Letter of Transmittal

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April 21, 2020

Dear Dr. Amish Patel and Professor Bruce Vrana,

The following report details a process design for the production of a protease produced by an extremophilic biocatalyst. The extremophilic biocatalyst in question is a bacteria *Natronomonas Pharaonis,* a bacterium that grows at high temperature (60°C), highly basic pH conditions and high salt concentrations. This bacterium produces a protease which is active at these temperatures and has use in potential high-grade detergent applications. This process uses several raw materials such as glucose and other biological nutrients to produce 4,500 metric tons of protease at 80% purity.

This process involves a seed train, followed by batch growth and then continuous production period by two 20,000 L bioreactors. The liquid media containing cells and extracellularly expressed product protein will undergo downstream purification, including centrifugation and ultrafiltration, to separate out cell mass and produce a purified product. The product will then be spray dried and packaged for a sale and distribution to detergent companies.

The economic analysis showed that the process was promising but further market research is required to most properly appraise the plant. The lack of obvious competitors show that the market is highly open but made estimating a product price point difficult. Upon consultant recommendation, economic analyses were conducted to ensure an IRR of 15%, yielding a product price of $36.00/kg. This price point made the project profitable but the net present value was low ($880,900) and made the project only profitable in 2036. It was found that these values were highly sensitive to the product price and so selling the product at a slightly higher price point would make the process significantly more profitable. Therefore, if further market research deems this possible, this process can be well recommended.

We appreciate the input and help throughout this project.

Sincerely,

Jazriel Ortega Sean Begane Adithya Sriram

Enzyme Production Using an Extremophilic Biocatalyst

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1. Abstract

Bio-detergents are useful in many markets because they contain enzymes that can break down proteins and in turn enhance stain removal. However, many of these enzymes deactivate at high temperatures so sterilization and stain removal must be done in two separate processes. This process produces extremozymes, enzymes derived from extremophilic microorganisms that have optimal activity and stability at harsh conditions, to be used in bio-detergents. The thermostable characteristics of this enzyme allow it to accomplish high levels of protein breakdown at temperatures conducive to sterilization. It is expected that this characteristic provides a 15% price premium over the leading incumbant. The extremophilic biocatalyst used for this process is *Natronomonas pharaonis* and the selected enzyme has optimal activity at 60°C and pH 10.0. The target production rate is 4,500 MT per year with the final product being 87% and selling for $36.00/kg. According to a 10-year profitability analysis, the predicted IRR is 15%. In 2021 the Net Present Value will be $880,900. In the third year of production the ROI will be 17.36%.

2. Introduction

2.1 Background

Bio-detergent is a term used to refer to laundry detergents that contain enzymes. These enzymes serve to breakdown proteins and in turn increase stain removal capabilities. Traditionally, these detergents are promoted to busy homes who benefit from running shorter, lower temperature washes to save time and money. The purpose of this project is to develop a bio-detergent that doesn't deactivate at high temperatures and can therefore be used for sanitation and stain removal, simultaneously. The market that will be targeted with this product is medical hospitals.

Enzyme detergents are fast growing as alternatives to synthetic detergents because of their low toxicity, biodegradability, high efficiency, enhanced cleaning properties and overall environmental friendliness. More and more bio-detergents are being looked at as the green alternative. The first patent for the use of enzymes in detergents was filed in 1913 by Dr. Otto Rohm but it was not commercialized because the enzyme could not be massed produced (Spooner, n.d.). The first detergent containing a bacterial enzyme went to market in 1956 and they have been increasing in popularity ever since. Every protease currently used in detergent is produced by the Bacillus bacteria strain because of its ability to produce large amounts of extracellular enzymes. In today's market Novozymes and Genencor International are the companies that produce 95% of the world's proteases (D. Kumar, 2008).

2.2 Project Goals

The goal of this project was to design a process that would produce 4500 metric tons of an extremophilic enzyme through the growth of a genetically modified alkaliphile, *Natronomonus pharaonis*. This particular strain was selected because of its thermostability and halostability. These characteristics are not found in the enzymes produced by the Bacillus genus, which is all the cleaning enzymes currently on the market today. This is believed to provide a 15% price premium over the leading bio-detergent incumbent.

2.3 Project Timeline

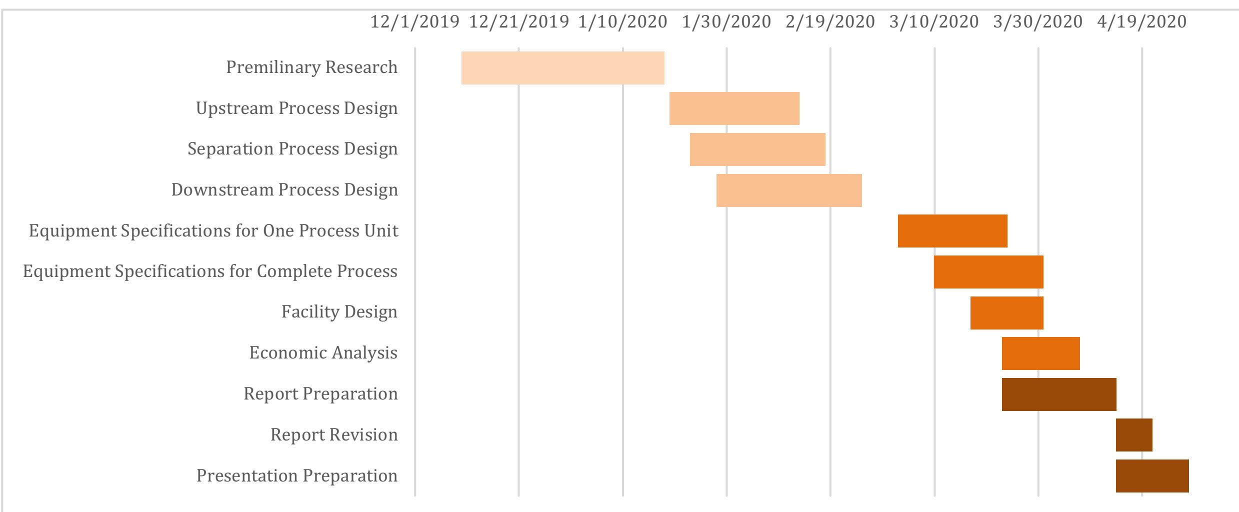

Figure 2.3. Gantt chart showing timeline for process design and report preparation

The first thing addressed in the design of the process was the bioreactor. Designing that required researching the bacteria strain, identifying its optimum growth parameters, developing a medium that would provide the necessary nutrients, and working backward from the total production to size the reactor. After these things were determined a seed train was developed, process flow diagram created, and heat exchangers and pumps designed around the process stages. Going into March, the remaining downstream equipment was designed, a plant location was determined, and the necessary raw materials were sourced. As April arrived, economic analysis and compilation of previous work was completed.

3. Innovation Map

N/A

4. Market and Competitive Assessment

4.1 Extremophiles

While enzymes have been used in cleaners for decades now, extremophilic enzymes, referred to as extremophiles, have yet to be capitalized on in this market. The commercial use of the Taq Polymerase brings in $80 million in sales each year, and is the first highly successful harvesting of an extremophile. Genencor International was the first company to introduce a detergent additive in the form of an extremophilic enzyme to the market and they did so in 1997.

A major bottleneck in the extremophile pipeline was the need for each company wanting to use one to successfully isolate it and test it to determine its ranges of activity. A biotech company in San Diego California began isolating and testing extremophiles for enzymatic activity at many different conditions and compiled various kits for purchase with the enzyme capabilities. This allowed companies to be able to afford working with extremozymes since they no longer had to fund the initial research stages (Pennisi, 1997).

Because of the promising future that enzymes offer, with a wide range of applications almost all providing an eco-friendly alternative to a current industry standard, the market continues to grow and demand for enzymes comes from many different markets.

4.2 Bio-Detergents

The advances in the enzyme market continued and by 2011 proteases accounted for 65% of the global enzyme market because of their popularity in detergents and dairy. Of this number, about 25% of the proteases are alkaline. In 2014 a market research report on the world enzyme market predicted a compound annual growth rate of 7.8% during the years 2015-2020. By 2020 the market would reach 6.3 Billion USD, meaning the market for proteases is about 4.1 Billion USD (Kanupriya Miglani Sharmaa, 2017). All of these proteases are currently produced using Bacillus strain of bacterium and nearly every substitution at each amino acid position has been patented. This makes further development of enzymes in this category extremely difficult and not very cost effective. The search for new extremophilic enzymes that can break into this market should be conducted outside of the Bacillus subtilis (D. Kumar, 2008).

4.3 This Product

The enzyme produced in this process uses a different genus of bacteria that has not been used for detergent additives before. The enzyme itself retains enzymatic activity at high temperatures and a wide range of pH's, meeting the desired characteristics of extremophilic detergent additives. This bacteria will be genetically modified to produce the enzyme extracellularly, offering the same convenience the Bacillus subtilis is able to provide. Because of the extremophilic nature a 15% price premium over the leading bio-detergent can be assumed. However, due to a lack of market research, the price was set to $36.00/kg to achieve an IRR of 15%. A sensitivity analysis on this price is presented in Section 21.1**.** The enzyme is not being used in pharmaceutical applications the percent purity of the final product is 87% which allowed for cut costs in the purification steps.

5. Customer Requirements

N/A

6. CTQ Variables – Product Requirements

N/A

7. Product Concepts

There is a possibility to modify the bacteria to produce other extracellular components that can be sold as well. While this would allow one process to offer multiple revenue streams, it would complicate the purifications and separation steps because the other component would not be necessary in the detergent industry. Additional separation steps would increase the cost of the process and would negatively impact the purities of both final products.

There was also the option to have the enzyme produced intracellularly and add cell lysis to the process but reduce the hydraulic load downstream. However, following the industry standard of using a bacterial strain that is capable of secreting extracellular enzymes, it was decided that an extracellular enzyme would lead to a more efficient process.

8. Superior Product Concepts

The final product will be an isolated extremophilic enzyme that can be sold to consumer goods manufacturers for use in detergents. It will have 87% purity and be the only product produced by the process. Focusing on isolating only one component of the cell allowed for a more streamlined and efficient process. Additionally, the decision to engineer the bacteria to secrete the enzyme extracellularly allowed for further simplification of the process by avoiding cell lysis and separation.

9. Competitive Patent Analysis

The genetic modification of bacterial cells for the purpose of producing a secondary, desired product is commonplace. The 1980 Supreme Court case *Diamond vs. Chakrabarty*, ruled that such genetically modified organisms can be patented. However, because all other biodetergent enzymatic additives are produced through the Bacillus subtilis, no patents were found to exist for the process described in this report.

Leading enzyme producers Genencor and Novozymes, may be working on a similar product and should patent lawyers find an existing patent that this process may be infringing on, 10% of profits would be offered to the patent owner.

Many patents were found for detergent compositions with enzymes and spray drying techniques of the final powder detergent, but all of these do not pertain to this particular process. It was worth noting that many of the formulation patents were held by Proctor and Gamble, who also own the two most popular detergent brands in the US, Tide and Gain, so that solidified the decision to attempt to draft an exclusivity contract with P&G to avoid patent fees.

10. Preliminary Process Synthesis

10.1 Raw Materials

The growth of bacteria is an aerobic process that requires a sizeable supply of raw materials. The elemental composition of the *Natronomus Pharaonis* bacteria strain was determined and scaled up to calculate the medium composition. Each element was provided in 25% excess, per the recommendation of the project author.

10.1.1 Alternative Carbon Sources

The carbon sources considered for this process were acetic acid and glucose. Glucose is more commonly produced in bulk and had a significantly cheaper price per metric ton ~$363 per metric ton**,** compared to ~$4535 per metric ton. In previous experiments where this bacteria was grown at lab scale, acetate was the most common carbon source that was used. However, it was determined that the colonies of extremophiles that grew in the presence of glucose could be selected for during the inoculation process which would make glucose viable as a food source. The inoculation process at the beginning of plant operation could be run repeatedly until such a colony was available, or until it is determined that acetate is required. Additionally, amino acids were considered as the carbon source as there were reports of amino acids being used as a nutrition source. This is most likely due to amino acids containing carbon in a carboxyl group, similar to acetate. However, these were extraordinarily expensive even compared to sodium acetate (e.g. price per metric ton for sodium glutamate was ~$36000).

