-butyrate kinase, phosphotransbutyrylase, α-ketoglutarate decarboxylase, aldehyde dehydrogenase, alcohol dehydrogenase or an aldehyde/alcohol dehydrogenase, wherein said exogenous nucleic acid is expressed in sufficient amounts to produce 1,4-butanediol (BDO).

13. The non-naturally occurring microbial biocatalyst of claim **12**, wherein said 4-HB biosynthetic pathway comprises 4-hydroxybutanoate dehydrogenase and succinyl-CoA synthetase and CoA-dependent succinic semialdehyde dehydrogenase or α-ketoglutarate decarboxylase.

14. The non-naturally occurring microbial biocatalyst of claim **12**, wherein said exogenous nucleic acid encodes 4-hydroxybutanoate dehydrogenase.

15. The non-naturally occurring microbial biocatalyst of claim **14**, further comprising a nucleic acid encoding an exogenous α-ketoglutarate decarboxylase.

16. The non-naturally occurring microbial biocatalyst of claim **12**, wherein said at least one exogenous nucleic acid further comprises two or more exogenous nucleic acids.

17. The non-naturally occurring microbial biocatalyst of claim **14**, further comprising a nucleic acid encoding an exogenous succinyl-CoA synthetase, exogenous CoA-dependent succinic semialdehyde dehydrogenase or exogenous succinyl-CoA synthetase and exogenous CoA-dependent succinic semialdehyde dehydrogenase.

18. The non-naturally occurring microbial biocatalyst of claim **12**, wherein said microbial organism lacks an endogenous 4-HB biosynthetic activity selected from 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase CoA-dependent succinic semialdehyde dehydrogenase and α-ketoglutarate decarboxylase.

19. The non-naturally occurring microbial biocatalyst of claim **12**, wherein said at least one exogenous nucleic acid further comprises a heterologous encoding nucleic acid.

20. The non-naturally occurring microbial biocatalyst of claims **14**, **15** or **17**, wherein said exogenous nucleic acid is expressed in sufficient amounts to produce monomeric 4-hydroxybutanoic acid.

21. The non-naturally occurring microbial biocatalyst of claim **12**, wherein said BDO biosynthetic pathway comprises aldehyde dehydrogenase, and alcohol dehydrogenase or aldehyde/alcohol dehydrogenase.

22. The non-naturally occurring microbial biocatalyst of claim **21**, wherein said BDO biosynthetic pathway further comprises 4-hydroxybutyrate:CoA transferase or 4-butyrate kinase and phosphotransbutyrylase.

23. The non-naturally occurring microbial biocatalyst of claim **21**, wherein said exogenous nucleic acid encodes an aldehyde/alcohol dehydrogenase.

24. The non-naturally occurring microbial biocatalyst of claim **21**, wherein said at least one exogenous nucleic acid further comprises two or more exogenous nucleic acids.

25. The non-naturally occurring microbial biocatalyst of claim **21** or **22**, wherein said microbial organism lacks an endogenous BDO biosynthetic activity selected from 4-hydroxybutyrate:CoA transferase, 4-butyrate kinase, phosphotransbutyrylase, aldehyde dehydrogenase, alcohol dehydrogenase and aldehyde/alcohol dehydrogenase.

26. The non-naturally occurring microbial biocatalyst of claim **21**, wherein said at least one exogenous nucleic acid further comprises a heterologous encoding nucleic acid.

27. The non-naturally occurring microbial biocatalyst of claim **12**, further comprising a substantially anaerobic culture medium.

28. The non-naturally occurring microbial biocatalyst of claim **25**, wherein said monomeric BDO is expressed at an intracellular concentration of at least about 5 mM.

29. The non-naturally occurring microbial biocatalyst of claim **28**, further comprising an intracellular concentration of said monomeric BDO of about 10 mM or more.

30. A method for the production of 4-HB, comprising culturing a non-naturally occurring microbial organism having a 4-hydroxybutanoic acid (4-HB) biosynthetic pathway comprising at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, or α-ketoglutarate decarboxylase under substantially anaerobic conditions for a sufficient period of time to produce monomeric 4-hydroxybutanoic acid (4-HB).

31. The method of claim **30**, wherein said 4-HB biosynthetic pathway comprises 4-hydroxybutanoate dehydrogenase and succinyl-CoA synthetase and CoA-dependent succinic semialdehyde dehydrogenase or α-ketoglutarate decarboxylase.

32. The method of claim **30**, wherein said exogenous nucleic acid encodes 4-hydroxybutanoate dehydrogenase.

33. The method of claim **32**, wherein said non-naturally occurring microbial organism further comprises a nucleic acid encoding an exogenous α-ketoglutarate decarboxylase.

34. The method of claim **30**, wherein said at least one exogenous nucleic acid further comprises two or more exogenous nucleic acids.

35. The method of claim **32**, further comprising a nucleic acid encoding an exogenous succinyl-CoA synthetase, exogenous CoA-dependent succinic semialdehyde dehydrogenase or exogenous succinyl-CoA synthetase and exogenous CoA-dependent succinic semialdehyde dehydrogenase.

36. The method of claim **30**, wherein said non-naturally occurring microbial organism lacks an endogenous 4-HB biosynthetic activity selected from 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase and α-ketoglutarate decarboxylase.

37. The method of claim **30**, wherein said at least one exogenous nucleic acid further comprises a heterologous encoding nucleic acid.

38. The method of claim **30**, wherein said monomeric 4-HB is expressed at an intracellular concentration of at least about 5 mM.

39. The method of claim **38**, further comprising an intracellular concentration of said monomeric 4-HB of about 10 mM or more.

40. The method of claim **30**, further comprising isolating 4-HB.

41. The method of claim **30**, further comprising a culture medium having a pH of about 7.5 or less.

42. The method of claim **41**, further comprising isolating γ-butyrolactone (GBL).

43. The method of claim **42**, wherein said isolation of GBL comprises separation of GBL.

44. The method of claim **43**, further comprising distillation to produce substantially pure GBL.

45. The method of claim **30** or **44**, further comprising chemical hydrogenation of 4-HB, GBL or a mixture thereof to produce 1-4 butanediol (BDO).

46. The method of claim **30** or **44**, further comprising chemical hydrogenation of 4-HB, GBL or a mixture thereof to produce tetrahydrofuran (THF).

47. A method for the production of BDO, comprising culturing a non-naturally occurring microbial biocatalyst, comprising a microbial organism having 4-hydroxybutanoic acid (4-HB) and 1,4-butanediol (BDO) biosynthetic pathways, said pathways comprising at least one exogenous nucleic acid encoding 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase, 4-hydroxybutyrate:CoA transferase, 4-butyrate kinase, phosphotransbutyrylase, α-ketoglutarate decarboxylase, aldehyde dehydrogenase, alcohol dehydrogenase or an aldehyde/alcohol dehydrogenase for a sufficient period of time to produce 1,4-butanediol (BDO).

48. The method of claim **47**, wherein said 4-HB biosynthetic pathway comprises 4-hydroxybutanoate dehydrogenase and succinyl-CoA synthetase and CoA-dependent succinic semialdehyde dehydrogenase or α-ketoglutarate decarboxylase.

49. The method of claim **47**, wherein said exogenous nucleic acid encodes 4-hydroxybutanoate dehydrogenase.

50. The method of claim **49**, further comprising a nucleic acid encoding an exogenous α-ketoglutarate decarboxylase.

51. The method of claim **47**, wherein said at least one exogenous nucleic acid further comprises two or more exogenous nucleic acids.

52. The method of claim **49**, further comprising a nucleic acid encoding an exogenous succinyl-CoA synthetase, exogenous CoA-dependent succinic semialdehyde dehydrogenase or exogenous succinyl-CoA synthetase and exogenous CoA-dependent succinic semialdehyde dehydrogenase.

53. The method of claim **47**, wherein said microbial organism lacks an endogenous 4-HB biosynthetic activity selected from 4-hydroxybutanoate dehydrogenase, succinyl-CoA synthetase, CoA-dependent succinic semialdehyde dehydrogenase and α-ketoglutarate decarboxylase.

54. The method of claim **47**, wherein said at least one exogenous nucleic acid further comprises a heterologous encoding nucleic acid.

55. The method of claims **49**, **50**, or **52**, wherein said exogenous nucleic acid is expressed in sufficient amounts to produce monomeric 4-hydroxybutanoic acid.

56. The method of claim **47**, wherein said BDO biosynthetic pathway comprises aldehyde dehydrogenase and alcohol dehydrogenase or an aldehyde/alcohol dehydrogenase.

57. The method of claim **56**, wherein said BDO biosynthetic pathway further comprises 4-hydroxybutyrate:CoA transferase or 4-butyrate kinase and phosphotransbutyrylase.

58. The method of claim **56**, wherein said at least one exogenous nucleic acid further comprises two or more exogenous nucleic acids.

59. The method of claim **56** or **57**, wherein said microbial organism lacks an endogenous BDO biosynthetic activity selected from 4-hydroxybutyrate:CoA transferase, 4-butyrate kinase, phosphotransbutyrylase, aldehyde dehydrogenase, alcohol dehydrogenase and aldehyde/alcohol dehydrogenase.

60. The method of claim **56**, wherein said at least one exogenous nucleic acid further comprises a heterologous encoding nucleic acid.

61. The method of claim **47**, further comprising a substantially anaerobic culture medium.

62. The method of claim **59**, wherein said monomeric BDO is expressed at an intracellular concentration of at least about 5 mM.

63. The method of claim **62**, further comprising an intracellular concentration of said monomeric BDO of about 10 mM or more.