10.1.2 Alternative Nitrogen Source

Rather than sparging ammonia gas through the reactor, adding ammonium chloride as a component of the growth media was considered. This was because references stated the use of ammonium chloride in the solution and also noted that the organism in question had uptake receptors for ammonium. However, upon recommendation from the consultant, due to standard practices and the benefit of added agitation from the gas flow, it was decided to use ammonia gas sparged into the reactor.

10.1.3 Alternative Oxygen Source

The most cost effective way to provide oxygen to the system would be to use pure air, so this was the first possibility that was considered. The problem statement provided an oxygen uptake rate of 250 mMol O_2/Liter/hr, which translated to 162 kg/hr of oxygen. The gas sparge rate into the reactor vessel was limited by a measurement of the volumetric flow rate per working volume of reactor (VVM). Upon recommendations from the consultant, the VVM was restricted to 0.1-0.2. A certain amount of this volumetric flow rate was to be dedicated to ammonia. If oxygen enriched air were to be used, the amount required would exceed to the allocated volumetric flow rate to meet the VVM requirement. Therefore it was decided that pure oxygen would be fed into the reactors.

10.2 Alternative Plant Locations

The primary plant locations that were considered were Lima, Ohio and Alexandria, Louisiana. As mentioned in Section 9, most of the patents for bio-detergent formulation are held by Proctor and Gamble so potential plant locations were by their detergent production sites. Upon further analysis it was decided to place the plant near the glucose source because 21,000 MT/yr of glucose would need to be transported to the plant and only 4500

MT/yr of product would be transported out of the plant. Discussing these limitations with Cargill, a starch supplier in the Midwest, they recommended the plant be placed in Dayton, Ohio because there is a Cargill distributor near there. In this way, transportation costs are minimized into and out of the plant.

10.3 Reactor Designs

The total working volume for the process to maintain peak cell density was calculated to be approximately 32000 L from the cell doubling time. The use of a single reactor vessel at this volume was considered as the price of a single larger reactor vessel was less than that of two 16000 L vessel. However, if only one production bioreactor was operating at a time, any bioreactor malfunctioning or contamination would completely halt production for that campaign. With two 16000 L vessels, the flow rate to the downstream process can be halved in the case of a required shutdown in one of the reactors, leading to half the product loss than if there was only one 32000 L vessel. As such, the cost of two reactors over one reactor is justifiable as a precautionary measure.

10.4 Campaign Schedule

Because of the genetic modification to the *N. pharaonis* cells in this process, the genetic stability over many generations lasts approximately 8 days. For this reason, a maximum of 8 days may pass from initial inoculation in the 2 L seed reactor to the end of the continuous production bioreactor operation. The two production bioreactors will operate in a slightly staggered manor so that plant operators have time between reactor startups, while still maximizing the time that the downstream process will operate at maximum flowrate.

The first campaign will begin with inoculation of the 2 L seed train reactor with a 1 mL aliquot of cells from the cell bank. It will take approximately 16 hours for the 2 L reactor to reach peak cell density, at which point the entire contents of the reactor will be transferred to the 2,000 L seed train reactor, and the 2 L reactor will be re-inoculated for a second growth period. When the 2,000 L reactor reaches peak cell density after 15 hours, the contents will be transferred to the first production scale bioreactor for 5 hours of batch growth. During these 5 hours, the second 2,000 L seed train inoculation will occur. After the 5 hours conclude and the first production scale reactor reaches peak cell density, it will begin continuous operation, with outlet from the bioreactor being fed to a holding tank. Approximately 10 hours later, the second 2,000 L run will end, and the second production reactor will begin batch operation. At 52 hours after the initial inoculation of the first 2 L reactor, the second production scale bioreactor will enter continuous operation and the downstream processing will begin. Each continuous campaign production phase will last 156 hours, or the amount of time to reach 8 days since the seed train for that production reactor was first inoculated. After the continuous operation ends, a 24 hour period of CIP and maintenance will begin for the reactor. The downstream process will cease after the holdup tank is completely drained and will then also begin CIP and maintenance until the next campaign begins. The beginning of the next campaign for the first production reactor will begin with inoculation of the 2 L seed train reactor 8 hours before the continuous production phase for the first reactor ends. This will ensure that the 2000 L reactor will reach peak cell density at the same time that the 24 hour CIP/maintenance period ends. The seed train schedule will match the first seed train schedule, leading to inoculation of the two production reactors at the same offset each time. The plant will operate for 95% of the year, or approximately 347 days, amounting to 38 production cycles for each reactor.

The campaign schedule for the first two cycles is depicted in Figure 10.4.

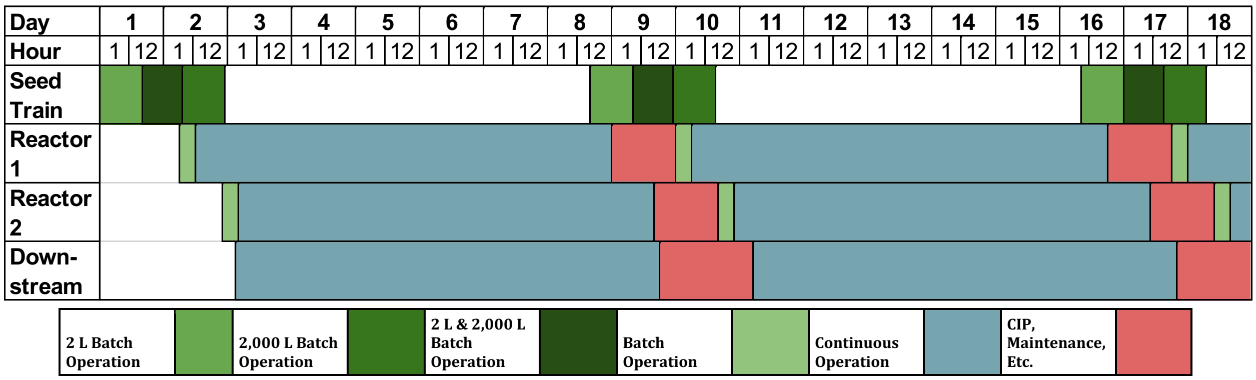

Figure 10.4. Campaign schedule showing operation modes for the Seed Train, Production Reactor, and Downstream process steps for the first two cycles.

11. Assembly of Data Base

11.1 Thermophysical Properties and Toxicity Data

All ASPEN simulations were done assuming the properties of water, which was a reasonable approximation as all the fluids in this process were either water or water with dissolved minerals and biological material. Chemical safety requirements were determined using the Material Safety Data Sheets. All of these MSDS's are included in Appendix C. Heat exchange calculations were done assuming the system had the same thermophysical propertied as water. The mass balance was done assuming elemental compositions of another halophilic bacteria, *Salinibacter* (Aharon Oren, 2002).

11.2 Cell Growth Kinetics

The cell growth kinetics for this fermentation process were provided in the project statement. The doubling time of the cell is 1.5 hours, and the peak product titer is 55 g/L with a bone dry mass cell density of 30 g/L. It was assumed that throughout the continuous portion of the fermentation process the concentration of cells were present at these densities. Upon discussion with the author is what decided that the total biomass density was 85 g/L and the enzyme was extracellular, with the product titer of 55 g/L. The seed train that was developed operates with a fed-batch structure. The peak oxygen uptake rate is 250 mMol O_2/Liter/hour and the enthalpy of fermentation is 14,400 KJ/Kg O_2 consumed. Each bioreactor campaign can run for 8 days before re-inoculation and there is a 95% process uptime. Finally, the dried biomass packaged bulk density is 0.7 grams/Liter.

11.3 Cost of Principle Chemicals

The primary raw materials in this process are glucose, oxygen, ammonia and the bacteria. Because the largest quantity to be purchased was glucose, Cargill was selected to supply it and placed the plant in Dayton, Ohio, in the same city as one of their distribution centers. It will be delivered by truck at $881.85/MT. Anhydrous ammonia will be purchased as compressed gas from Nutrien and delivered by rail from their distribution center in Northbrook, Illinois. Because of the high oxygen uptake rate of this cellular growth process it was decided to purchase an oxygen generator and supply oxygen to the process that way. The one time cost of the bacteria was determined to be $376 per 5 mL of cell broth as priced on the American Type Culture Collection website for a for-profit purchase. Costs for the other components outlined in Aharon Oren, 2002 were determined by searching Alibaba for bulk quantities of each. The cost of water and utilities, including but not limited to, cooling water, steam, electricity, and process water were estimated using the cost factors for utilities in *Product and Process Design Principles, Fourth Edition.* These costs are outlined in Section 19.2.

The final product for sale is 87% of the desired enzyme by weight. This enzyme's extremophilic properties offer it a 15% price premium over the leading incumbent. The leading incumbent is supplied by either Novozyme, DSM, or Genencor, the three largest enzyme suppliers in the world. The prices at which they sell to consumer goods companies are not public and thus were not available for use in determining the price of this product. The base case for this project appraisal was at a product price point of $36.00/kg to return an IRR of 15%. A sensitivity analysis for this price can be found in Section 21.1. The costs of all outlined materials is shown in Table 11.1.

Table 11.1 Costs of all discussed raw materials

Material	**Cost (USD/MT)**	**Minimum Order**
Glucose	$881.85	N/A
Ammonia	$473	N/A
Sulfur	$49	25 MT
Sodium Phosphate	$1000	N/A
Sodium Chloride	$67.25	23 MT
Potassium Hydroxide	$900	25 MT
Magnesium Chloride	$300	5 MT
Boric Acid	$700	25 MT

12. Process Flow Diagrams and Material Balances

12.1 General Flow Sheet

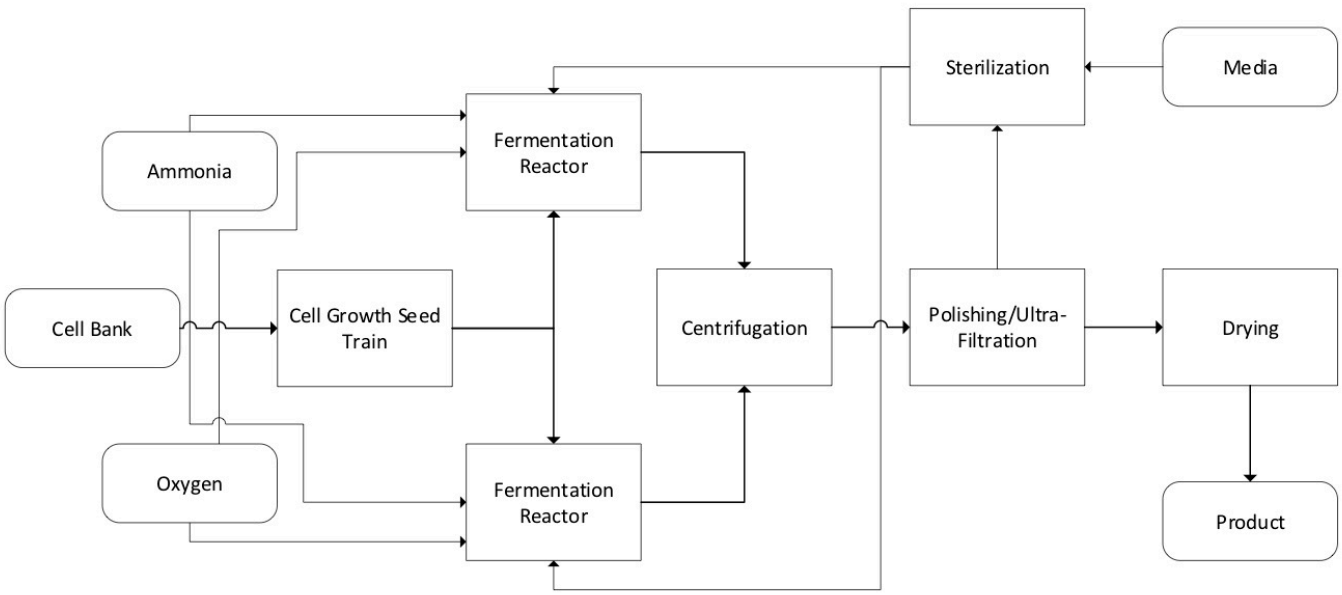

Figure 12.1. General flow sheet for the process including all main reactants, process steps, and final products.

12.2 Seed Reactor Process Flow Diagram

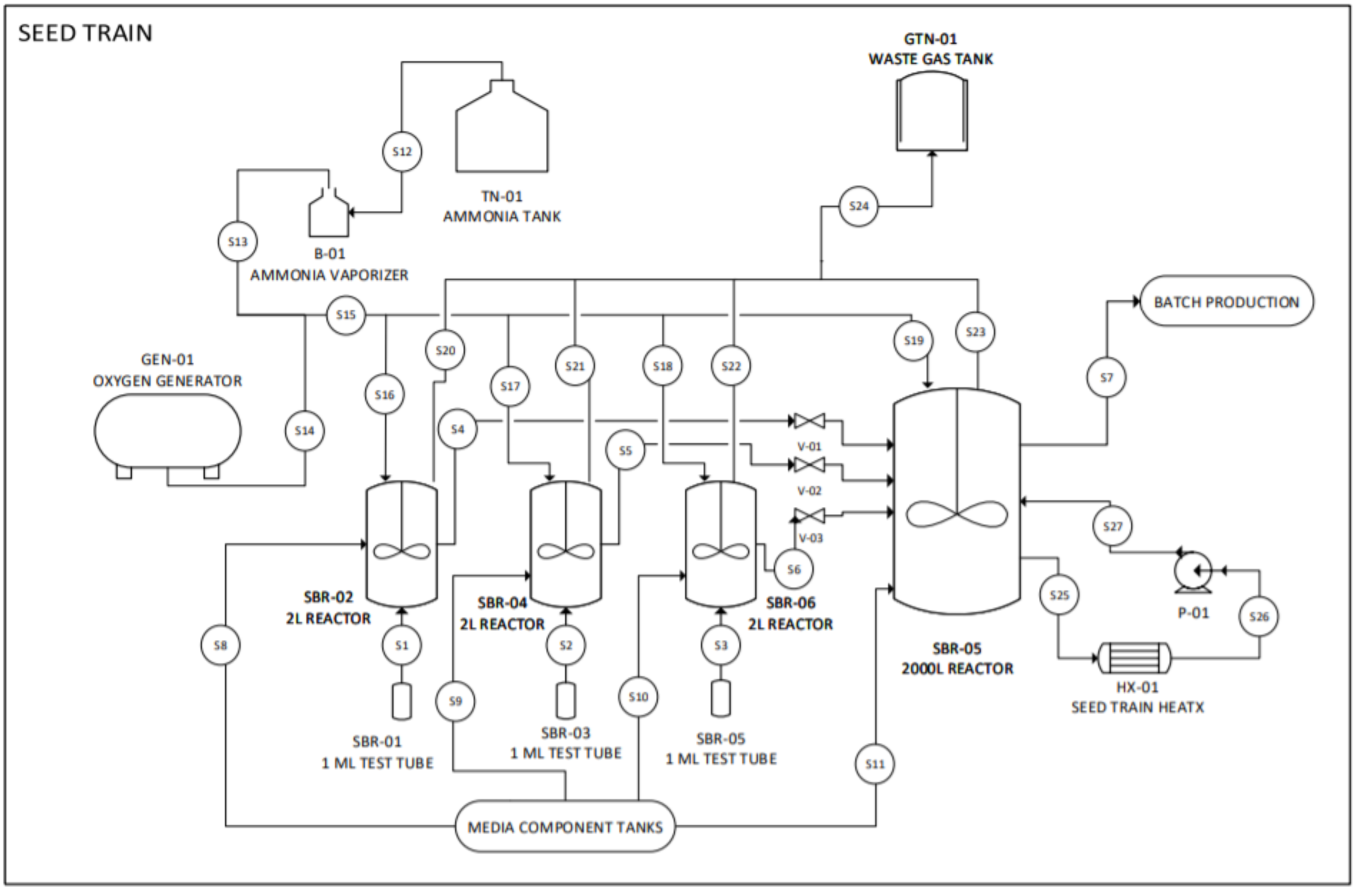

Figure 12.2. Process flow diagram for seed train including all main reactants and process steps.

12.3 Seed Reactor Material Balances

Table 12.3.1 Material balance for 2L seed train batch reactors

2L REACTOR 1									
Stream	**S12**	**S13**	**S14**	**S15**	**S1**	**S8**	**S16**	**S4**	**S20**
Description	**Ammonia tank**	**Vaporized ammonia**	**Generated oxygen**	**Oxygen/ammonia mix**	**From aliquot**	**Media in**	**Gases in**	**Cells out**	**Waste gas**
Temperature	32.0	66.0	66.0	66.0	32.0	32.0	66.0	60.0	60.0
Pressure	1.2	1.4	1.4	1.4	1.0	1.2	1.4	1.2	1.2
Total Mass (g/batch)	5116.9	5116.9	242720.8	247837.6	1.0	2126.6	263.9	1754.6	163.6
Component Mass (g/batch)									
Water	0.0	0.0	0.0	0.0	1.0	1617.5	0.0	1618.5	0.0
Glucose	0.0	0.0	0.0	0.0	0.0	151.2	0.0	0.0	0.0
Carbon Waste	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.0
Sodium Phosphate	0.0	0.0	0.0	0.0	0.0	4.8	0.0	1.0	0.0
Sulfur	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.1	0.0
Sodium Chloride	0.0	0.0	0.0	0.0	0.0	328.6	0.0	65.7	0.0
Potassium Hydroxide	0.0	0.0	0.0	0.0	0.0	14.8	0.0	3.0	0.0
Magnesium Chloride	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.1	0.0
Boric Acid	0.0	0.0	0.0	0.0	0.0	8.6	0.0	8.6	0.0
Oxygen	0.0	0.0	242720.8	242720.8	0.0	0.0	258.8	0.0	51.8
Ammonia	5116.9	5116.9	0.0	5116.9	0.0	0.0	5.1	0.0	1.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	110.8
Cells	0.0	0.0	0.0	0.0	0.0	0.0	0.0	48.6	0.0
Product Protein	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other Extracellular Protein	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

2L REACTOR 2					
Stream	**S2**	**S9**	**S17**	**S5**	**S21**
Description	**From aliquot**	**Media in**	**Gases in**	**Cells out**	**Waste gas**
Temperature	32.0	32.0	66.0	60.0	60.0
Pressure	1.0	1.2	1.4	1.2	1.2
Total Mass (g/batch)	1.0	2126.6	263.9	1754.6	163.6
Component Mass (g/batch)					
Water	1.0	1617.5	0.0	1618.5	0.0
Glucose	0.0	151.2	0.0	0.0	0.0
Carbon Waste	0.0	0.0	0.0	9.1	0.0
Sodium Phosphate	0.0	4.8	0.0	1.0	0.0
Sulfur	0.0	0.4	0.0	0.1	0.0
Sodium Chloride	0.0	328.6	0.0	65.7	0.0
Potassium Hydroxide	0.0	14.8	0.0	3.0	0.0
Magnesium Chloride	0.0	0.7	0.0	0.1	0.0
Boric Acid	0.0	8.6	0.0	8.6	0.0
Oxygen	0.0	0.0	258.8	0.0	51.8
Ammonia	0.0	0.0	5.1	0.0	1.0
Carbon Dioxide	0.0	0.0	0.0	0.0	110.8
Cells	0.0	0.0	0.0	48.6	0.0
Product Protein	0.0	0.0	0.0	0.0	0.0
Other Extracellular Protein	0.0	0.0	0.0	0.0	0.0

2L REACTOR 3					
Stream	**S3**	**S10**	**S18**	**S6**	**S22**
Description	**From aliquot**	**Media in**	**Gases in**	**Cells out**	**Waste gas**
Temperature	32.0	32.0	66.0	60.0	60.0
Pressure	1.0	1.2	1.4	1.2	1.2
Total Mass (g/batch)	1.0	2126.6	263.9	1754.6	163.6
Component Mass (g/batch)					
Water	1.0	1617.5	0.0	1618.5	0.0
Glucose	0.0	151.2	0.0	0.0	0.0
Carbon Waste	0.0	0.0	0.0	9.1	0.0
Sodium Phosphate	0.0	4.8	0.0	1.0	0.0
Sulfur	0.0	0.4	0.0	0.1	0.0
Sodium Chloride	0.0	328.6	0.0	65.7	0.0
Potassium Hydroxide	0.0	14.8	0.0	3.0	0.0
Magnesium Chloride	0.0	0.7	0.0	0.1	0.0
Boric Acid	0.0	8.6	0.0	8.6	0.0
Oxygen	0.0	0.0	258.8	0.0	51.8
Ammonia	0.0	0.0	5.1	0.0	1.0
Carbon Dioxide	0.0	0.0	0.0	0.0	110.8
Cells	0.0	0.0	0.0	48.6	0.0
Product Protein	0.0	0.0	0.0	0.0	0.0
Other Extracellular Protein	0.0	0.0	0.0	0.0	0.0

Table 12.3.2 Material balance for 2000L seed train batch reactor

2000L REACTOR									
Stream	**S4/S5/S6**	**S11**	**S19**	**S7**	**S23**	**S24**	**S25**	**S26**	**S27**
Description	**From 2L reactor**	**Media in**	**Gases in**	**Cells out**	**Waste gas**	**Total waste gas**	**To heatx**	**To heatx pump**	**From heatx**
Temperature (C)	60.0	60.0	52.0	60.0	60.0	60.0	60.0	60.0	32.0
Pressure (bar)	1.2	1.2	1.4	1.2	1.2	1.2	1.4	1.2	1.2
Total Mass (kg/batch)	1865.4	2125.8	247.0	1754.6	160.1	207.8	6347.3	6347.3	6347.3
Component Mass (kg/batch)									
Water	1618.5	1616.9	0.0	1618.5	0.0	0.0	5855.0	5855.0	5855.0
Glucose	0.0	151.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbon Waste	9.1	0.0	0.0	9.1	0.0	0.0	32.8	32.8	32.8
Sodium Phosphate	1.0	4.8	0.0	1.0	0.0	0.0	3.5	3.5	3.5
Sulfur	0.1	0.4	0.0	0.1	0.0	0.0	0.3	0.3	0.3
Sodium Chloride	65.7	328.5	0.0	65.7	0.0	0.0	237.7	237.7	237.7
Potassium Hydroxide	3.0	14.8	0.0	3.0	0.0	0.0	10.7	10.7	10.7
Magnesium Chloride	0.1	0.7	0.0	0.1	0.0	0.0	0.5	0.5	0.5
Boric Acid	8.6	8.6	0.0	8.6	0.0	0.0	31.1	31.1	31.1
Oxygen	0.0	0.0	241.9	0.0	48.4	203.7	0.0	0.0	0.0
Ammonia	0.0	0.0	5.1	0.0	1.0	4.1	0.0	0.0	0.0
Carbon Dioxide	110.8	0.0	0.0	0.0	110.7	0.0	0.0	0.0	0.0
Cells	48.6	0.0	0.0	48.6	0.0	0.0	175.6	175.6	175.6
Product Protein	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other Extracellular Protein	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