* * * * *

Appendix F: Equipment Specifications

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    - Oil Fired Direct Vent Boilers. L
    - Oil Fired Steel Hydronic Package
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      - Standing Pilot Models.
    - Packaged Oil Boilers--Water
    - Packaged Oil Fired Boiler, Steam
    - Packaged Oil Fired Boiler, Water
    - Semi-Packaged Boilers, Water.
    - Semi-Packaged Oil Fired Boiler-S
    - Small Cast Iron, Oil Fired Water
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  - Ultimate Boilers.
  - Utica Boilers.
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- Cleaning Kits
- Coils
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- Complete Split System AC only
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- Complete Split System Heat Pumps
- Digital Thermostats
- Duct, Registers & Grilles.
- Ductless Mini Split
- Fireplace Packages
- Floor Furnaces
- Gas Furnaces
- Generators
- Geothermal Heat Pump
- Hanging Furnaces
- Heat Strips
- Humidifiers
- HVAC Parts
- Installation Supplies and Kits
- Oil Fired Furnace
- Outside Wood Furnace
- Packaged Air Conditioners
- Packaged Dual Fuel
- Packaged Gas & Electric
- Packaged Heat Pumps
- Package Terminal AC (PTAC)
- Pellet / Multi-Fuel Units
- Pool and Spa Heat Pump
- Portable Air Conditioning
- Portable Heater
- Refrigerant
- Split System Air Conditioners
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- Superior Fuel Savings and Simplified Service Requirements.
- A rear drafthood installs in low overhead areas and allows for clearance and flexibility for existing piping.
- Honeywell controls. High quality brand name controls assure reliability and availability.
- Step-opening gas valve. This type of gas valve enables the boiler to start on low fire.
- Industrial quality pressuretrol for accurate, trouble-free control.
- Rugged cast iron sections.

Made in the USA.

Other Standard Features :

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- Combustible Floor Certified.
- Steam Pressure Gauge.
- Pressure Limit Control
- Safety Relief Valve.
- Low Water Cut Off.
- High Limit.
- Thermostat Isolation Relay.
- Transformer and Junction Box.
- Vent Damper.
- 120V Power switch
- 100% Shut-off Combination.
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- Stainless Steel Burners.
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- Flame Roll-Out Switch

- Factory tested.

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Product Detail

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MFG. PART #:	PIN7SNI-ME2
SUPPLIER:	INGRAM'S WATER & AIR EQUIPMENT
A.F.U.E.%:	82.1
DIMENSIONS H x W x D:	40-1/4 x 27-1/2 x 25"
FLUE:	7"
WEIGHT:	620
PIPING CONNECTIONS RETURN:	2"
PIPING CONNECTIONS SUPPLY:	2"
I=B=R NET RATINGS STEAM BTUH:	130,000
I=B=R NET RATINGS STEAM SQ.FT.:	542
D.O.E. CAPACITY BTUH:	173,000

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No order cancellation fee	✓	✓	✗	✗
Contractor Assistance Available	✓	✓	✗	✗
Friendly and Helpful Service Before and AFTER the sale	✓	✗	✗	✗
Family-Owned Business Dedicated to Helping the Consumer	✓	✗	✗	✗
Lowest Out the Door the Price Guarantee	✓	✗	✗	✗
Experienced HVAC Personnel on Staff	✓	✗	✗	✗
(A) Rated with the Better Business Bureau	✓	✗	✓	✗
Free Duct sizing Help	✓	✓	✗	✓
24/7 Live Chat Support	✓	✗	✗	✗

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- Phone (USA) 1-877-788-8387
- Phone (Outside USA) 760-788-8387
- Fax: 760-896-6999
- Sales: sales@roconsumables.com
- Website: www.roconsumables.com
- Website: www.raindancewatersystems.com
- Website: www.RainDanceH2O.com
- Residential and commercial water treatment systems

RO Consumables

Water Treatment Made In The USA
(Division of RainDance Water Systems)

Commercial Reverse Osmosis to solve your toughest water treatment challenges.
Choose from our Commercial Water Filters and Commercial Reverse Osmosis - From 400gpd To 1,000,000gpd

Commercial:
Home | Commercial Reverse Osmosis | Light Commercial Reverse Osmosis | EDI - Electrodeionization | Pharmaceutical Water Filters | Reverse Osmosis Quote | Iron and Manganese Removal | Chlorine and Chemical Removal | Multi-Media Filters |Water Treatment Chemicals | Reverse Osmosis Membrane Cleaners | Reverse Osmosis Antiscalants | Reverse Osmosis Membranes | Commercial Water Filters | Commercial Filter Housings |Water Treatment Chemical Quote | Reverse Osmosis Membrane Quote || Water Filter Quote | Filter Housing Quote |

Residential:
Whole House Water Filters - Water Softeners | Iron Filters | Custom Water Softeners | No Salt Water Conditioners For City Water | Non Chemical Whole House Water Filter For Wells |

Specializing in integrated high recovery commercial reverse osmosis and commercial water treatment systems to meet virtually any water purification requirement. Traditional applications for our products include purification of potable water supplies, bottled water production, desalination, industrial process water preparation, and high purity water systems, including pharmaceutical systems.

Why a commercial reverse osmosis from RainDance Water Systems? Because we can customize absolutely everything tailored to the customer's water chemistry and ambient operating conditions. A reverse osmosis unit for Japan will be significantly different from one sent to Alaska. If your application requires custom water treatment please use our hassle free online quote form or choose from our most popular cost effective, user friendly commercial reverse osmosis systems listed below. Choose from our Skid Mount, Vertical Mount, Horizontal Mount, Wall Mount, Compact and Portable RO systems.

Ready-Made and Custom-Made Water Treatment Equipment Built For Your Specific Business
Featuring Commercial Reverse Osmosis Systems, Reverse Osmosis Membrane Cleaning Services, Qualified R.O. Tech Support
TSM-Series: 400gpd-1,500gpd ~TV-Series: 2000gpd-12,000gpd ~ TH-Series: 15,000gpd-24,000gpd ~ TP-Series: 35,000gpd-1,000,000gpd

Business to Business Savings/Discounts - We can match the correct reverse osmosis system to your specific application. We offer a wide variety of water treatment equipment options from Filtration to Softening to Purification to Conditioning to help your business save money. Our Business to Business Discount Program is designed to provide Commercial Businesses with exclusive reverse osmosis system offers, discounts and value-added opportunities from RainDance Water Systems. Contact our Reverse Osmosis Water Specialists today and ***Save*** on your next water filter equipment purchase. Contact Email: Sales@ROConsumables.com

Running any of our R.O. systems listed below at 100% duty cycle (24/7) will significantly shorten the life of the pump/motor so it is not recommended. Generally, these reverse osmosis systems will do well if run at 75% (3 hours on, 1 hour off for example) or less duty cycle. The usual sizing formula for a R.O. System is to make it twice the actual needed water flow. If the usage needs 1000 GPD then a 2000 GPD system would be appropriate. This also allows some excess system capacity in case the usage requirement rises later on.

Let RainDance Water Systems provide you the best possible water for your business or farm - A small sample of our water treatment customer base includes: The U.S. Army, The U.S. Environmental Protection Agency (EPA), The U.S. Fish and Wildlife Federation, The United States Coast Guard, Federal Aviation Administration, Lockeed Martin, Gaffney-Kroese Supply Corp, Washington St. National Park Service, San Diego State University, Arizona State University, Palomar College, Miasole, Trico Products Corporation, Affinity Flavors, Snake River Power Plant, South Placer Municipal Utility District, Berkley Surgical Corporation, Abengoa Energy of NE, Advanced Marine PTE., Quinlan Texas Elementary School, Hunter Industries, Sonance Corp., Owens Brigam Medical, 1st Choice GMAC Realty, Century 21 Realty, Coldwell Banker Realty, Austin Productions, Fairfield Country Club, Auer Precision Inc., Deer Park Monastery,Global Food Technologies, Oral Bio Tech, Earthbound Farms, Old Country Vineyards, Fairbanks Farms, Golden Eagle Thoroughbred Horse Farm, Buckridge Plantation and Stables, Just to name a few.

We offer commercial water filter solutions for distilleries, breweries, wineries, vineyards, micro-breweries, purified ice and beverage companies.

We offer sodium salts, total dissolved solids, and silica water filtration through commercial reverse osmosis, nano filtration and ultrafiltration systems for agriculture including palm trees, tomatoes, avocados, almonds, nut farms, berries, citrus fruits, grapes, green houses, orchards, and irrigation filtration system for farms.

We have successfully treated water for Dairy, Cattle, Poultry, Swine, Horse, and many other livestock applications. If your farm or ranch is in need of a water filtration system let RainDance Water Systems help you design the correct water purification filter for your application. For more information please e-mail us at sales@raindancewatersystems.com

We offer reverse osmosis water purification solutions for manufacturing including bottled water stores, pharmaceutical, electronic industry, process water, chemical industry, electroplating industry, electrical power generating, polymer solutions and more.

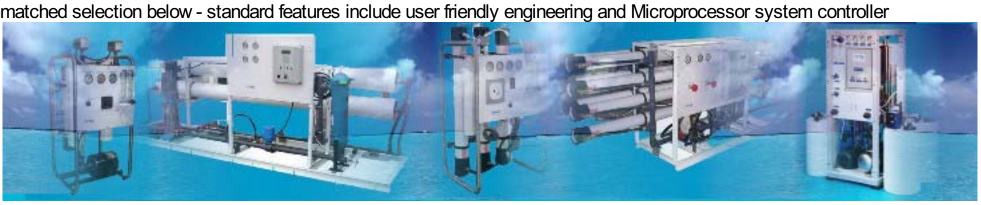
Let, RainDance Water Systems solve your water treatment needs. Typical applications of a Commercial Reverse Omsosis Filter from RainDance Water Systems are:

- Water Treatment From 150gpd to 1,000,000gpd
- Agriculture-Dairy, Cattle, Horse, Swine
- Schools And Work Shops
- Green Houses- Orchards, Groves
- Food & Beverage Industry
- Hemodialysis
- Pharmaceutical
- Electronic Industry
- Process Water
- Chemical Industry
- Electroplating Industry
- Electrical Power Generating
- Polymer Solutions
- And many More
- For Sea Water Purification Please See Our Sea Water Reverse Osmosis
- Water Store Business's Please Click Here: Water Store Equipment

- Semiconductor, Power generation , Industrial boiler feed makeup, Electronics, Pharmaceuticals, Biotechnology, Chemical manufacturing, Laboratories See our EDI Electrodeionization Systems
- Membrane softening and membrane technologies see our NanoFiltration Systems
- TSM Series Commercial Reverse Osmosis Water Purifiers - great performance, visual appeal, user friendly, and stainless steel throughout. TSM reverse osmosis applications include micro breweries, supermarket produce and food preparation operations, misting and humidification systems, car wash facilities, and many other businesses that must have consistent high quality water for their daily operations. The key factors of reliability, serviceability, and consistent performance with minimum user intervention are achieved in the solid design of the TSM systems.

Not all reverse osmosis systems are created equal! RainDance Water Systems offers top of the line state of the art commercial reverse osmosis systems for your factory, business, and farm. See what sets our reverse osmosis filters apart from all others and why companies and home owners from all over the Globe have chosen RainDance Water Systems for their water treatment needs.

See and compare our unmatched selection below - standard features include user friendly engineering and Microprocessor system controller



TV Series Reverse Osmosis Water Filter Systems

Each TV-RO unit is equipped with casters for maximum portability

TV Series reverse osmosis systems are designed for applications such as glassware, rinsing, beverage, solution preparation, and numerous other scientific, commercial and industrial applications. Water purified by reverse osmosis has had often greater than 95%of dissolved ions, and 99% of most contaminants removed.

TV Series commercial reverse osmosis systems are designed to filter well water or city tap water. Typical applications include: Agriculture, Livestock, Green Houses, Wineries, Orchards, Groves, Food, Ice,and Beverage Industry, Bottled Water Stores, Pharmaceutical, Electronic Industry, Process Water, Chemical Industry, Electroplating Industry, Electrical Power Generating, Polymer Solutions, Perfect for low mineral and contaminant filtration applications and more.

Why is pretreatment needed? For the preservation of the efficiency and life span of a Reverse Osmosis System (RO) installation, a sufficient pre-treatment is required. A proper selection of pre-treatment methods for feed water will improve affectivity and extend the life span of the system by preventing or minimizing iron, manganese chlorine/organic fouling, scaling and membrane plugging. If your application is using **well water** please fax (1-760-896-6999) or email sales@roconsumables.com your water analysis. Once your well water test has be reviewed we can add the proper pretreatment. If the feed water you are treating is from a **city municipal water treatment plant** (tap water) email sales@roconsumables.com the name of your water district or provider and we will let you know what type of pretreatment that is required.

New TV-Series Exclusive: High Efficiency Hard Water & Chlorine Pretreatment With Our Dual-Media Water Softener Chlorine Filter:
The RainDance Water Systems ****RDWS-DMCATAC-125-10** - Space Saving Compact Design Dual Water Softener & Chlorine Pre-Treatment System. Reduce costs and conserve resources - This system combines two technologies in two separate media chambers in one tank that provide the necessary hard water, chlorine, and organic pretreatment prior to the TV Series Reverse Osmosis System. Media Tank Dimensions: 10"x54" Total Height 64", Brine Tank 18"x40", Power: 110v, Chrome Tank Jacket, Digital Metered Control Valve - Regenerates on water usage for greater efficiency, 1" Connections With Bypass Valve, Flow Rate: 12gpm. We save you time - This system is delivered to your business or jobsite with the media tank & valve fully assembled. * Special Package Price is available with the purchase of any TV Series Reverse Osmosis. Both TV Reverse Osmosis and **RDWS-DMCATAC-125-10** must be purchased at the same time. No exceptions. ***Special R.O. Pretreatment Package Price: $595.00 Free Shipping Within The Continental US.**

RDWS-DMCATAC-125-10 **- This pretreatment system can also be customized to provide well water iron pre-filtration

Vertical Mount 2,000gpd - 12,000gpd Water Production
TV Series Commercial / Industrial Reverse Osmosis Water Purifiers

TV SERIES QUALITY COMMERCIAL REVERSE OSMOSIS - User Friendly, Compare Standard Features Listed Below:
TV series are designed for commercial /industrial applications where floor space is at a premium. All major system elements are mounted within a sturdy tubular stainless steel frame, welded for long term rigidity and open for easy access to all components. The TV system is a compact, heavy duty R.O. water purifier for users requiring 2000 to 12000 gallons per day water production. TV systems are fully equipped with the instruments and controls needed for reliable long term operation.

All 2,000gpd Through 12,000gpd TV Series Reverse Osmosis Systems Include Our Following "BEST" Features:
.. Stainless steel frame for long lasting durability
.. Structural ABS control panel
.. Casters for mobile portability - Each TV-RO unit is equipped with casters for maximum portability
.. On / Off Switch
.. Float Switch For Atmospheric Tank
.. 20" Prefilter: **High Chemical Absorptive Capacity, Significantly Reduces Chlorine, Volatile Organic Compounds (VOC), and TOC.**
.. Quick connect brine and product connections
.. Feed and membrane vessel pressure gauges
.. Product water flowmeter
.. Brine and recirculation flow meters with integral needle valves
.. Low pressure cut off
.. High pressure switch

.. Patented PureFlush membrane flush feature - Clean water flushes the membrane upon start-up
.. Low energy, thin film membrane element(s)
.. "The TV series of R.O. water treatment systems are equipped with a **microprocessor based controller** which monitors several functional conditions and regulates operation of the high pressure pump and control valves. The controller is connected to sensors which, depending on their state, allow the cyclic production of purified water or prevent operation due to abnormal conditions. The standard configuration of the controller monitors feed water supply pressure for minimum level, product water storage tank level for system start/stop conditions and product water conductivity for maximum set point value and for digital front panel display. Additional optional parameters that the controller is capable of monitoring include main pump high pressure set point, low feed water tank level and membrane flush cycle occurrence and duration. Front panel buttons and digital display allow operator adjustment of set points and flush parameters."
.. *TV High Recovery - Reduce Waste, Uses Less Water - 50%, 60%, up to 70% *Recovery Depending On Water Chemistry.

Better Value, Better Features: New 2011 TV-Reverse Osmosis "On-The-Spot" Premium Features Now Included:

.. Water Alarm: now included with every TV Series Reverse Osmosis Water Purification Order. Portable water leak detection/water alarm system - Helps detects water leaks and moisture. Sounds a loud alarm when water is detected. Includes 6ft of wire attached to the sensor and up to 100 feet of additional wire can be spliced into the line. Perfect for monitoring from your office or work area.
.. Multipurpose Water Quality Tester: All-In-One waterproof **on the spot tester** offers high accuracy **Electrical Conductivity (EC) / Total Dissolved Solids (TDS) and Temperature** measurements in a single tester! No more switching between meters for your routine measurements. The waterproof Combo (it even floats) has a large easy-to-read, dual-level LCD and automatic shut-off. EC/TDS readings are automatically compensated for the effects of temperature (ATC). Fast, efficient, accurate and portable, the Combo Electrical Conductivity / Total Dissolved Solids (TDS) and Temperature tester brings you all the features you've asked for and more! TDS is the sum of the mineral salts in water and if too high can result in objectionable taste, cloudy ice, interference with the flavor of foods and beverages and scale left behind in pipes, machinery, glass, etc.
.. Chlorine, Hardness, Iron, and pH Test Kit: On the spot easy testing. Includes frequently measured water quality parameters in one rugged kit - Total Chlorine, Hardness as $CaCO_3$, Iron, pH.

TV-RO SPECIFICATIONS:
.. Dimensions: 22"W x 25"D x 49"H (2-4KGPD), 29"W x 25"D x 49"H (6-12KGPD)
.. Weight: 140 -200 lbs. depending on model
.. Output*: TV-2000, 4000, 6000, 8000, 10000, & 12000 GPD models
.. Membrane: Low energy, high rejection 4040 thin film type
.. Dissolved Solids Rejection: 98%
.. Prefilter: 10 micron polypropylene depth type
.. Power Req.: 120V, 1 Ph. to 460V 3 Ph.
.. Connections: Feed - 3/4"FPT, product and tank - 1/2" tube
.. Operating Parameters: Max TDS-5000ppm, Total iron is less than 0.3ppm, Manganese is less than 0.05ppm, Water hardness below 5 gpg.
.. FREE Shipping within the continental US

Contact us today for great savings! Save now on all TV-RO Systems
We invite you to take advantage of our best prices and specials of the year. Our Representatives are happy to discuss your water purification needs. To get started, simply fill out our TV-RO Quote Form below or email us at sales@roconsumables.com , fax at (760) 896-6999, or toll-free at (877) 788-8387. We look forward to serving you.

TV Series Commercial Reverse Osmosis Specifications

Description

TV-2000

	TV-2000	TV-4000	TV-6000 - 12000
Frame	Welded stainless steel tube		
Membranes	Low energy, thin-film (high rejection option)		
Vessels	PVC (stainless steel option)		
Pump	Brass Positive Displacement (SS option)	SS Multi-stage Centrfugal	
Gauges	Filter inlet/outlet, Vessel inlet/outlet, SS case, bronze internals, glycerine filled		
Valves	Brine and Recirculation control valves		
Switches	Low pressure cut-out		
Filters	10 micron, 20" pre-filter and housing		
Electrical*	110/220V 50/60Hz 1-phase 220/380/460V, 50/60Hz 3-ph.		220/380/460V 50/60Hz 3-phase
Connections Feed/Drain/Product	3/4" FPT/1/2" QC/1/2" QC		3/4" FPT/1/2" hose/1/2" hose
Control System	Microprocessor based		
Standard Panel Instruments	TDS Monitor, Recirculation flowmeter, Brine flowmeter, Product flowmeter		

*For 220/380/480 voltage/phase options and power specifications, please see the Electrical Datasheet

Specifications

Production rate and TDS rejection are based on membrane performance after 24 hours at 115 psig (10.3bar) net operating pressure, 77°F (25°C), pH 7.5, 15% recovery on feed water containing 1,500 ppm TDS. Flow tolerance is +/- 15%

Potential membrane foulants such as Iron, Manganese and Hydrogen Sulfide must be removed from the feed stream prior to the system.