12.4 Batch Process Flow Diagram

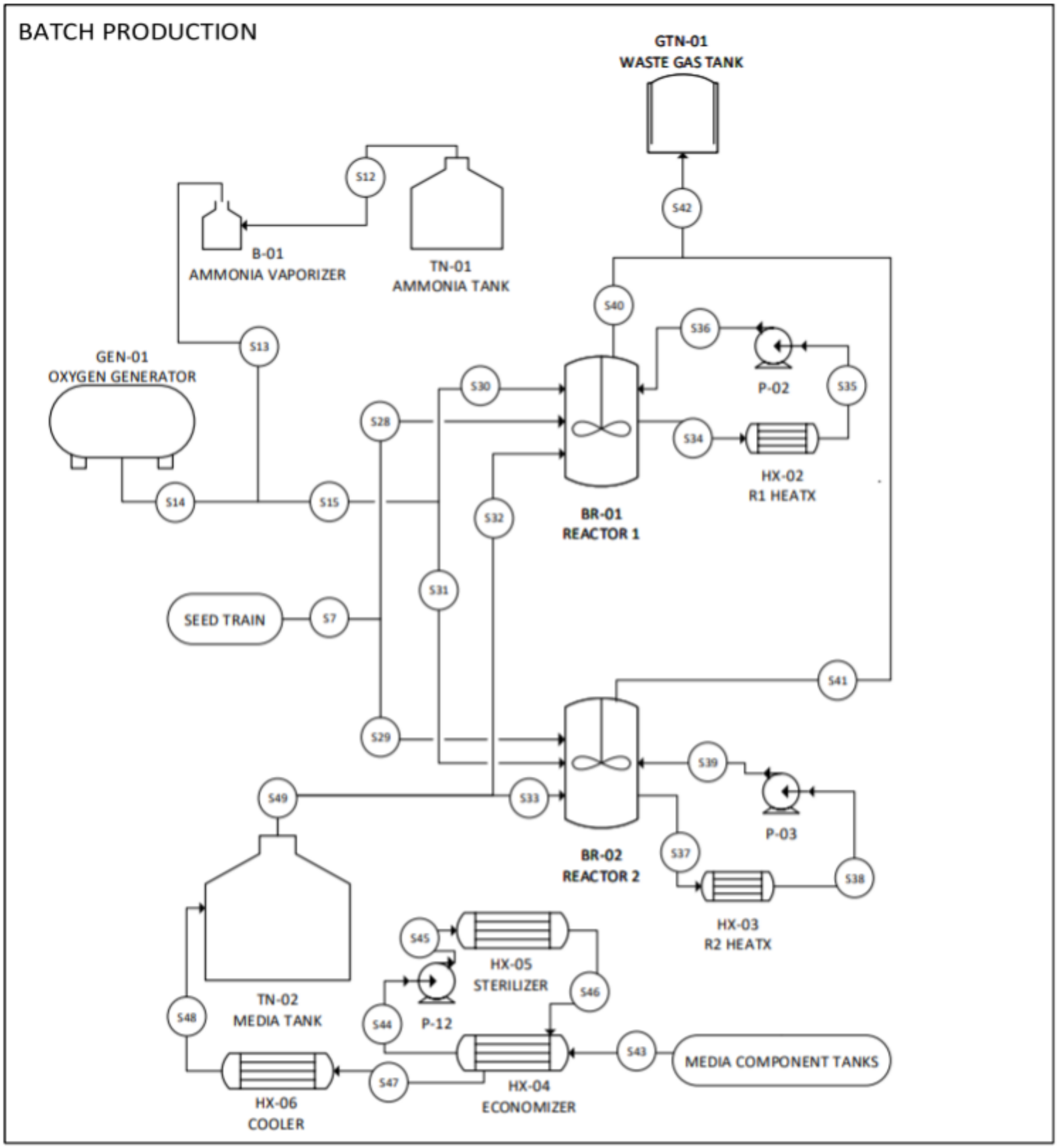

Figure 12.4. Process flow diagram for batch process including all main reactants, process steps and final products.

Table 12.4.1 Material balances for media tank during batch operation

MEDIA TANK							
Stream	**S43**	**S44**	**S45**	**S46**	**S47**	**S48**	**S49**
Description	**From component tanks**	**From economizer**	**To sterilizer**	**To economizer**	**To cooler**	**From cooler**	**From media tank**
Temperature	25.0	63.0	63.0	140.0	106.0	32.0	32.0
Pressure	1.2	1.2	3.6	3.6	3.6	1.2	1.2
Total Mass (kg/batch)	38817.2	38817.2	38817.2	38817.2	38817.2	38817.2	38817.2
Component Mass (kg/batch)							
Water	29133.0	29133.0	29133.0	29133.0	29133.0	29133.0	29133.0
Glucose	2723.2	2723.2	2723.2	2723.2	2723.2	2723.2	2723.2
Carbon Waste	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sodium Phosphate	85.1	85.1	85.1	85.1	85.1	85.1	85.1
Sulfur	7.8	7.8	7.8	7.8	7.8	7.8	7.8
Sodium Chloride	6439.7	6439.7	6439.7	6439.7	6439.7	6439.7	6439.7
Potassium Hydroxide	260.0	260.0	260.0	260.0	260.0	260.0	260.0
Magnesium Chloride	12.7	12.7	12.7	12.7	12.7	12.7	12.7
Boric Acid	155.8	155.8	155.8	155.8	155.8	155.8	155.8
Oxygen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cells	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Product Protein	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other Extracellular Protein	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 12.4.2 Material balances for 20,000 L reactors in batch operation

REACTOR 1						
Stream	**S7**	**S28**	**S12**	**S13**	**S14**	**S15**
Description	**From seed train**	**Cells in**	**Ammonia tank**	**Vaporized ammonia**	**Generated oxygen**	**Oxygen/ammonia mix**
Temperature	60.0	60.0	32.0	66.0	66.0	66.0
Pressure	1.2	1.2	1.2	1.4	1.4	1.4
Total Mass (kg/batch)	3509.2	1754.6	102.0	102.0	1613.0	1715.0
Component Mass (kg/batch)						
Water	3237.0	1618.5	0.0	0.0	0.0	0.0
Glucose	0.0	0.0	0.0	0.0	0.0	0.0
Carbon Waste	18.1	9.1	0.0	0.0	0.0	0.0
Sodium Phosphate	1.9	1.0	0.0	0.0	0.0	0.0
Sulfur	0.2	0.1	0.0	0.0	0.0	0.0
Sodium Chloride	131.4	65.7	0.0	0.0	0.0	0.0
Potassium Hydroxide	5.9	3.0	0.0	0.0	0.0	0.0
Magnesium Chloride	0.3	0.1	0.0	0.0	0.0	0.0
Boric Acid	17.2	8.6	0.0	0.0	0.0	0.0
Oxygen	0.0	0.0	0.0	0.0	1613.0	1613.0
Ammonia	0.0	0.0	102.0	102.0	0.0	102.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0
Cells	97.1	48.6	0.0	0.0	0.0	0.0
Product Protein	0.0	0.0	0.0	0.0	0.0	0.0
Other Extracellular Protein	0.0	0.0	0.0	0.0	0.0	0.0

REACTOR 1 (CONT.)						
Stream	**S30**	**S32**	**S40**	**S34**	**S35**	**S36**
Description	**Gases in**	**Media in**	**Waste gas**	**To heatx**	**To heatx pump**	**From heatx**
Temperature	66.0	32.0	60.0	60.0	32.0	32.0
Pressure	1.4	1.2	1.2	1.2	1.2	1.2
Total Mass (kg/batch)	857.5	19408.6	1169.1	17542.2	17542.2	17542.2
Component Mass (kg/batch)						
Water	0.0	14566.5	0.0	16185.0	16185.0	16185.0
Glucose	0.0	1361.6	0.0	0.0	0.0	0.0
Carbon Waste	0.0	0.0	0.0	90.7	90.7	90.7
Sodium Phosphate	0.0	42.5	0.0	8.7	8.7	8.7
Sulfur	0.0	3.9	0.0	0.8	0.8	0.8
Sodium Chloride	0.0	3219.8	0.0	657.1	657.1	657.1
Potassium Hydroxide	0.0	130.0	0.0	26.6	26.6	26.6
Magnesium Chloride	0.0	6.4	0.0	1.3	1.3	1.3
Boric Acid	0.0	77.9	0.0	86.5	86.5	86.5
Oxygen	806.5	0.0	161.3	0.0	0.0	0.0
Ammonia	51.0	0.0	10.2	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	997.6	0.0	0.0	0.0
Cells	0.0	0.0	0.0	485.6	485.6	485.6
Product Protein	0.0	0.0	0.0	0.0	0.0	0.0
Other Extracellular Protein	0.0	0.0	0.0	0.0	0.0	0.0

REACTOR 2								
Stream	**S29**	**S31**	**S33**	**S41**	**S42**	**S37**	**S38**	**S39**
Description	**Cells in**	**Gases in**	**Media in**	**Waste gas**	**Total waste gas**	**To heatx**	**To heatx pump**	**From heatx**
Temperature	60.0	66.0	32.0	60.0	60.0	60.0	32.0	32.0
Pressure	1.2	1.4	1.2	1.2	1.2	1.2	1.2	1.2
Total Mass (kg/batch)	1754.6	857.5	19408.6	1169.1	2338.3	17542.2	17542.2	17542.2
Component Mass (kg/batch)								
Water	1618.5	0.0	14566.5	0.0	0.0	16185.0	16185.0	16185.0
Glucose	0.0	0.0	1361.6	0.0	0.0	0.0	0.0	0.0
Carbon Waste	9.1	0.0	0.0	0.0	0.0	90.7	90.7	90.7
Sodium Phosphate	1.0	0.0	42.5	0.0	0.0	8.7	8.7	8.7
Sulfur	0.1	0.0	3.9	0.0	0.0	0.8	0.8	0.8
Sodium Chloride	65.7	0.0	3219.8	0.0	0.0	657.1	657.1	657.1
Potassium Hydroxide	3.0	0.0	130.0	0.0	0.0	26.6	26.6	26.6
Magnesium Chloride	0.1	0.0	6.4	0.0	0.0	1.3	1.3	1.3
Boric Acid	8.6	0.0	77.9	0.0	0.0	86.5	86.5	86.5
Oxygen	0.0	806.5	0.0	161.3	322.6	0.0	0.0	0.0
Ammonia	0.0	51.0	0.0	10.2	20.4	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	997.6	1995.3	0.0	0.0	0.0
Cells	48.6	0.0	0.0	0.0	0.0	485.6	485.6	485.6
Product Protein	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other Extracellular Protein	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

12.6 Continuous Process Flow Diagram

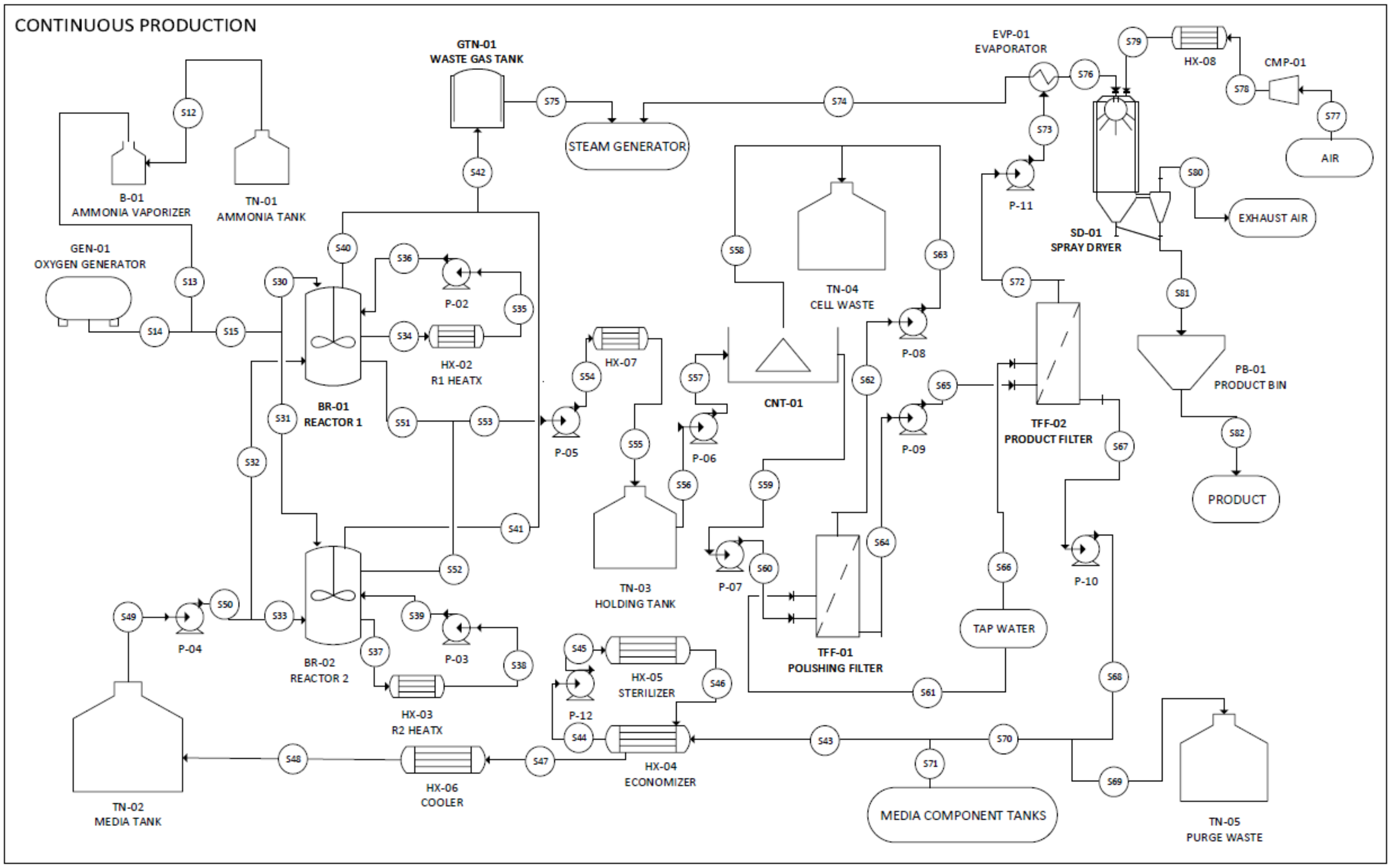

Figure 12.6. Process flow diagram for batch process including all main reactants, process steps and final products.

12.7 Continuous Process Material Balance

Table 12.7.1 Material Balance for Media Tank in Continuous Operation

MEDIA TANK								
Stream	**S43**	**S44**	**S45**	**S46**	**S47**	**S48**	**S49**	**S50**
Description	**From component tanks**	**From economizer**	**To sterilizer**	**To economizer**	**To cooler**	**From cooler**	**From media tank**	**To reactors**
Temperature	25.0	63.0	63.0	140.0	106.0	32.0	32.0	32.0
Pressure	1.2	1.2	3.6	3.6	3.6	1.2	1.2	1.2
Total Mass (kg/hr)	21788.2	21788.2	21788.2	21788.2	21788.2	21788.2	21788.2	21788.2
Component Mass (kg/hr)								
Water	14958.1	14958.1	14958.1	14958.1	14958.1	14958.1	14958.1	14958.1
Glucose	3507.9	3507.9	3507.9	3507.9	3507.9	3507.9	3507.9	3507.9
Carbon Waste	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Sodium Phosphate	42.6	42.6	42.6	42.6	42.6	42.6	42.6	42.6
Sulfur	19.9	19.9	19.9	19.9	19.9	19.9	19.9	19.9
Sodium Chloride	3036.5	3036.5	3036.5	3036.5	3036.5	3036.5	3036.5	3036.5
Potassium Hydroxide	136.5	136.5	136.5	136.5	136.5	136.5	136.5	136.5
Magnesium Chloride	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7
Boric Acid	80.0	80.0	80.0	80.0	80.0	80.0	80.0	80.0
Oxygen	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cells	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Product Protein	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Other Extracellular Protein	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 12.7.2 Material Balance on Bioreactor in Continuous Operation

REACTOR 1					
Stream	**S12**	**S13**	**S14**	**S15**	**S30**
Description	**Ammonia tank**	**Vaporized ammonia**	**Generated oxygen**	**Oxygen/ammonia mix**	**Gases in**
Temperature	32.0	66.0	66.0	66.0	66.0
Pressure	1.2	1.4	1.4	1.4	1.4
Total Mass (kg/hr)	53.4	53.4	323.7	377.1	188.6
Component Mass (kg/hr)					
Water	0.0	0.0	0.0	0.0	0.0
Glucose	0.0	0.0	0.0	0.0	0.0
Carbon Waste	0.0	0.0	0.0	0.0	0.0
Sodium Phosphate	0.0	0.0	0.0	0.0	0.0
Sulfur	0.0	0.0	0.0	0.0	0.0
Sodium Chloride	0.0	0.0	0.0	0.0	0.0
Potassium Hydroxide	0.0	0.0	0.0	0.0	0.0
Magnesium Chloride	0.0	0.0	0.0	0.0	0.0
Boric Acid	0.0	0.0	0.0	0.0	0.0
Oxygen	0.0	0.0	323.7	323.7	161.9
Ammonia	53.4	53.4	0.0	53.4	26.7
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0
Cells	0.0	0.0	0.0	0.0	0.0
Product Protein	0.0	0.0	0.0	0.0	0.0
Other Extracellular Protein	0.0	0.0	0.0	0.0	0.0

REACTOR 1 (CONT.)						
Stream	**S32**	**S40**	**S34**	**S35**	**S36**	**S51**
Description	**Media in**	**Waste gas**	**To heatx**	**To heatx pump**	**From heatx**	**From reactor**
Temperature	32.0	60.0	60.0	32.0	32.0	60.0
Pressure	1.2	1.2	1.2	1.2	1.2	1.2
Total Mass (kg/hr)	10894.1	1522.2	7820.3	7820.3	7820.3	8546.6
Component Mass (kg/hr)						
Water	7479.1	0.0	6843.5	6843.5	6843.5	7479.1
Glucose	1754.0	0.0	0.0	0.0	0.0	0.0
Carbon Waste	0.0	0.0	51.8	51.8	51.8	56.6
Sodium Phosphate	21.3	0.0	3.9	3.9	3.9	4.3
Sulfur	9.9	0.0	1.8	1.8	1.8	2.0
Sodium Chloride	1518.2	0.0	277.8	277.8	277.8	303.6
Potassium Hydroxide	68.3	0.0	12.5	12.5	12.5	13.7
Magnesium Chloride	3.3	0.0	0.6	0.6	0.6	0.7
Boric Acid	40.0	0.0	36.6	36.6	36.6	40.0
Oxygen	0.0	32.4	0.0	0.0	0.0	0.0
Ammonia	0.0	26.7	0.0	0.0	0.0	0.0
Carbon Dioxide	0.0	1463.2	0.0	0.0	0.0	0.0
Cells	0.0	0.0	205.3	205.3	205.3	224.4
Product Protein	0.0	0.0	376.4	376.4	376.4	411.3
Other Extracellular Protein	0.0	0.0	10.0	10.0	10.0	11.0

REACTOR 2								
Stream	**S31**	**S33**	**S41**	**S42**	**S37**	**S38**	**S39**	**S52**
Description	**Gases in**	**Media in**	**Waste gas**	**Total waste gas**	**To heatx**	**To heatx pump**	**From heatx**	**From reactor**
Temperature	66.0	32.0	60.0	60.0	60.0	32.0	32.0	60.0
Pressure	1.4	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Total Mass (kg/hr)	188.6	10894.1	1522.2	3044.5	7820.3	7820.3	7820.3	8546.6
Component Mass (kg/hr)								
Water	0.0	7479.1	0.0	0.0	6843.5	6843.5	6843.5	7479.1
Glucose	0.0	1754.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbon Waste	0.0	0.0	0.0	0.0	51.8	51.8	51.8	56.6
Sodium Phosphate	0.0	21.3	0.0	0.0	3.9	3.9	3.9	4.3
Sulfur	0.0	9.9	0.0	0.0	1.8	1.8	1.8	2.0
Sodium Chloride	0.0	1518.2	0.0	0.0	277.8	277.8	277.8	303.6
Potassium Hydroxide	0.0	68.3	0.0	0.0	12.5	12.5	12.5	13.7
Magnesium Chloride	0.0	3.3	0.0	0.0	0.6	0.6	0.6	0.7
Boric Acid	0.0	40.0	0.0	0.0	36.6	36.6	36.6	40.0
Oxygen	161.9	0.0	32.4	64.7	0.0	0.0	0.0	0.0
Ammonia	26.7	0.0	26.7	53.4	0.0	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	1463.2	2926.3	0.0	0.0	0.0	0.0
Cells	0.0	0.0	0.0	0.0	205.3	205.3	205.3	224.4
Product Protein	0.0	0.0	0.0	0.0	376.4	376.4	376.4	411.3
Other Extracellular Protein	0.0	0.0	0.0	0.0	10.0	10.0	10.0	11.0

Table 12.7.3 Material Balance on Holding Tank TN-03 in Continuous Operation

HOLDING TANK				
Stream	**S53**	**S54**	**S55**	**S56**
Description	**Total from reactors**	**To heatx**	**To holding tank**	**From holding tank**
Temperature	60.0	60.0	32.0	32.0
Pressure	1.2	1.2	1.2	1.2
Total Mass (kg/hr)	17093.1	17093.1	17093.1	17093.1
Component Mass (kg/hr)				
Water	14958.1	14958.1	14958.1	14958.1
Glucose	0.0	0.0	0.0	0.0
Carbon Waste	113.2	113.2	113.2	113.2
Sodium Phosphate	8.5	8.5	8.5	8.5
Sulfur	4.0	4.0	4.0	4.0
Sodium Chloride	607.3	607.3	607.3	607.3
Potassium Hydroxide	27.3	27.3	27.3	27.3
Magnesium Chloride	1.3	1.3	1.3	1.3
Boric Acid	80.0	80.0	80.0	80.0
Oxygen	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0
Cells	448.7	448.7	448.7	448.7
Product Protein	822.7	822.7	822.7	822.7
Other Extracellular Protein	21.9	21.9	21.9	21.9

Table 12.8.3 Material Balance on Centrifuge in Continuous Operation

CENTRIFUGE			
Stream	**S57**	**S58**	**S59**
Description	**To centrifuge**	**Cell waste**	**From centrifuge**
Temperature	32.0	32.0	32.0
Pressure	1.2	1.2	1.2
Total Mass (kg/hr)	17093.1	1332.8	15760.3
Component Mass (kg/hr)			
Water	14958.1	798.5	14159.6
Glucose	0.0	0.0	0.0
Carbon Waste	113.2	6.0	107.1
Sodium Phosphate	8.5	0.5	8.1
Sulfur	4.0	0.2	3.8
Sodium Chloride	607.3	32.4	574.9
Potassium Hydroxide	27.3	1.5	25.8
Magnesium Chloride	1.3	0.1	1.3
Boric Acid	80.0	4.3	75.7
Oxygen	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0
Cells	448.7	444.3	4.5
Product Protein	822.7	43.9	778.8
Other Extracellular Protein	21.9	1.2	20.8

Table 12.7.4 Material Balance on Filtration Steps in Continuous Operation

45 kDa ULTRAFILTRATION					
Stream	**S60**	**S61**	**S62**	**S63**	**S64**
Description	**To polishing filter**	**Crossflow water**	**Retentate waste**	**To waste tank**	**Permeate product**
Temperature	32.0	32.0	32.0	32.0	32.0
Pressure	1.2	1.2	1.2	1.2	1.2
Total Mass (kg/hr)	15760.3	25200.0	14331.9	14331.9	26628.4
Component Mass (kg/hr)					
Water	14159.6	25200.0	14159.6	14159.6	25200.0
Glucose	0.0	0.0	0.0	0.0	0.0
Carbon Waste	107.1	0.0	10.7	10.7	96.4
Sodium Phosphate	8.1	0.0	0.8	0.8	7.3
Sulfur	3.8	0.0	0.4	0.4	3.4
Sodium Chloride	574.9	0.0	57.5	57.5	517.4
Potassium Hydroxide	25.8	0.0	2.6	2.6	23.3
Magnesium Chloride	1.3	0.0	0.1	0.1	1.1
Boric Acid	75.7	0.0	7.6	7.6	68.2
Oxygen	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0
Cells	4.5	0.0	4.5	4.5	0.0
Product Protein	778.8	0.0	77.9	77.9	700.9
Other Extracellular Protein	20.8	0.0	10.3	10.3	10.5

8 kDa TANGENTIAL FLOW FILTRATION								
Stream	**S65**	**S66**	**S67**	**S68**	**S69**	**S70**	**S71**	**S72**
Description	**To product filter**	**Crossflow water**	**Permeate media**	**To sterilization loop**	**Purge**	**From purge**	**Media components**	**Retentate product**
Temperature	32.0	32.0	32.0	32.0	32.0	32.0	32.0	32.0
Pressure	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Total Mass (kg/hr)	26628.4	25200.0	25855.8	25855.8	10508.4	15347.4	6517.0	25962.4
Component Mass (kg/hr)								
Water	25200.0	25200.0	25200.0	25200.0	10241.9	14958.1	0.0	25200.0
Glucose	0.0	0.0	0.0	0.0	0.0	0.0	3507.9	0.0
Carbon Waste	96.4	0.0	82.0	82.0	33.3	48.7	0.0	14.5
Sodium Phosphate	7.3	0.0	6.2	6.2	2.5	3.7	38.9	1.1
Sulfur	3.4	0.0	2.9	2.9	1.2	1.7	18.2	0.5
Sodium Chloride	517.4	0.0	439.8	439.8	178.7	261.0	2775.5	77.6
Potassium Hydroxide	23.3	0.0	19.8	19.8	8.0	11.7	124.8	3.5
Magnesium Chloride	1.1	0.0	1.0	1.0	0.4	0.6	6.1	0.2
Boric Acid	68.2	0.0	57.9	57.9	23.5	34.4	45.6	0.0
Oxygen	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cells	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Product Protein	700.9	0.0	37.4	37.4	15.2	22.2	0.0	663.5
Other Extracellular Protein	10.5	0.0	8.9	8.9	3.6	5.3	0.0	1.6