	TV-2000	TV-4000	TV-6000	TV-8000	TV-10000	TV-12000
Capacity	2000 GPD ($7.6m^3/d$)	4000 GPD ($15.1m^3/d$)	6000 GPD ($22.7m^3/d$)	8000 GPD ($30.2m^3/d$)	10000 GPD ($37.8m^3/d$)	12000 GPD ($45.3m^3/d$)
Membranes	1	2	3	4	5	6
Nominal/Maximum Operating Pressure	150/200 psi (10.3/13.8 bar)					
Min. Conc. Flow - Discharge + Recirc	3 gpm ($0.68m^3/h$)			6-9 gpm ($1.36-2.04m^3/h$)		
Nominal Recovery (with Recirc Valve)	31% (70%)	48% (70%)	58% (70%)	48% (70%)	54% (70%)	58% (70%)
Typical TDS Rejection	98%					
Max Feed Temp	113°F (45°C)					
Feed pH	3-10					
Feed Chlorination	Dechlorination reqd. if >0.1 ppm					
Maximum Feed TDS	5,000 ppm					
Motor Rating	1.0-1.5HP	1.0-1.5HP	1.5-2HP		2.0-3.0HP	3.0-5.0HP

All 2,000gpd Through 12,000gpd TV Series Reverse Osmosis Systems Include Custom Features Listed Above

Get a same day quote with our TV Reverse Osmosis Online Quote Form

Part #	Gallons Per Day	# of Elements	Motor Hp	Price Guide 
TV-RO-2000	2000	1	1.0-1.5	**TV-2000: $5,898.00** **[Add to Cart] [View Cart]** Order Online or Call Toll Free 1-877-788-8387 Free Shipping Within the Continental US Click For Online TV-RO Quote
TV-RO-4000	4000	2	1.0-1.5	**TV-4000: $6,898.00** **[Add to Cart] [View Cart]** Order Online or Call Toll Free 1-877-788-8387 Free Shipping Within the Continental US Click For Online TV-RO Quote
TV-RO-6000	6000	3	1.5-2.0	**TV-6000: $7,898.00** **[Add to Cart] [View Cart]** Order Online or Call Toll Free 1-877-788-8387 Free Shipping Within the Continental US Click For Online TV-RO Quote
				TV-8000: $8,898.00 **[Add to Cart] [View Cart]**

TV-RO-8000	8000	4	1.5-2.0	Order Online or Call Toll Free 1-877-788-8387 Free Shipping Within the Continental US Click For Online TV-RO Quote
TV-RO-10,000	10000	5	2.0-3.0	**TV-10,000: $9,898.00** **[Add to Cart] [View Cart]** Order Online or Call Toll Free 1-877-788-8387 Free Shipping Within the Continental US Click For Online TV-RO Quote
TV-RO-12,000	12000	6	3.0-5.0	**TV-12000: $10,898.00** **[Add to Cart] [View Cart]** Order Online or Call Toll Free 1-877-788-8387 Free Shipping Within the Continental US Click For Online TV-RO Quote

TV LARGE VOLUME REVERSE OSMOSIS STORAGE TANKS:
Needed to store water from reverse osmosis system
Note: Our TV reverse osmosis tanks include complete float switches for storage tanks (top tank for shut off)

Approx. 35" dia x 81" ht. 300 Gallon Storage Tank (green) (Designed to fit through standard doorways)	Model: TK-300/Float Price: $995.00ea *Free Shipping [Add to Cart] [View Cart]
Approx. 47" diameter and 78" high 550 Gallon Storage Tank (green)	Part #: LCX-TK-ST550 Price: $1,195.00ea *Free Shipping [Add to Cart] [View Cart]

TV REVERSE OSMOSIS REPRESSURE SYSTEM:
Draws water out of the TV storage tank to your application

Re-pressure pump rated @ 10gpm (110v) Designed for athmospheric storage tanks listed above.	Model: RP-10GPM Price: $695.00ea *Free Shipping [Add to Cart] [View Cart]

TV-Reverse Osmosis Pre-Filtration & Post-Filtration Option List: RainDance Water Systems Package Pricing Savings
Hard Water Pretreatment, pH Correction, UV Disinfection, Iron, Manganese, Hydrogen Sulfide Gas Prefiltration

HARD WATER PRETREATMENT: NO CHLORIDE DISCHARGE, NO BACKWASHING Calcium and magnesium (limescale) - two of the hardest minerals for a reverse osmosis membrane to remove. Our automatic antiscalant feed system will provide hard water treatment and extend the life of your reverse osmosis membrane and, thus, improve the efficiency of your reverse osmosis water filter. **Water Softener Alternative: Hard Water Treatment (NO-Salt Discharge)** antiscalant feed system - Pretreats and protects the membrane from hard water scale and silica. Antiscalant injection method is preferable to brine regenerating softeners as it is lower maintenance, economical, and is perfect for areas of the country that have water softener chloride discharge limits. The antiscalant formulation has been certified by the National Sanitation Foundation **(NSF) under ANSI/NSF Standard 60** for use in producing potable water. **Model: TOM-CFP-V3000** **Protects ro membrane from hard water scale, Calcium Carbonate (CaCO3),Calcium Sulfate (CaSO4),Barium Sulfate (BaSO4) Strontium Sulfate (SrSO4), Calcium Fluoride (CaF), and Silica (SiO2).** Features:Power: 110v, Automatic antiscalant injection pump with solution tank	**Model: TOM-CFP-V3000** Price: $995.00ea *Free Shipping [Add to Cart] [View Cart]
Post TV-RO pH CORRECTION SYSTEM: Increase your pH Designed to keep your ro water **non-corrosive and protect any copper plumbing**. **Model: RDWS-PH-MAX** How it works: The cylinder is installed into the water supply as near to the source as possible so that all the water from that point onwards is neutralised. Water passes into the cylinder and permeates through the pH correction media before passing out into the main water line again. The media dissolves into the water until the pH is raised to approximately 7 or 7.2. Once this level the water is neutral and no more media dissolves.	Ask about our best Packaged pH Correction Pricing when purchased with our TV Reverse Osmosis Please call 1-877-788-8387 or Email: Sales@ROConsumables.com
Model: S12Q-PA UV System This compact line of ultraviolet disinfection systems is ideally suited for point of use filtration, RO pre or post disinfection or with a myriad of other applications requiring the flexibility this design offers. The hard glass germicidal lamps provide an economical way of treating water requiring a 4-log (99.99%) reduction of bacteria and virus and protozoan cysts (Giardia lamblia and Cryptosporidium). This process is accomplished without adding any harmful chemicals to your water.	Ask about our best Packaged UV Pricing when purchased with our TSM or TV Reverse Osmosis Please call 1-877-788-8387 or Email: Sales@ROConsumables.com
Well Water Pre-Filtration: **Model: Iron Max-125** This stand alone system provides up to 15 ppm Iron (Ferrous & Ferric) , 7 ppm Hydrogen Sulfide, and 3ppm of Manganese Filtration. This pretreatment process is accomplished without adding any harmful chemicals to your water.	Ask about our best Packaged Iron Max-125 Pricing when purchased with our TSM or TV Reverse Osmosis Please call 1-877-788-8387 or Email: Sales@ROConsumables.com
Twin Alternating Continuous Water Softener - Hard Water & Iron Removal Pretreatment: **Model: Twin Iron Eater-10** The perfect RO pretreatment for both iron and hard water This system provides up to 25 ppm of Iron (Ferrous & Ferric) and treats up to 110 grains of hard water. ● For use on city municipal water and well water sources. ● Protects TV-RO System from hard water scale, rust, and iron fouling.	

- EZ set-up - We deliver this unit with the tanks and media already assembled. Just fasten valve to the tanks. Includes innovative second tank quick connection.
- Installing this twin alternating water softener prior to the TV reverse osmosis system ensures maximum performance, consistent quality and quantity of water.
- Provides uninterrupted soft water pretreatment as your TV reverse osmosis makes water.
- Salt and water savings by using 100% capacity of the tank in service, before switching to the second tank.
- Regenerates immediately for continuous soft water pretreatment.
- The Alternating Twin Softener can regenerate anytime, day or night, without hard water breakthrough. Thus, eliminating the need for pre-treatment lockout during regeneration.
- Regenerates with soft water and keeps system clean for optimum operating efficiency.
- Proven technology and performance

Ask about our best Packaged Twin Iron Eater Pricing when purchased with our TSM or TV Reverse Osmosis

Please call 1-877-788-8387 or Email: Sales@ROConsumables.com

High Performance Reverse Osmosis Signature State Of The Art Stainless Steel TSM RO Series

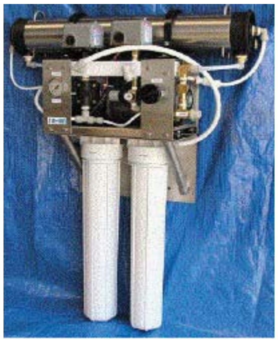
Stainless steel is used throughout; in frame, pressure vessels and fastening hardware to provide the structural strength and corrosion resistance appropriate for a commercial appliance.

Perefect Choice - compact wall mount water filter solutions for coffee shops, cafe's, restaurants, spot-free rinse, labs, and small business.

Need additional information ? Use our TSM Reverse Osmosis Same Day Quote Form

TSM STAINLESS STEEL SERIES
REVERSE OSMOSIS SYSTEM QUICK INSTALL GUIDE - EASY START-UP EASY MAINTENANCE
1. Mount or place the TSM reverse osmosis unit at the desired

Wall Mount / Shelf Mount 400gpd - 1500gpd Water Production
TSM Series Commercial Reverse Osmosis Water Purifiers



TSM Reverse Osmosis System Shown With Attached Pretreatment (Included Free For A Limited Time Only)

TSM QUALITY COMMERCIAL R.O. - User Friendly !
TSM series of commercial Reverse Osmosis water purifiers built to suit the needs and requirements of commercial pure water users.
This includes restaurants, coffee stores, convenience stores, micro breweries, supermarket produce and food preparation operations, misting and humidification systems, car wash facilities, and many other businesses that must have consistent high quality water for their daily operations. The key factors of reliability, servicability, and consistent performance with minimum user intervention are achieved in the solid design of the TSM systems. Stainless steel is used throughout; in frame, pressure vessels and fastening hardware to provide the structural strength and corrosion resistance appropriate for a commercial appliance.

The TSM system design is optimized for either **shelf mount or wall mount installation**. The four models; 400 GPD, 800 GPD, 1200 GPD and 1500 GPD all occupy the same very compact footprint. This allows flexibility in accomodating the limited space available in most commercial utility equipment locations. Quick connect tube fittings on all system ports further simplifies installation and service. Most important, all TSM models incorporate the most reliable combination of proven reverse osmosis hydraulic design and state of the art Thin Film membrane elements to provide the long term performance expected by commercial users.