Table 12.7.5 Material Balance on Evaporator in Continuous Operation

EVAPORATOR				
Stream	**S73**	**S74**	**S75**	**S76**
Description	**To evaporator**	**To steam generator**	**From waste gas tank**	**To spray dryer**
Temperature	32.0	100.0	60.0	32.0
Pressure	1.2	1.2	1.2	1.2
Total Mass (kg/hr)	25962.4	24880.0	3044.5	512.7
Component Mass (kg/hr)				
Water	25200.0	24880.0	0.0	320.0
Glucose	0.0	0.0	0.0	0.0
Carbon Waste	14.5	0.0	0.0	14.5
Sodium Phosphate	1.1	0.0	0.0	1.1
Sulfur	0.5	0.0	0.0	0.5
Sodium Chloride	77.6	0.0	0.0	77.6
Potassium Hydroxide	3.5	0.0	0.0	3.5
Magnesium Chloride	0.2	0.0	0.0	0.2
Boric Acid	0.0	0.0	0.0	0.0
Oxygen	0.0	0.0	64.7	57.9
Ammonia	0.0	0.0	53.4	0.0
Carbon Dioxide	0.0	0.0	2926.3	0.0
Cells	0.0	0.0	0.0	0.0
Product Protein	663.5	0.0	0.0	0.0
Other Extracellular Protein	1.6	0.0	0.0	37.4

Table 12.7.6 Material Balance on Spray Dryer/Product Bin in Continuous Operation

SPRAY DRYER/PRODUCT BIN							
Stream	**S76**	**S77**	**S78**	**S79**	**S80**	**S81**	**S82**
Description	**To spray dryer**	**Air**	**Compressed air**	**Heated air**	**Exhaust air**	**To product bin**	**Product**
Temperature	32.0	28.9	76.0	160.0	80.0	32.0	32.0
Pressure (bar)	1.2	1.0	1.5	1.5	1.5	1.4	1.0
Total Mass (kg/hr)	1082.4	1012.8	1012.8	1012.8	1332.8	762.4	762.4
Component Mass (kg/hr)							
Water	320.0	12.8	12.8	12.8	332.8	0.0	0.0
Glucose	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbon Waste	14.5	0.0	0.0	0.0	0.0	14.5	14.5
Sodium Phosphate	1.1	0.0	0.0	0.0	0.0	1.1	1.1
Sulfur	0.5	0.0	0.0	0.0	0.0	0.5	0.5
Sodium Chloride	77.6	0.0	0.0	0.0	0.0	77.6	77.6
Potassium Hydroxide	3.5	0.0	0.0	0.0	0.0	3.5	3.5
Magnesium Chloride	0.2	0.0	0.0	0.0	0.0	0.2	0.2
Boric Acid	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Air	0.0	1000.0	1000.0	1000.0	1000.0	0.0	0.0
Oxygen	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ammonia	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carbon Dioxide	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cells	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Product Protein	663.5	0.0	0.0	0.0	0.0	663.5	663.5
Other Extracellular Protein	1.6	0.0	0.0	0.0	0.0	1.6	1.6

13. Process Descriptions

13.1 Seed Train

To successfully run this process with a continuous fermenter the cells must be grown starting from lab scale to manufacturing scale. This process will be started with a 1 mL test tube with a cell density of 30 g/L and eventually grown to two 20,000 L bioreactors with an equivalent cell density, as well as 55 g/L of extracellular product protein. The seed train is comprised of 2 L bioreactors in triplicate and a 2000 L bio-reactor.

Incrementally growing the cells through a seed train is an important factor in quality control. The small bioreactors being grown in triplicate help with this subject, the lab staff will check the contents of the bioreactor before moving the contents to the next reactor and, should there be an issue, they will use one of the other 2L reactors to inoculate the larger one.

13.1.1 Biobank

In order to ensure that new cells are always ready to inoculate batches whenever necessary, a cell bank will be created. Biobanking helps preserve cell lines for further use and keep operating costs lows (Cryopreservation Guide: The basics of cellular cryopreservation for research & clinical use, 2017). The cell colonies grown on the agar plate will undergo extensive testing to ensure they are not contaminated and then will be used to fill 1 mL test tubes. Each of these will be frozen at -80°C to stop growth until they are needed to inoculate a new batch. When the cell bank is near empty, one sample from the master cell bank will be used to generate more samples in the working cell bank.

13.1.2. 2 L Bioreactors

A triplicate of 2L bioreactors will be used as the first step in the seed train. 1 mL samples of the cells will be used to inoculate the bioreactors and all raw materials will be fed to the bioreactors at the start of fermentation. It will take 16 hours for the contents of the reactor to reach peak cell density. The reactors have a total working volume of 80% which is 1.6 liters each. The final concentration of biomass in the reactor will be 85 g/L, resulting in 136 grams of biomass. A gas vent will be used to prevent pressure buildup and all gas will be sent to a scrubber to collect any cell debris before releasing to the atmosphere. The cells will be tested for contamination and then the contents from the most ideal reactor will be used to inoculate the 2000 L reactor.

13.1.3. 2000 L Bioreactor

A 2000 L bioreactor will be the second step in the seed train. The contents from one of the 2 L bioreactors will be used to initiate cell growth and all raw materials will be fed to the bioreactors at the start of fermentation. It will take 15 hours for the reactor contents to reach peak cell density. The reactor will have a total working volume of 80% which is 1600 liters. The final concentration of biomass in the reactor will be 85 g/L, resulting in 136 kg of biomass. A gas vent will be used to prevent pressure buildup and all gas will be sent to a scrubber to collect any cell debris before releasing to the atmosphere. An external heat exchanger will be used to ensure the bioreactor temperature remains at 60°C. The cells will be tested for contamination and then entirety of the contents will be fed to the continuous reactor.

13.2 Feed Material Storage

Many various feed materials will need to be introduced to the process to promote cell growth, enable enzyme creation, regulate pH and provide foam management. All of these materials must be stored safely to ensure safe operation of all process components and no delays in operations. The storage tank design specifications are outlined in Section 15.1.

13.2.1 Glucose Storage

Glucose will be delivered every 3 days to the plant from Dayton, Ohio via truck at a price of $881.85/MT. Glucose is the only source of carbon for the process and is fed to the large bioreactors at a rate of 84 MT/day. Running out of glucose, due to delays in delivery or any other types of accidents, would result in having to shut down and restart the 8 day campaign which would interfere with downstream processes. Because of this glucose will be stored in two 350,000 L storage tanks, each one holding enough dextrose to supply one bioreactor for 6 days.

13.2.2 Ammonia Storage

Anhydrous ammonia will be delivered via rail from the Nutrien distribution center in Northbrook, Illinois. It will be delivered once a week in the form of compressed liquefied gas. A 2 week supply of the anhydrous ammonia will be stored in a 100 L tank, providing 30% for evaporation. According to the SDS provided in Appendix C, the ammonia can be safely stored up to 120°F, so the expected range of this system will be between 0°F and 100°F. This corresponds to a pressure range of 1.1 bar to 13.7 bar, respectively (Properties of Ammonia, n.d.). The tank will have a floating roof to comply with EPA regulations requiring a floating roof when the vapor pressure of the liquid exceeds 0.27 bar at maximum temperature.

13.2.3 Media Storage

Media will be introduced to the system through a sterilization process then proceed to a storage tank to be held and fed to the bioreactors. This tank will be made of 316L stainless steel and will be a horizontal pressure vessel of volume 11000 gallons maintained at 1.2 bar and 32 °C. There will be a control loop purging the recycled media when necessary to ensure the tank does not overflow curing the 24 hour period one bioreactor is undergoing CIP and to prevent carbon buildup.

13.3 Gas Feed Generation

For cell growth, sources of oxygen and nitrogen are required in a form that cells can easily uptake. As such, gaseous oxygen and ammonia must be sparged into the bioreactors at every stage of cell growth and production in order to ensure cells can grow to and maintain peak cell density. In order to produce these gases, process units are required.

13.3.1 Oxygen Generator

Due to the high oxygen uptake rate needed for cell growth pure oxygen will be used as the oxygen source for the cell growth. 7.8 MT/day of pure oxygen gas is required when the process is running continuously, and as such, it was determined that instead of purchasing oxygen from a supplier, it would be more economically feasible to generate oxygen on site using an oxygen generation skid. An oxygen generator operates by separating air into its primary constituents of oxygen and nitrogen by capturing and expelling the nitrogen, while allowing the oxygen gas to continuing to the rest of the process. Furthermore, the oxygen generator will pressurize the oxygen gas before sending it to the bioreactors. In order to sparge the oxygen through the bottom of the reactor, the pressure of the gas must be greater than the sum of the operation pressure of 1.0 bar, and the hydrostatic pressure of 0.42 bar caused by a liquid height of 4.27 m in the reactor. Thus, the oxygen gas will be pressurized to 1.43 bar by the oxygen generation skid.

13.3.2 Ammonia Vaporizer

Because the ammonia will be delivered and stored in a liquid, anhydrous form, a vaporizer is required in order to vaporize the anhydrous ammonia and produce the required flow rate of ammonia gas into the reactor at the required pressure. 1.3 MT/day of ammonia gas will be required at a pressure of 1.43 bar by the same calculation as to determine the oxygen gas pressure. An ammonia vaporizer that can process the required flow rate and pressure will be installed directly following the ammonia storage tank.

13.4 Batch Bioreactor Growth

Following the seed train growth, the two 20,000 L production scale bioreactors will be inoculated with the contents of the 2000 L bioreactor, with staggered schedules which are outlined in Section 10.4. Cell growth will take approximately 5 hours, at which point the cells will reach peak cell density of 30 g/L and produce extracellular product protein at a concentration of 55 g/L.

13.5 Continuous Bioreactor Production

Once peak cell density and peak product titer are reached, the continuous process will then begin, consisting of constant flow rates in and out of the bioreactor. Flowing in will be a gaseous ammonia mixed with oxygen, a cooled recycle stream, and a media stream. The outlets will consist of a gas stream which contains excess air, oxygen, ammonia and carbon dioxide, and a liquid product stream which will be fed to a centrifuge. Each bioreactor has a campaign period of 8 days after which it will be emptied of all its contents and they too will continue downstream. The bioreactor will then undergo a clean in place process and start a new growth period. Because of the staggered schedules a new campaign is started every 4 days.

13.5.1 20,000 L Bioreactors

Two 20,000 L bioreactors will be used semi-continuously to meet the production goal specified in the project statement. They will have 80% working volume, equating to approximately 16,000 L each. They will be kept at 1.22 bar which is slightly above atmospheric pressure to ensure that, should a leak occur, components from the surrounding air will not enter the bioreactors. An external heat exchanger will be used to ensure the bioreactors remain at 60°C and the peak cell density of 30 g/L will be conserved throughout the campaign. The bioreactor will require 12.6 kW of agitation.

13.5.2 Cooling Recycle Stream Heat Exchangers

Because this fermentation process is highly exothermic, producing 512 kW of heat will be created in each bioreactor and cooling will be necessary to ensure the bioreactors remain at 60°C. This will be accomplished with the use of external countercurrent shell and tube heat exchangers which will cool a portion of the bioreactor contents to 32°C and then return them to the bioreactors. A valve at the bottom of the reactor will vent the pressure of the liquid and the piping length will be adjusted such that the liquid is delivered to the exchanger at approximately 1.2 bar. The hot fluid is located on the shell side and is entering at 140 °F (60 °C) and is leaving at 77 °F (25 °C) with negligible pressure drop. The hot fluid is entering at a flow rate of 7821 kg/hr. Cooling water is entering on the tube side at 80 °F and leaving at 120 °F. The cooling water has flow rate 9854 kg/hr. The heat exchanger is built assuming 0.0005 ft^2 hr °F btu^{-1}. The product of the overall heat transfer coefficient and area was found to be 62298.7 btu hr^{-1} $°F^{-1}$. The heat exchanger will be constructed out of 316L stainless steel to withstand the high pH of the media being cooled. The shell's inside and outside diameter are 8.407 in and 8.625 in respectively. There are 44 tubes with 2 passes, and the tubes have length 183.1 in with outside diameter and pitch 0.75 in and 0.9375 respectively. The tube pattern is 30-triangular, and they are located in the baffle window. There are 22 baffles that are spaced 7.67 in apart center to center. The baffles are single segmental and cut horizontally. There are 3 shells in series.

13.6 Holding Tank System

13.6.1 Holding Tank

A holding tank catches the effluent coming from the bioreactors to maintain consistency for the downstream processing equipment. It is a horizontal pressure vessel with agitation designed to hold a volume greater than that of the working volumes of the two bioreactors, and it delivers fluid to the downstream at a constant flow rate.

13.6.2 Holding Tank Heat Exchangers

The suspension containing the product must be cooled from 60 °C in order to remain compatible with the downstream processes. This heat exchanger is placed in order to deliver the suspension at 25 °C to the holding tank TN-03. The hot fluid is entering on the shell side at a flow rate of 40807 lb/hr (18318 kg/hr). It is entering at 140 °F (60 °C) and leaving at 77 °F (25 °C). The inlet pressure is 1.2 bar and the pressure drops to 1.09

bar. Chilled water enters at 45 °F on the tube side and leaves at 90 °F. The chilled water flow rate is 57119 lb/hr (25437 kg/hr). This heat exchanger is also built with 0.0005 ft^2 hr °F btu^{-1}. The product of the overall heat transfer coefficient and the area was found to be 6.45×10^{7} btu hr^{-1} $°F^{-1}$. The heat exchanger will be constructed out of 316L stainless steel to withstand the high pH of the media being cooled. The shell's inside and outside diameter are 8.407 in and 8.625 in respectively. There are 118 tubes with 1 pass, and the tubes have length 195 in with outside diameter and pitch 0.5 in and 0.625 respectively. The tube pattern is 30-triangular, and they are located in the baffle window. There are 24 baffles that are spaced 7.7 in apart center to center. The baffles are single segmental and cut horizontally.

13.7 Centrifugation

In order to separate the live cell mass from the liquid suspension containing the product protein, a centrifuge is required. It was decided that a disc stack centrifuge will be used as disc stack centrifuges can be run continuously to process large volumetric flow rates, and they are commonly used to separate biomass from fermentation broths (Thomas, 2014). Disc stack centrifuges contain multiple conical discs in the bowl of the device and as the suspension is fed to the bottom of the bowl, the centrifugal force pushes solids outward and down the discs as the liquid suspension rises and exits the top of the centrifuge.

Approximately 15,000 L/h of cell suspension will be fed to the centrifuge from the holding tank containing 450 kg/hr of live cells that need to be removed before the liquid containing the product protein can undergo further purification. Based on consultant recommendation, it is assumed that the centrifuge will remove 99% by mass of cells, and the resulting waste slurry will contain 1/3 cells by mass and 2/3 water, protein, and media constituents by mass.

13.8 Ultrafiltration

After 99% of the cell mass is removed in with the centrifuge, the liquid product will continue to a series of ultrafiltration steps. The first step will remove the remaining cell debris and most extracellular components larger than the desired enzyme. This will improve the purity of the final product and also help protect the following tangential flow filtration step from large particles that can clog the membrane and lead to membrane fouling. The tangential flow filtration step will be optimized to ensure 87% and the retentate of this step will be sent to the spray dryer.

13.7.1 45kDa Ultrafiltration

The first filtration step will consist of 45 kDa membranes in with a total membrane area of 30 m^2. The system used for this process will be the ProStak tangential flow filtration unit which allows for high product recovery and low hold up volume. The membranes are hydrophilic polyvinylidene difluoride which allows them to have lower protein binding and therefore better separation. They are also functional in the pH range 2-11, which will offer maximum functionality in this system. The cross flow rate, area, and transmembrane pressure will be optimized at lab scale using the MilliporeSigma Single Pass Tangential Flow Filtration system and scaled up to ensure 90% separation. The permeate of this step will be sent on to the second filtration unit.

13.7.2 8 kDa Tangential Flow Filtration

The second filtration step will consist of 8 kDa Biomax membranes which are engineered for high flux, high yield and high reliability. With functionality over pH 1-14 and low protein binding, these membranes will offer sufficient separation in this process. The system is linearly scalable so the cross flow rate, area, and transmembrane pressure will be optimized at lab scale to reach 87% separation. It will then be scaled up to manufacturing scale. The lab scale system used will be the Pellicon 2 Mini. The Biomax membranes will be used in these Pellicon 2 cassettes and scaled up in a Cogent Process Scale Tangential Flow Filtration System. All brochures for these systems can be found in Appendix D.

13.8 Recycle Sterilization

In order to maintain proper cell growth in the bioreactors the recycle streams returning to them must be sterilized. Upon consultant recommendation, a loop containing an economizer, sterilizer and cooler will be used to complete this task. The recycle streams will consist of the permeate from the tangential flow filtration process unit and the water crossflow from the gas scrubber.

13.8.1 Economizer

The economizer is a heat exchanger designed to utilize the heat from the sterilized stream (i.e. fluid leaving the sterilizer heat exchanger) to heat up the incoming media stream which is to be recycled. Fluid will enter at 25 °C and leave at 63 °C. This will be a shell and tube heat exchanger constructed out of 316L stainless steel. The tubes will be 0.75 in in outer diameter and will have pitch 0.9375. The tube pattern will be 30-triangular and they will be located in the baffle windows. The baffle type will be single segmental and they will be cut horizontally. The shell inside and outside diameter respectively will be

6.407 in and 6.625 in. They will be 153.5 in long and there will be 27 tubes and only 1 pass. The baffles will be spaced 11.6 in apart center to center and there will be 12 baffles.

13.8.2 Sterilizer

The fluid leaving the economizer will be pressurized to 3.62 bar before entering a second shell and tube heat exchanger. This fluid will then be heated to 140 °C using 150 psig steam. The exchanger will be constructed out of 316L stainless steel. The tubes will be 0.5 in in outer diameter and will have pitch 0.625. The tube pattern will be 30-triangular and they will be located in the baffle windows. The baffle type will be single segmental and they will be cut horizontally. The shell inside and outside diameter respectively will be 29.53 in and 30.08 in. They will be 59.1 in long and there will be 1870 tubes and only 1 pass. The baffles will be spaced 6.5 in apart center to center and there will be 6 baffles.

13.8.3 Cooler

The cooler will be designed to cool the fluid leaving the economizer down to a low temperature so that it may be stored in a holding tank. The fluid will enter the cooler at 106 °C and will be cooled to 32 °C using cooling water. The exchanger will be constructed out of 316L stainless steel. The tubes will be 0.75 in in outer diameter and will have pitch 0.9375. The tube pattern will be 30-triangular and they will be located in the baffle windows. The baffle type will be single segmental and they will be cut horizontally. The shell inside and outside diameter respectively will be 16.73 in and 17.13 in. They will be 230.3 in long and there will be 247 tubes and only 1 pass. The baffles will be spaced 4.2 in apart center to center and there will be 52 baffles.

13.9 Drying

Following the ultrafiltration step, the purity of the solution is high enough at 87% product protein by mass that liquid stream will undergo two drying steps to produce a solid powder product. The goal of these two drying steps is to remove all water from the product stream and granulize the product.

13.9.1 Evaporator

As the product stream from the 8 kDa tangential flow filtration step contains 25,200 kg/hr of water, the majority of this water must first be evaporated off using a multiple effect evaporator before a spray dryer can be used to produce a product powder. The multiple effect evaporator functions as a series of evaporator bodies, where in the first effect, steam is used to evaporate a portion of the water in the feed stream, thus concentrating the feed and condensing the steam. The vapor from the first effect is then used to evaporate more water in a second effect held at a lower pressure. By using effects at progressively lower pressures, externally heated steam is only required to evaporate liquid in the first effect and all subsequent effect rely on vapor from the previous. Through this method, latent heat is conserved and the amount of steam required to vaporize a given amount of water is equal. A three effect evaporator is most common in industrial multiple effect evaporator processes as a balance between space costs and efficiency and will be used in this process. The evaporator will be used to concentrate the product stream to contain 320 kg/hr of water, equal to the evaporative capacity of the spray dryer.

13.9.2 Spray Dryer

Once the product stream, which will at this point in the process be a slurry due to the high ratio of protein to water, exits the evaporator with the desired water content, it will be sent to a spray dryer. As the product slurry is fed through an atomizer to produce small droplets of product, heated compressed air will be fed co-current to the atomized droplets in order to vaporize the remaining water leaving solid granules of protein. The spray dryer will function in a co-current manner so that the air at the highest temperature will come into contact with the droplets containing the most liquid as not to denature the protein. As per US Patent 4,233,405, the inlet air will be heated to the recommended temperature of 160°C and the outlet temperature of the exhaust air will be at 80°C (United States of America Patent No. 4,233,405, 1979). The water content of the inlet air is estimated at 50% relative humidity at the average high temperature in the summer in the plant location of Dayton, Ohio, that is 0.0128 kg water/kg air. The flow rate of inlet air will be 1000 kg/hr, which was determined as approximately 125% of the air required to remove all water if the outlet air were at 100% relative humidity at the outlet temperature. Once the water is removed, 762 kg/hr of product powder will produced at a purity of 87% desired protein.

13.10 Final Packaging

Following the spray dryer, the product protein will need to be collected and packaged for shipment. The product powder will be collected in a carbon steel vertical holding bin with a bin activator to ensure a constant flow of product powder out of the vessel. The bin activator accomplishes this by vibrating the powder throughout the vessel to ensure a steady mass flow of product. The product powder will be collected in super sacks that will be filled and sealed for distribution. The bulk density of the dried protein product is 0.7 kg/L and 762.4 kg/hr of product will be produced, so product will need to be packaged at a rate of 1089 L/hr.

14. Energy Balance and Utility Requirements

14.1 Annual Utility Requirements

Table 14.1 Annual utility costs for this process.

Utility	**Annual Demand**	**Annual Cost**
Cooling Water	149,590,153 kg	$4,051
Chilled Water	162,267,706 kg	$81,134
Process Water	341,830,404 kg	$92,572
150 psig Steam	1,254,039,855 kg	$19,186,810
Electricity	13,210,702 kg	$924,729

14.2 Utility Requirements for Equipment

Table 14.2 Annual energy requirements and associated costs for process units.

Equipment Code	Cooling Water (kg/hr)	Chilled Water (kg/hr)	Process Water (kg/hr)	Medium Pressure Steam (kg/hr)	Electricity (kW)	Hours Uptime per Year
TN-01	0.0	0.0	0.0	0.0	0.0	8322.0
TN-02	0.0	0.0	14958.1	0.0	8.8	8322.0
B-01	0.0	0.0	0.0	0.0	20.0	8322.0
GEN-01	0.0	0.0	0.0	0.0	74.6	8322.0
SBR-01	0.0	0.0	0.0	0.0	0.0	0.0
SBR-03	0.0	0.0	0.0	0.0	0.0	0.0
SBR-05	0.0	0.0	0.0	0.0	0.0	0.0
SBR-02	0.0	0.0	0.0	0.0	0.3	1847.5
SBR-04	0.0	0.0	0.0	0.0	0.3	1847.5
SBR-06	0.0	0.0	0.0	0.0	0.3	1847.5
SBR-07	0.0	0.0	0.0	0.0	0.6	1344.5
HX-01	20.0	0.0	0.0	0.0	0.0	1344.5
CMP-01	0.0	0.0	0.0	0.0	13.4	6782.3
BR-01	0.0	0.0	0.0	0.0	12.6	7242.7
BR-02	0.0	0.0	0.0	0.0	12.6	7242.7
P-02	0.0	0.0	0.0	0.0	0.4	7242.7
P-03	0.0	0.0	0.0	0.0	0.4	7242.7
HX-02	9854.0	0.0	0.0	0.0	0.0	7242.7
HX-03	9854.0	0.0	0.0	0.0	0.0	7242.7
GTN-01	0.0	0.0	0.0	0.0	0.0	8322.0
P-05	0.0	0.0	0.0	0.0	0.0	6782.3
HX-07	0.0	23925.0	0.0	0.0	0.0	6782.3
TN-03	0.0	0.0	0.0	0.0	8.8	6782.3
P-06	0.0	0.0	0.0	0.0	1.7	6782.3
CNT-01	1010.0	0.0	0.0	0.0	22.0	6782.3
TN-04	0.0	0.0	0.0	0.0	962.0	6782.3
P-07	0.0	0.0	0.0	0.0	1.5	6782.3
TFF-01	0.0	0.0	25200.0	0.0	22.0	6782.3

P-08	0.0	0.0	0.0	0.0	1.3	6782.3
P-09	0.0	0.0	0.0	0.0	2.5	6782.3
TFF-02	0.0	0.0	25200.0	0.0	22.0	6782.3
P-10	0.0	0.0	0.0	0.0	2.4	6782.3
HX-04	0.0	0.0	0.0	0.0	0.0	7242.7
P-12	0.0	0.0	0.0	0.0	2.4	7242.7
HX-05	0.0	0.0	0.0	158718.0	0.0	7242.7
HX-06	0.0	0.0	0.0	0.0	0.0	7242.7
P-11	0.0	0.0	0.0	0.0	43.6	6782.3
EVP-01	0.0	0.0	0.0	13482.6	37.3	6782.3
SD-01	0.0	0.0	0.0	0.0	5.0	6782.3
PB-01	0.0	0.0	0.0	0.0	7.5	6782.3
HX-08	0.0	0.0	0.0	1923.0	0.0	6782.3
TN-05	0.0	0.0	0.0	0.0	653.0	8322.0
CIP System	0.0	0.0	0.0	0.0	14.9	1711.1

15. Equipment List and Unit Descriptions

Below are descriptions and designs of all major equipment used in the process. For the equipment that was to be ordered from a particular vendor, the vendor and the price quoted are given. All other equipment was costed using the equipment costing spreadsheet or the price was quoted by advice from consultants, with the exception of heat exchangers. Except for HX-01, all heat exchangers were designed and costed in ASPEN and in the design of the exchangers, the property changes due to dissolved contents were neglected.