TSM FEATURES
All TSM Reverse Osmosis systems include the following important quality features.
• Stainless Steel Frame & Pressure Vessels
• Thin Film Composite Membranes
• High Performance 3/4 HP Motors
• Positive Displacement Rotary Vane Pump
• Integral Hydraulic Manifold Assembly
• Fast Flush Control
• Low Feed Pressure Cutout Switch
• Tank Pressure Control Switch
• Delrin Orifice or Teflon Tube Flow Control
• Feed Inlet Solenoid Valve
• SS Needle Valve Pressure Control
• Product Tank Pressure Relief Valve
New 2011 Separate Feature: Water Alarm now included with every TSM Reverse Osmosis Water Purifier Order. Alarm can be floor or wall mounted. Includes 6ft of wire attached to the sensor and up to 100 feet of additional wire can be spliced into the line. Perfect for monitoring from your office or work area.
• Includes: TDS Meter -Tester. The Only Way To Know If Your Reverse Osmosis RO Membrane Is Performing Correctly - Tests for TDS - Total Dissolved Solids. A Must For All RO System Owners. Compare Incoming Feed Water To Treated Water. TDS is the sum of the mineral salts in water and if too high can result in objectionable taste, cloudy ice, interference with the flavor of foods and beverages and scale left behind in pipes, machinery, glass, etc.
• All Systems Wet Tested Before Shipping
Operating Parameters: Max TDS-2500ppm, Total iron is less than 0.3ppm, Manganese is less than 0.05ppm, Water hardness below 5 gpg.
FREE SHIPPING ON ALL TSM REVERSE OSMOSIS WITHIN THE CONTINENTAL UNITED STATES.

OVERSEAS SHIPPING AVAILABLE OR HAVE YOUR FREIGHT FORWARDER PICK-UP
NO CHARGE FOR EXPORT CRATING

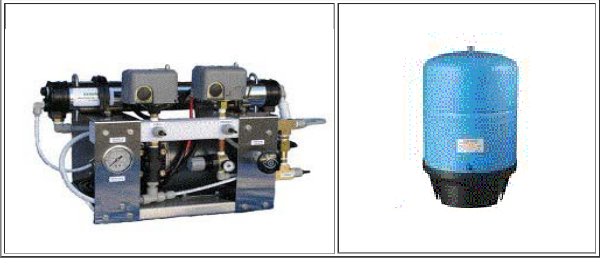
RainDance Water Systems Exclusive *Limited Time Only* TSM Stainless Steel RO Series FREE Pre-Treatment Special

Protect Your Investment, Save Money, Avoid Unnecessary Downtime! - LIMITED TIME FREE SPECIAL

INCLUDED FREE *Dual 20" sediment / carbon filter pretreatment system included **FREE** with every TSM reverse osmosis system order. Attaches directly to the base of the TSM RO system creating a unitized wall mount assembly. The Pre-filter components are mounted within a sturdy, heavy gauge stainless steel frame with easy access to inlet and outlet connections.connections.

FREE Integrated Dual Sediment/Carbon Prefilter System Included.
Provides Reverse Osmosis Membrane Protection from Premature Fouling (loss of rejection & production).
Consists of (1) Sediment Filter Cartridge + (1) Carbon Block Filter Cartridge.
Sediment Filter is rated @ 5 microns and filters Suspended Solids (dirt, rust, sediment, etc).
Carbon Filter Significantly Reduces Chlorine and Organic Compounds.
Heavy gauge stainless steel frame with fasteners to attach to the TSM RO System,
wall mountable as support for TSM-RO system, 2 each 20" Slim Line pre-filters,
Feed shut-off ball valve and EZ-Wrench for no-hassle pre-filter maintenance.

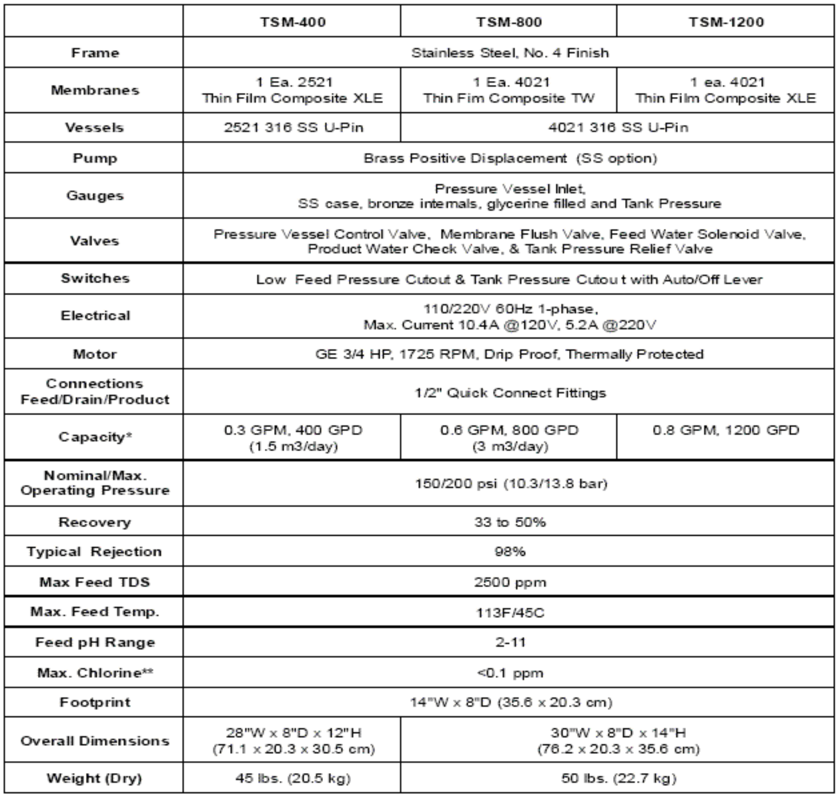
Need additional information ? Use our TSM Reverse Osmosis Same Day Quote Form



Premium Stainless Steel TSM-800 Reverse Osmosis Water Filter Shown With 40 Gal Pre-Charged Pressure Tank

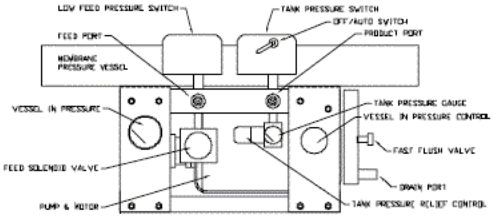
Compact - Perfect For Restaurants, Cafe's, Bars, Coffee, Tea, Ice Supply, Labs, Small Business

TSM Series Commercial Reverse Osmosis Specifications



	TSM-400	TSM-800	TSM-1200
Frame	Stainless Steel, No. 4 Finish		
Membranes	1 Ea. 2521 Thin Film Composite XLE	1 Ea. 4021 Thin Fim Composite TW	1 ea. 4021 Thin Film Composite XLE
Vessels	2521 316 SS U-Pin	4021 316 SS U-Pin	
Pump	Brass Positive Displacement (SS option)		
Gauges	Pressure Vessel Inlet, SS case, bronze internals, glycerine filled and Tank Pressure		
Valves	Pressure Vessel Control Valve, Membrane Flush Valve, Feed Water Solenoid Valve, Product Water Check Valve, & Tank Pressure Relief Valve		
Switches	Low Feed Pressure Cutout & Tank Pressure Cutou t with Auto/Off Lever		
Electrical	110/220V 60Hz 1-phase, Max. Current 10.4A @120V, 5.2A @220V		
Motor	GE 3/4 HP, 1725 RPM, Drip Proof, Thermally Protected		
Connections Feed/Drain/Product	1/2" Quick Connect Fittings		
Capacity*	0.3 GPM, 400 GPD (1.5 m3/day)	0.6 GPM, 800 GPD (3 m3/day)	0.8 GPM, 1200 GPD
Nominal/Max. Operating Pressure	150/200 psi (10.3/13.8 bar)		
Recovery	33 to 50%		
Typical Rejection	98%		
Max Feed TDS	2500 ppm		
Max. Feed Temp.	113F/45C		
Feed pH Range	2-11		
Max. Chlorine**	<0.1 ppm		
Footprint	14"W x 8"D (35.6 x 20.3 cm)		
Overall Dimensions	28"W x 8"D x 12"H (71.1 x 20.3 x 30.5 cm)	30"W x 8"D x 14"H (76.2 x 20.3 x 35.6 cm)	
Weight (Dry)	45 lbs. (20.5 kg)	50 lbs. (22.7 kg)	

* Product flow rates based on flow to atmosphere. When using a bladder pressure tank, product flow rate will reduce as tank back pressure increases.
** Feed water containing chlorine must be dechlorinated by carbon pre-filtration or other means.



Signature Stainless Steel TSM Series Commercial Reverse Osmosis Price Guide

A must have for consistent high quality water - Stainless steel (not aluminum) **is used throughout; in frame, pressure vessels**

and fastening hardware to provide the structural strength and corrosion resistance appropriate for a commercial appliance.

Need additional information ? Use our TSM Reverse Osmosis Same Day Quote Form

Part #	Gallons Per Day	# of Elements	Motor Hp	Voltage	Price
TSM-RO-400	400GPD	1	3/4	110/220	**TSM-400: $2,998.00** **[Add to Cart] [View Cart]** *Free shipping within the continental US E-Mail Or Call Toll Free 1-877-788-8387
TSM-RO-800	800GPD	1	3/4	110/220	**TSM-800: $3,198.00** **[Add to Cart] [View Cart]** *Free shipping within the continental US E-Mail Or Call Toll Free 1-877-788-8387
TSM-RO-1200	1,200GPD	1	3/4	110/220	**TSM-1200: $3,298.00** **[Add to Cart] [View Cart]** *Free shipping within the continental US E-Mail Or Call Toll Free 1-877-788-8387
TSM-RO-1500	1,500GPD	1	3/4	110/220	**TSM-1500: $3,498.00** **[Add to Cart] [View Cart]** *Free shipping within the continental US TSM-1500 Overall Dimensions: 45" W x 8" D x 14" HT Spec Sheet available upon request Email: Sales@RainDanceWaterSystems.com

TSM Series SS Reverse Osmosis can be used with your existing atmospheric storage tank & pressure tanks Or choose from our options list below

TSM REVERSE OSMOSIS STORAGE TANKS:
Needed to store water from reverse osmosis system
Note: Our TSM reverse osmosis tanks include complete float switches for storage tanks (top tank for shut off)

Approx. 35" dia x 81" ht. 300 Gallon Storage Tank (green) (Designed to fit through standard doorways)	Model: TK-300/Float Price: $995.00ea *Free Shipping [Add to Cart] [View Cart]
Approx. 47" diameter and 78" high 550 Gallon Storage Tank (green)	Part #: LCX-TK-ST550 Price: $1,195.00ea *Free Shipping [Add to Cart] [View Cart]

TSM REPRESSURE SYSTEM:
Draws water out of the TSM storage tank to your application

Re-pressure pump rated @ 10gpm (110v) Designed for athmospheric storage tanks listed above.	Model: RP-10GPM Price: $695.00ea *Free Shipping [Add to Cart] [View Cart]

TSM Pre-Filtration & Post-Filtration Option List: Take Advantage Of Great Savings With RDWS Package Pricing
Hard Water Pretreatment, pH Correction, UV Disinfection, Iron, Manganese, Hydrogen Sulfide Gas Prefiltration

HARD WATER PRETREATMENT: NO CHLORIDE DISCHARGE, NO BACKWASHING Calcium and magnesium (limescale) - two of the hardest minerals for a reverse osmosis membrane to remove. Our automatic antiscalant feed system will provide hard water treatment and extend the life of your reverse osmosis membrane and, thus, improve the efficiency of your reverse osmosis water filter. **Water Softener Alternative: Hard Water Treatment (NO-Salt Discharge)** antiscalant feed system - Pretreats and protects the membrane from hard water scale and silica. Antiscalant injection method is preferable to brine regenerating softeners as it is lower maintenance, economical, and is perfect for areas of the country that have water softener chloride discharge limits. The antiscalant formulation has been certified by the National Sanitation Foundation **(NSF) under ANSI/NSF Standard 60** for use in producing potable water. **Model: TOM-CFP-V3000 (protects ro membrane from hard water scale 5gpg to 200gpg)** Features:Power: 110v, Automatic antiscalant injection pump with solution tank	**Model: TOM-CFP-V3000** Price: $995.00ea *Free Shipping [Add to Cart] [View Cart]
Post TSM-RO pH CORRECTION SYSTEM: Increase your pH. Designed to keep your ro water non-corrosive and protect any copper plumbing. 4” x 20” Big Blue Calcite Cartridge mounted separately. Capacity 24,000GAL. Includes housing, bracket, wrench and one 20"cartridge	**Model: CCPH-20-BB** Price: $195.00ea *Free Shipping [Add to Cart] [View Cart]
4” x 20” Big Blue Calcite Replacement Cartridge. Capacity 24,000GAL.	**Replacement Cartridge Model: CCPH-20-BBRPF** Price: $40.00ea *Free Shipping [Add to Cart] [View Cart]

Model: S12Q-PA UV System This compact line of ultraviolet disinfection systems is ideally suited for point of use filtration, RO pre or post disinfection or with a myriad of other applications requiring the flexibility this design offers. The hard glass germicidal lamps provide an economical way of treating water requiring a 4-log (99.99%) reduction of bacteria and virus and protozoan cysts (Giardia lamblia and Cryptosporidium). This process is accomplished without adding any harmful chemicals to your water.	Ask about our best Packaged UV Pricing when purchased with our TSM or TV Reverse Osmosis Please call 1-877-788-8387 or Email: Sales@ROConsumables.com
Well Water Pre-Filtration: **Model: Iron Max-125** This stand alone system provides up to 15 ppm Iron (Ferrous & Ferric) , 7 ppm Hydrogen Sulfide, and 3ppm of Manganese Filtration. This pretreatment process is accomplished without adding any harmful chemicals to your water.	Ask about our best Packaged Iron Max-125 Pricing when purchased with our TSM or TV Reverse Osmosis Please call 1-877-788-8387 or Email: Sales@ROConsumables.com
Twin Alternating Continuous Water Softener - Hard Water & Iron Removal Pretreatment: **Model: Twin Iron Eater-10** The perfect RO pretreatment for both iron and hard water This system provides up to 25 ppm of Iron (Ferrous & Ferric) and treats up to 110 grains of hard water. • For use on city municipal water and well water sources. • Protects TV-RO System from hard water scale, rust, and iron fouling. • EZ set-up - We deliver this unit with the tanks and media already assembled. Just fasten valve to the tanks. Includes innovative second tank quick connection. • Installing this twin alternating water softener prior to the TV reverse osmosis system ensures maximum performance, consistent quality and quantity of water. • Provides uninterrupted soft water pretreatment as your TV reverse osmosis makes water. • Salt and water savings by using 100% capacity of the tank in service, before switching to the second tank. • Regenerates immediately for continuous soft water pretreatment. • The Alternating Twin Softener can regenerate anytime, day or night, without hard water breakthrough. Thus, eliminating the need for pre-treatment lockout during regeneration. • Regenerates with soft water and keeps system clean for optimum operating efficiency. • Proven technology and performance	Ask about our best Packaged Twin Iron Eater Pricing when purchased with our TSM or TV Reverse Osmosis Please call 1-877-788-8387 or Email: Sales@ROConsumables.com

*** Free Shipping Within The Continental US**

RainDance Water Systems Custom TV-RO Complete Skid Series Commercial Reverse Osmosis

Our RDWS-TV-RO Skid Mount Series reverse osmosis systems are designed for applications such as glassware, rinsing, beverage, solution preparation, and numerous other scientific, commercial and industrial applications. Water purified by reverse osmosis has had often greater than 95%of dissolved ions, and 99% of most contaminants removed.

We offer commercial skid water filter solutions for distilleries, breweries, wineries, vineyards, micro-breweries, purified ice and beverage companies.

We offer sodium salts, total dissolved solids, and silica water filtration through commercial reverse osmosis, nano filtration and ultrafiltration systems for agriculture including palm trees, tomatoes, avocados, almonds, nut farms, berries, citrus fruits, grapes, green houses, orchards, and irrigation filtration system for farms.

We offer bacteria, nitrate, sulfate, salt water filtration systems for livestock including horse farms, cattle, pig, chicken, turkey, sheep, duck, poultry farms, dairy farms, dog kennels, exotic animal farms.

We offer complete reverse osmosis water purification skid mount solutions for manufacturing including bottled water stores, pharmaceutical, electronic industry, process water, chemical industry, electroplating industry, electrical power generating, polymer solutions and more.

(All Skid Mount Reverse Osmosis Systems Will Vary In Appearances & Color)

COMPLETE WATER TREATMENT SKIDS - WE INVITE COMPARISONS TO ANYTHING ON THE MARKET!

A must for high sodium and high TDS waters. Unlike, most multi-piece water treatment systems that are scattered throughout your home, business and well house our mobile skid mount unit contains all pretreatment, reverse osmosis, pump, re-pressure system, **NON-AGGRESSIVE water with our built-in skid mount pH neutralizer system** - used to protect copper plumbing and NSF Certified UV on a single skid mount base frame with wheels for easy positioning. And most important our Skid Series reverse osmosis systems are user friendly.

Create the ultimate water supply throughout your entire business or home with our user friendly **Salt Free Complete Skid Mount Series** reverse osmosis system. Each **Skid Mount Series** reverse osmosis system includes: Complete pre-treatment for the removal of iron, manganese, hydrogen sulfide gas, suspended solids, and hard water. This state of the art skid mount reverse osmosis system also includes our pH neutralizing system designed to keep your water non-corrosive. **See our standard features listed below.** All components - (excluding storage tank) are contained on our skid mount platform with wheels - for easy positioning. **Enjoy the benefits of filtered water water at every tap as well as "soft water" without the use softener salt.** Our Skid Mount Reverse Osmosis Systems are among the most water efficient systems in the industry. With the use of concentrate water recirculation our systems can recover 50% to 60% of the feed water as usable product water. Most other systems only recover 30% to 40% of the feed water. Note: Recovery will vary depending on water chemistry.

Once our **Skid Mount RO** is installed and feed water is made available to it, the Storage Tank will begin to fill with product water. The product water will continue to fill the Storage Tank until the tank float switch closes and shuts the entire system off. At the same time, while the tank is filling, concentrate water will begin to flow into the drain system. The concentrate water will continue to flow to the drain system until the tank float switch closes and shuts the entire system off. When there is a demand for water, the Repressurization System is activated and draws product water from the Storage Tank. The product (RO) water is then delivered with the on skid re-pressure system to your home or business. If enough product water is used to the point where the float switch is reopened, the Skid Mount RO Sysem is automatically reactivated and begins to replace the product water that has been depleted.

Why a RainDance Water Systems Top Of The Line Skid Mount Series reverse osmosis system?

Our total skid reverse osmosis systems are designed for the customer who wants a state of the art, user friendly, multi-filtration system to filter and to provide premium water filtration. Reverse Osmosis is the same process that major water, beverage bottling, and pharmaceutical companies use. These systems are designed to filter brackish water, high levels of sodium and total dissolved solids (TDS), iron, manganese, and more. If your well has salt water intrusion problems and high TDS, this is the only solution. Unlike, most multi-piece water treatment systems that are scattered throughout your home or business and well house **our mobile skid mount unit contains all pretreatment, reverse osmosis, pumps, UV on a single skid base frame with wheels for easy positioning. And most important our Skid Series reverse osmosis systems are user friendly.**

One more reason that sets a RainDance Water Systems custom skid reverse osmosis apart from the other guys:

A Must Have For Your Skid Mount Water Treatment System! All Skid RO Series reverse osmosis systems now include a state of the art **microprocessor based controller** which monitors several functional conditions and regulates operation of the high pressure pump and control valves. The controller is connected to sensors which, depending on their state, allow the cyclic production of purified water or prevent operation due to abnormal conditions. The standard configuration of the controller monitors feed water supply pressure for minimum level, product water storage tank level for system start/stop conditions and product water conductivity for maximum set point value and for digital front panel display. Additional optional parameters that the controller is capable of monitoring include main pump high pressure set point, low feed water tank level and membrane flush cycle occurrence and duration. Front panel buttons and digital display allow operator adjustment of set points and flush parameters."Perfect for serviceman or do it yourselfer

Our RDWS-TV-RO Skid Mount Series reverse osmosis systems are designed for applications such as whole house, glassware, rinsing, beverage, solution preparation, and numerous other scientific, commercial and industrial applications. Water treated by reverse osmosis has had often greater than 95%-99% of dissolved ions removed.

RDWS-TV-RO Skid Mount Series reverse osmosis applications include: Whole House, Agriculture, Livestock, Green Houses- Orchards, Groves, Food, Ice,and Beverage Industry, Bottled Water Stores, Pharmaceutical, Electronic Industry, Process Water, Chemical Industry, Electroplating Industry, Electrical Power Generating, Polymer Solutions and more..

COMPARE OUR RDWS-TV-RO-SKID FEATURES - WE INVITE COMPARISONS TO ANYTHING ON THE MARKET!

RO Skid Mount Series Include NSF 55 Class A Certified UV Treatment

Assurance: On Skid UV Ultraviolet Water Treatment carries NSF/ANSI Standard 55, Class A Certification. These units are ideal for use in any UV application where a third-party validated unit is specified.

RDWS-Skid-RO Series are preplumbed and prewired in most cases with one inlet connection, one drain connection, one water use connection, and one electrical connection to allow for fast hassle free installation. From the delivery of the equipment to your facility to the set up and use of water - In hours not days!

RainDance Water Systems Custom TV-RO Skid Series Standard Features

- **All components** (excluding storage tank) located and contained on our skid mount platform with wheels - for easy positioning.
- Approx. System dimensions: 34"W x 96"L x 75" high (1000gpd -12000gpd) Designed to fit through most standard door ways.
- Single point power connection for 220V, 60 Hz, 1-Phase power. Custom and overseas power connections available.
- Includes on skid **(NO-Salt Discharge) Hard Water Treatment** antiscalant feed system - Pretreats and protects the membrane from hard water scale and silica. Antiscalant injection method is preferable to brine regenerating softeners as it is lower maintenance, economical, and is perfect for areas of the country that have chloride discharge limits. The antiscalant formulation has been certified by the National Sanitation Foundation **(NSF) under ANSI/NSF Standard 60** for use in producing potable water.
- Includes on skid **(NO Salt)** iron, manganese, hydrogen sulfide gas, and suspended solids well water pretreatment system or substitute our (No Salt) chlorine removal system for city water sources.
- Automatic Flush on start up - Fast clean-flushes membrane each time the unit starts.
- Includes a **microprocessor based controller -** Controller includes digital product water conductivity indicator, Controller monitors feed water pressure, pump output pressure, product water conductivity, product water storage tank level and performs membrane flush on startup. Low feed pressure, high pump pressure and high product water conductivity result in alarm state indications. System control panel includes prefilter in and out pressure gauges, pressure vessel in and out pressure gauges, product water flow meter, recirc. flow meter with control valve and concentrate flow meter with control valve."Perfect for serviceman or do it yourselfer."
- Includes 1- 20" BB sediment prefilter to remove dirt and sediment
- Includes 1- 20" BB carbon prefilter
- Prefilter outlet equipped with pressure switch for use by system controller to detect low feed pressure and clogged prefilter condition.
- Includes a Thin Film Composite Membrane. Rejection rate (98% +). High feed water recovery rate (50% +).
- Includes additional testing monitor - Portable waterproof tester offers high accuracy pH, electrical conductivity (EC), total dissolved solids (TDS) and temperature measurements in a single tester. Allows you to test untreated and post treated water.
- Includes on skid **Post pH correction of product water. Designed to keep your ro water non-corrosive and protect any copper plumbing.**
- Includes 1- 35" dia. x 81" ht. 300 gallon storage tank. For ease of handling and ability to get through doors we use a multiple array of tanks. Product water storage tank is green, with 16" manway, 2" bottom inlet/outlet (bushed to 1.25"). Two ea. Pump Up float switches installed in tank.
- Includes 12gpm @ 50psi fully automatic electric pump (constant pressure) re-pressure system - Draws water out of the storage tank.
- Includes on skid chemical free ultraviolet UV system. **Ultraviolet Water Treatment carries NSF/ANSI Standard 55, Class A Certification**. These units are ideal for use in any UV application where a third-party validated unit is specified.
- Each skid mount reverse osmosis is fully assembled and tested before it leaves the plant.
- For optimum Skid RO Series performance customers must have at least 7gpm @ 30psi available. Note: Our Skid RO Series contains complete pretreatment for chlorine and hard water (calcium) typically associated with municipal water sources. If Skid RO is to be used on well water we offer the following guidelines: Operating Parameters: Max TDS-3500ppm, Total iron is less than 5ppm, Manganese is less than 3ppm, H2S less than 10ppm, Water hardness below 60gpg, pH 6.5 -8.5, No iron bacteria. Calculations of production capabilities are based on a feed water temperature of 60 Deg. F. Lower temperatures may result in slightly lower production levels.
- Unlike most companies , we are able to tailor our Skid RO systems to your needs. To ensure the longest trouble free system life and best possible treated water quality, we encourage you to fax (760-896-6999) or E-mail your water chemistry report or let us know the name of your municipal water district provider. You may also send us a water sample for evaluation.
- FREE SHIPPING ON ALL RO SKID REVERSE OSMOSIS WITHIN THE CONTINENTAL UNITED STATES.
- OVERSEAS SHIPPING AVAILABLE OR HAVE YOUR FREIGHT FORWARDER PICK-UP

Custom RDWS-TV-RO-Skid Reverse Osmosis Systems Selection & Pricing

Model #	Description	Price
RDWS-TV-RO-SKID-1000	Complete skid mount 1000 gallon per day reverse osmosis system includes standard features and 1 - 35" dia x 80" ht 300 gallon storage tank	Price $13,175.00 Free Shipping Available To Order Call Toll Free

	Click Here For Our No-Hassle TV-RO-SKID Quote	1-877-788-8387 Fax: 760-896-6999
RDWS-TV-RO-SKID-2000	Complete skid mount 2000 gallon per day reverse osmosis system includes standard features and 1 - 35" dia x 80" ht 300 gallon storage tank Click Here For Our No-Hassle TV-RO-SKID Quote	Price $14,975.00 Free Shipping Available To Order Call Toll Free 1-877-788-8387 Fax: 760-896-6999
RDWS-TV-RO-SKID-4000	Complete skid mount 3000 gallon per day reverse osmosis system includes standard features and 1 - 35" dia x 80" ht 300 gallon storage tank Click Here For Our No-Hassle TV-RO-SKID Quote	Price $15,575.00 Free Shipping Available To Order Call Toll Free 1-877-788-8387 Fax: 760-896-6999
RDWS-TV-RO-SKID-6000	Complete skid mount 6000 gallon per day reverse osmosis system includes standard features and 1 - 35" dia x 80" ht 300 gallon storage tank Click Here For Our No-Hassle TV-RO-SKID Quote	Special Price $17,804.00 Free Shipping Available To Order Call Toll Free 1-877-788-8387 Fax: 760-896-6999
RDWS-TV-RO-SKID-8000	Complete skid mount 9000 gallon per day reverse osmosis system includes standard features and 1 - 35" dia x 80" ht 300 gallon storage tank Click Here For Our No-Hassle TV-RO-SKID Quote	Special Price $23,804.00 Free Shipping Available To Order Call Toll Free 1-877-788-8387 Fax: 760-896-6999
RDWS-TV-RO-SKID-10000	Complete skid mount 9000 gallon per day reverse osmosis system includes standard features and 1 - 35" dia x 80" ht 300 gallon storage tank Click Here For Our No-Hassle TV-RO-SKID Quote	Special Price $25,804.00 Free Shipping Available To Order Call Toll Free 1-877-788-8387 Fax: 760-896-6999
RDWS-TV-RO-SKID-12000	Complete skid mount 12000 gallon per day reverse osmosis system includes standard features and 1 - 35" dia x 80" ht 300 gallon storage tank Click Here For Our No-Hassle TV-RO-SKID Quote	Special Price $27,804.00 Free Shipping Available To Order Call Toll Free 1-877-788-8387 Fax: 760-896-6999

Not all reverse osmosis systems are created equal! RainDance Water Systems offers top of the line state of the art reverse osmosis systems for your home, factory, business, and farm. See what sets our reverse osmosis filters apart from all others and why companies and home owners from all over the Globe have chosen RainDance Water Systems for their water treatment needs.

Shipping and Exporting is our specialty. To ensure a safe and proper delivery for your new water treatment equipment we offer export crating and packaging for all overseas orders. We can use your freight forwarder, ship freight collect or we can find the best shipping rates from our facility. For our domestic U.S. orders we offer free shipping any where in the continental US and offer crating or pallet packaging for a safe easy delivery.

INDUSTRIAL REVERSE OSMOSIS WATER FILTRATION

Industrial High Capacity TP Series Industrial Reverse Osmosis 24,000 to 300,000 GPD (16-208gpm) Pricing Guide

RainDance Water Systems specializes in creating specific RO systems to meet unique customer needs. The Horizontal product line allows for large volumes and specialized water treatment. These custom engineered systems can handle a broad range of industrial, commercial and agricultural applications.

Output: 24,000-1,000,000 gal/day Ideal for: Surface, Well, City, and Brackish Water

Integrated Treatment Systems - Industrial Reverse Osmosis Water Filtration Systems
In many past projects, we have had the responsibility of integrating multiple water treatment and process functions with RO membrane purification on an integrated skid system. Such integrated processes have included bulk media prefiltration, ozonation, UV, antiscalant injection, post pH correction injection as well as specific customer components. These specialized integrated systems come on a unitized skid frame with central water and power connections.
These systems have been produced for applications such as humidification control, environmental fog and misting, hothouse/agriculture, food processing, bio-manufacturing, power plant process water, potable water production and others. When a complete system approach is needed to provide the solution to a water treatment requirement, we can deliver the solution.

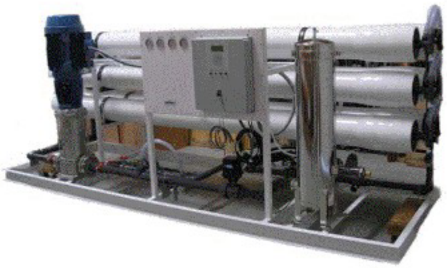
High purity water treatment systems have included both Reverse Osmosis/Deionizing Resin (RO/DI) as well as Reverse Osmosis/Electrodeionization (RO/EDI) systems. These systems produce output water up to 18 megohms and have been employed in chemical processes, medical device cleaning and semiconductor manufacturing. Engineers are well acquainted with the special considerations required in the design and implementation of such systems as to materials, instrumentation, safeguards and controls necessary to provide a reliable and dependable system product.

2.8 GPM Integrated System for Food Process

Features

TP Series industrial reverse osmosis systems are designed to deliver 24,000 to 300,000 GPD (16-208 gpm).



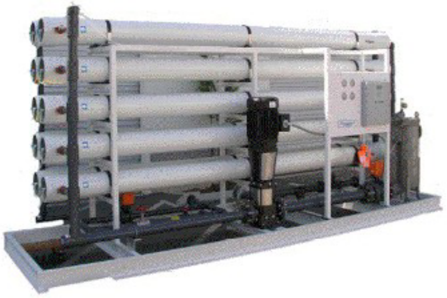
100,000 GPD System

- ABS control panel, NEMA 4X electrical enclosure, UL/CSA
- Microprocessor control: pre-treat lockout, low feed pressure, product tank full, high TDS
- Microprocessor monitoring: product TDS
- System status and warning lights
- Gauges: Vessel array in/out and filter in/out. SS gauges with bronze internals, glycerin filled and mounted on front panel
- Valves: 316SS brine and recirculation valves, globe-type, mounted in the last vessel brine line
- Prefilter: PP or SS bag or multi-cartridge depending on system size
- Pump: 316SS main pump wetted parts
- Frame: Powder coated steel construction on levelers
- Schedule 80 PVC plumbing
- Flow monitoring for product, brine and recirculation

System Options

- Feed pH, TDS and temperature monitoring
- Pump: high pressure for TDS feed streams >5000 ppm
- Gauges: 316SS case and internals, glycerine-filled
- Flushing systems: high flow feed water or product water replacement of brine
- Blending system: filtered feed water into product stream
- Filters: SS (304 or 316) feed water filter housing for bags or mutiple 5-30 micron, 2.5"cartridges
- Frame casters

Support Options



260,000 GPD System

- Pre treatment
  - Chemical injection for chlorine removal, antiscalant, coagulant
  - UV sterilization
  - GACF auto-backwash filtration
  - Cartridge or bag filtration
  - Softener
  - Clarifier or centrifuge separators
- Buffer tank
- ORP meter for chlorine monitoring
- Boost pump
- Post treatment
  - Chemical injection for chlorine
  - UV sterilization
  - GAC auto-backwash filtration
  - Cartridge or bag filtration
  - pH control
- Product storage: atmospheric or pressure
- Pressurizer systems

TP Series High Capacity Industrial Reverse Osmosis

Available in 24,000, 30,000, 40,000, 50,000, 60,000, 70,000, 80,000, 90,000, 100,000 UP TO 1,000,000GPD Capacities.

<Click Here TP Industrial Series Request Information & Price Quote>

COMMERCIAL / INDUSTRIAL
HORIZONTAL REVERSE OSMOSIS WATER FILTRATION ON CASTER WHEELS

Commercial High Capacity TH Series Commercial Reverse Osmosis 12,000 to 24,000 GPD (8-16gpm) Pricing Guide
(Pricing includes all State Of The Art standard features listed below)

Features

TH Series commercial reverse osmosis systems are designe to deliver 12,000 to 24,000 GPD (8-16 gpm).

- ABS control panel, NEMA 4X electrical enclosure, UL/CS
- Microprocessor control: pre-treat lockout, low feed pressu product tank full, high TDS
- Microprocessor monitoring: product TDS
- System status and warning lights
- Gauges: Vessel array in/out (2) and filter in/out (2). SS gauges with bronze internals, glycerin filled and mounted on front panel
- Valves: 316SS brine and recirculation valves, globe-type, mounted in the last vessel brine line, on front panel
- Prefilter: Big Blue with 1.5" FPT ports for a 4.5" x 20", 10-micron element
- Pump: 304SS main pump wetted parts
- Frame: Powder coated steel construction on levelers
- Schedule 80 PVC plumbing and brass hose fittings
- TEFC Motor
- PVC Vessels
- Low energy membrane elements
- Flow monitoring for product, brine and recirculation

Commercial High Capacity TH Series Commercial Reverse Osmosis 12,000 to 24,000 GPD (8-16gpm) Pricing Guide

Performance Specifications

Product Rate	8 - 16 gpm (1.8 - 3.6 m³/h)
Rejection	95-98%
Recovery (without recycle)	30-65%
Design Temperature	68 °F (20°C)

System Specifications

Membrane Type	Thin-film, spiral wound, low energy
Number of Membranes	6-12
Membrane element size	4" x 40"
Array	2x1 to 4x2
Number of pressure vessels	3-6 rated at 225 psi (15.5 bar)
Membranes per vessel	2
Size of pressure vessels	4"ID x 84"L (10 x 213 cm)
Pump	Goulds SVB (304 SS), vertical 1.25" pipe Victaulic-type inlet/outlet
Motor	3 to 7.5HP, TEFC
Electrical	High/low voltage, 50/60Hz, 3-phase
Overall Dimensions	95"W x 30"D x 46.5"H (241 x 76 x 118 cm)
Weight	450 to 750 lbs. (204 to 340 kg)

Design Basis

Water pressure	Minimum 15 psi (1.03 bar)
Feed TDS	5,000 ppm max
Temperature range	±10°F(5.6°C) design temperature
Chlorine level	Dechlorination reqd. if >0.1 ppm
Turbidity	<1 NTU
SDI	3 max

TH Series High Capacity Commercial Reverse Osmosis 12,000-24,000gpd

TH Series Request Information & Price Quote

All TH Series commercial reverse osmosis have caster wheels for easy positioning and/or relocation.

We pride ourselves on our workmanship and our attention to detail.To ensure our customers that our systems will perform to their utmost potential ***WE RECOMMEND FAXING AN EXISTING WATER ANALYSIS OR HAVING YOUR WATER TESTED TO DETERMINE WHICH IS THE BEST SYSTEM FOR YOUR WATER CHEMISTRY.*** Once that recommendation is made, you can have complete faith that your system will work at its peak potential over a long period of time. You can choose to buy a reverse osmosis system "off the rack" and take your chances or you can let us tailor make a system based upon your specific needs. This is what sets RainDance Water Systems commercial reverse osmosis systems apart from all the rest.

Customer Assurance - **If you have a current water analysis report, please fax it to us @ 760-896-6999**, E-mail or send at least a 16oz sample of unfiltered water in a leak proof bottle to:

Please send sample and this form to:
RainDance Water Systems Attn: Commercial Water Sample
1672 E. Main St., Suite E, PMB 312, Ramona, California 92065

Once your feed water has been analyzed, we will provide you with a firm system recommendation.

The only way to ensure that a commercial reverse osmosis system will be both optimally effective and efficient is to plan & design each system on a complete breakdown of the raw water to be treated. RainDance Water Systems will thoroughly analyze your current water analysis or a submitted water sample before making a recommendation. Once that recommendation is made, you can have complete faith that your system will work at its peak potential over a long period of time. You can choose to buy a reverse osmosis system "off the rack" and take your chances or you can let us tailor make a system based upon your specific needs. This is what sets RainDance Water Systems commercial reverse osmosis systems apart from all the rest.

Our customers include numerous companies, manufacturers, cattle & dairy farms, and households around the world - United States, Spain, Japan, Canada, Taiwan, Indonesia, Malaysia, United Kingdom, Cayman Islands, and the Bahamas who require water treatment and pure water applications.

RainDance Water Systems customers include: The United States Coast Guard, Washington St. National Park Service, San Diego State University, Palomar College, Quinlan Texas Elementary School, Hunter Industries, Sonance Corp., Owens Brigam Medical, 1st Choice GMAC Realty, Century 21 Realty, Coldwell Banker Realty, Austin Productions, Fairfield Country Club, and Auer Precision Inc. just to name a few.

RainDance Water Systems
P.O. Box 2312 Ramona, Ca.

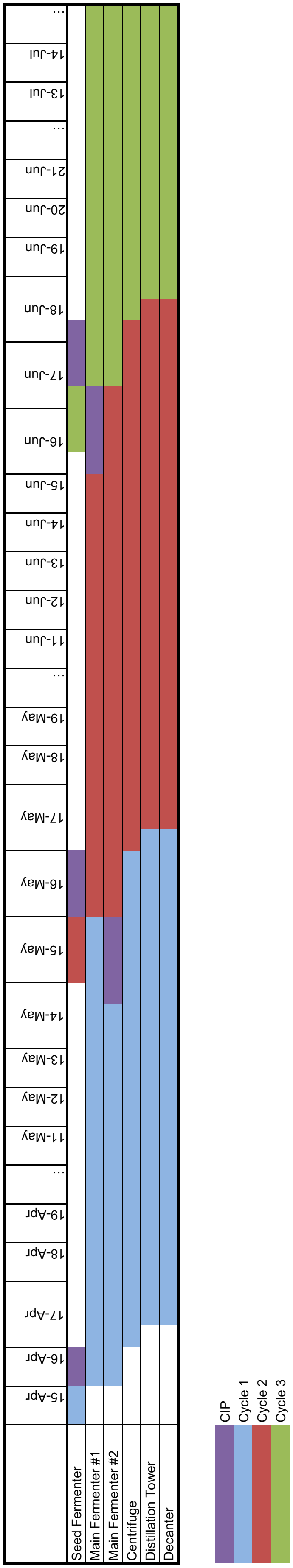
- Phone (USA) 760-788-8387
- Phone (Outside USA) 760-788-8387
- Fax: 760-896-6999
- Sales: sales@roconsumables.com
- Website: www.roconsumables.com
- Website: www.raindancewatersystems.com

Commercial Water Treatment Systems:

Home | Commercial Reverse Osmosis | Light Commercial Reverse Osmosis | EDI - Electrodeionization | Pharmaceutical Water Filters | Reverse Osmosis Quote | Iron and Manganese Removal | Chlorine and Chemical Removal | Multi-Media Filters |Water Treatment Chemicals | Reverse Osmosis Membrane Cleaners | Reverse Osmosis Antiscalants | Reverse Osmosis Membranes | Commercial Water Filters | Commercial Filter Housings |Water Treatment Chemical Quote | Reverse Osmosis Membrane Quote || Water Filter Quote | Filter Housing Quote | Nano Filtration

ROConsumables.com one stop for commercial reverse osmosis solutions, storage tanks, replacement filters, reverse osmosis membranes, water treatment systems

Appendix G: Gantt Chart



Gantt Chart of Process Scheduling