15.1 Feed Process Units

15.1.1 Glucose Storage

Two 350,000 L stainless steel storage tanks with a cone roof will be used to store a supply of dextrose. The tanks will be kept at room temperature and atmospheric pressure. Each tank will be able to supply dextrose to a bioreactor for 6 days. The tanks were costed using the Equipment Costing Spreadsheet and are estimated to cost $395,739 each. Because liquid methanol is highly flammable and the resulting vapor can be toxic, the storage tank will comply with storage safety regulations.

15.1.2 Ammonia Storage (TN-01)

A 100 L stainless steel storage tank with a floating roof will be used to store a 2 week supply of anhydrous ammonia in the form of compressed liquefied gas. The tank will be able to operate from 0°F to 100°F with pressures ranging from 1.1 bar to 13.7 bar, respectively. The tanks were costed using the Equipment Costing Spreadsheet and came out to $10,573. The tank is significantly smaller than the range covered by the correlation provided so this value was extrapolated. The tank will have 30% head space to account for expansion during the hotter months. The tank will also comply with ammonia storage safety regulations since it is a highly toxic substance.

15.1.3 Media Tank (TN-02)

A 44,480 L pressure vessel made from 316L stainless steel will be used to store media before it is fed to the two bioreactors. It will be a horizontal pressure vessel maintained at 1.2 bar and 25 °C. It will have a diameter of $D = 10\ ft$ and length of $L = 20\ ft$. It will be built with earthquake and weather maintenance and have an elasticity $E = 30 \times 10^6 psi$. It will a corrosion thickness of 0.125 in and have a density of 490 lb/ft^3. From the equipment costing spreadsheet, the purchase cost of the vessel is $102427 and the bare module cost is $426097. There will be a control loop prior to the sterilization unit that feeds the tank. The function of the control loop is to measure the media level in the tank and ensure it does not overflow during the 24 hour windows in which one bioreactor is undergoing CIP and the tank is only feeding one reactor. The loop will trigger a purge stream of media during this time, which will also help mitigate carbon buildup. The vessel will have agitation at a rate of 8.76 kW and the agitation system will cost $6240.

15.1.4 Ammonia Vaporizer (B-01)

For ammonia vaporization, a PowerXP-120AA unit from Algas-SDI will be used. The required ammonia vaporization load during continuous operation is 53 kg/hr, and the vaporization capacity for the 120AA model is 54 kg/hr. Because of the relatively small vaporization rate, the vaporizer is electrically powered at 20 kW. The vaporizer contains a finned aluminum heat exchanger that is electronically supplied thermal energy. The vessel that contains the vaporized ammonia is pressurized to approximately 18 bar, allowing for the pressure to be dropped to the required 1.4 bar before the ammonia gas reaches the bioreactors. The ammonia vaporizer will cost $20,000.

15.1.5 Oxygen Generator (GEN-01)

A PRISM T-Series VSA Oxygen Generator will be used for production of pure oxygen gas from air. The T-Series has a maximum generation of rate of 23 MT/day, so the system will easily be able to provide for the 7.8 MT/day oxygen requirement during continuous bioreactor operation. The T-Series is a vacuum swing adsorption (VSA) oxygen generator, meaning that the system separates oxygen from nitrogen in air through a molecular sieve that adsorbs nitrogen gas, allowing pure oxygen to continue forward. The VSA functions differently from other adsorbent generators as it operates by drawing the air in through an internal vacuum, removing the need for a pressurized gas feed. The modular design of the skid allows for significant flexibility in installation. The skid also is capable of completely continuous operation by drawing from a product buffer tank during points in the generation cycle where the adsorbent must be regenerated. The T-Series skid will cost $1,300,000 and require 74.6 kW of power for operation.

15.2 Seed Train Process Units

15.2.1 1 mL Test Tubes (SBR-01, SBR-03, SBR-05)

Fisherbrand Sterile Plastic Culture Tubes will be used to store the genetically modified cells in the cell bank. They are tested for sterility and claim no chance of contamination. They are made of clear polysterine, will have screw on caps, and will be stored in a freezer at -80°C. They will be purchased in bulk with a case of 1000 in total costing $521.50.

15.2.2 2 L Bioreactors (SBR-02, SBR-04, SBR-06)

The 2 L bioreactors operating in triplicate are the first step in the seed train. These reactors will be Biostat A units from Sartorius Stedim. The bioreactor has automatic aeration system and pH monitoring. It also has an integrated chiller, eliminating the need for external cooling water. This reactor is optimized for fed-batch processes, allowing you to insert substrate profiles and automate external pumps with a PID controller. The purchase cost is $25,000 and the power requirement is 300 W for each reactor.

15.2.3 2000 L Bioreactor (SBR-07)

The next step in the seed train scale up is the 2000 L bioreactor. This model will be the Biostat STR also from Sartorius Stedim. This bioreactor comes with automated raw material feed and cell bleed controls that respond to changes in pH, foam levels, and oxygen and carbon dioxide concentrations. The Flexsafe STR bags have a resin formulation that leads to cell viability and higher cell densities. The cost for the reactor is $560,000 and each bag is an additional $3000.

15.2.4 Seed Train Heat Exchanger (HX-01)

For the 2000 L bioreactor an external heat exchanger is necessary to maintain the internal temperature at 60°C throughout the fermentation process. This heat exchanger will be a double pipe heat exchanger due to the small amount of liquid flowing through it. The hot fluid, i.e. the media from the 2000 L bioreactor, will enter at 60 °C and 1.2 bar and leave at 28 °C. The cool fluid will be cooling water which enters at 26.66 °C and 1 bar and leaves at 48.88 °C. The pressure drop across both ends is negligible. It will be constructed out of stainless steel and have a heat transfer area of 2.02 ft^2. The purchase cost is $5113.

15.3 Continuous Process Units

15.3.1 20,000 L Bioreactors (BR-01, BR-02)

Two 20,000 L bioreactors will be used for the continuous fermentation portion of the process. The reactors have a working volume of a little more than 80% the total volume, totaling in 16185 L, and are made of Stainless Steel 316L. The reactors have a gas inlet stream at the base and a gas vent stream at the top that will feed to the on-site boiler. A porous metal sparger will be used to help oxygenate the contents of the bioreactor. A pitch blade impeller will be used to provide 12.6 kW of power to sufficiently mix the components in the bioreactor and maximize cell growth. There is an external heat exchanger that is used to maintain the internal bioreactor temperature at 60°C. The system will be kept at 1.2 bar to prevent any leaks to the surrounding environment. They will be run on staggered 8 day campaigns and each have 24 hours designated for a clean in place process. Each reactor will cost $285,000 and each agitator will cost $125,000. They will be supplied by Paul Mueller Company. A proportional integral controller will measure the temperature inside the bioreactor and adjust the flowrate of liquid heading into the cooling heat exchanger. This will also control the flow rate during the batch stage of the process.

15.3.2 Heat Exchanger Pumps (P-02, P-03)

Pumps will follow each heat exchanger in the cooling loop to flow the liquid back into each reactor vessel at a pressure of 1.2 bar. These are designed assuming 100 ft head loss before entering the heat exchanger. These will be centrifugal pumps constructed out of stainless steel and will be designed to accommodate 100 ft of head loss, delivering the fluid at 1.2 bar. It is assumed to have an efficiency of 0.9 and has a power requirement of 0.38 kW. Each pump will cost $15641, leading to a bare module cost for each pump of $51616.

15.3.3 Heat Exchangers (HX-02, HX-03)

A shell and tube heat exchanger will be used externally to each bioreactor in order to cool the contents down at such a rate that the heat duty exchanged will maintain the bioreactors at 60 °C. Furthermore, there will be a control loop regulating the flow rate of media through the heat exchanger to ensure that the reactor temperature is maintained. Media will be entering the shell side at a pressure of 1.2 bar and temperature of 60 °C with flow rate 7821 kg/hr. It will be leaving the exchanger at 32 °C. Cooling water at 26.66 °C (80 °F) will enter the tube side at a flow rate of 9854 kg/hr and will leave the heat exchanger at 48.88 °C (120 °F). The pressure drop across both sides of the exchanger was negligible. The exchanger was designed with 0.0005 ft^2 hr °F/btu and the product of the overall heat transfer coefficient and the heat transfer area was 62299 btu hr^{-1} $°F^{-1}$. The exchanger will be constructed out of 316L stainless steel. The tubes will be 0.75 in in outer diameter and will have pitch 0.9375. The tube pattern will be 30-triangular and they will be located in the baffle windows. The baffle type will be single segmental and they will be cut horizontally. The shell inside and outside diameter respectively will be 8.407 in and 8.625 in. They will be 183.1 in long and there will be 44 tubes and 2 passes. The baffles will be spaced 7.67 in apart center to center and there will be 22 baffles. There are 3 shells in series. Each exchanger will cost $47769. The cooling water is available at $0.027/$m^3$.

15.3.4 Waste Gas Tank (GNT-02)

This waste gas tank will house the waste gases from the bioreactors before the gas mixtures are sent to the steam generator and then ultimately the gas outlet. It is designed to hold one batch cycle's worth of material. It will be a large storage vessel with inlet flowrate 3045 kg/hr. The volume of the storage vessel is 422000 gallons and it will have a floating roof. The purchase cost is $357516.

15.3.5 Pump to Holding Tank (P-05)

This pump is designed to deliver the effluent from each bioreactor, after the streams have merged, to a heat exchanger and subsequently a holding tank. The pump will be designed to accommodate 100 ft of head loss and deliver the liquid to the holding tank at 1.2 bar. It will be a centrifugal pump constructed out of stainless steel. The purchase cost will be $9085 and the bare module cost will be $29981. The power requirement will be 1.65 kW with an efficiency of 0.9.

15.3.6 Holding Tank Heat Exchanger (HX-07)

A shell and tube heat exchanger will be used to cool the effluent of the bioreactors down to 25 °C. Media will be entering the shell side at a pressure of 1.2 bar and temperature of 60 °C with flow rate 17093 kg/hr. It will be leaving the exchanger at 25 °C. Chilled water at 7.22 °C (45 °F) will enter the tube side at a flow rate of 23925 kg/hr and will leave the heat exchanger at 32.22 °C (90 °F). The pressure drop across both sides of the exchanger was negligible. The exchanger was designed with 0.0005 ft^2 hr °F/btu and the product of the overall heat transfer coefficient and the heat transfer area was 59611 btu hr^{-1} $°F^{-1}$. The exchanger will be constructed out of 316L stainless steel. The tubes will be 0.5 in in outer diameter and will have pitch 0.625. The tube pattern will be 30-triangular and they will be located in the baffle windows. The baffle type will be single segmental and they will be cut horizontally. The shell inside and outside diameter respectively will be 8.41 in and 8.625 in. They will be 189.0 in long and there will be 118 tubes and only 1 pass. The baffles will be spaced 7.7 in apart center to center and there will be 22 baffles. Each exchanger will cost $16940. Chilled water is available at $1.50/ton-day.

15.3.7 Holding Tank (TN-03)

This holding tank will have the same specifications as that of the media holding tank. It will be a 44,480 L pressure vessel made from 316L stainless steel designed to hold the effluent coming from the two bioreactors before being sent to the downstream process. It will be a horizontal pressure vessel maintained at 1.2 bar and 25 °C. It will have a diameter of $D = 10\ ft$ and length of $L = 20\ ft$. It will be built with earthquake and weather maintenance and have an elasticity $E = 30 \times 10^6$ psi. It will a corrosion thickness of 0.125 in and have a density of 490 lb/ft^3. From the equipment costing spreadsheet, the purchase cost of the vessel is $102427 and the bare module cost is $426097. The vessel will have agitation at a rate of 8.76 kW and the agitation system will cost $6240.

15.3.8 Pump to Centrifuge (P-06)

This pump is designed to deliver the liquid from the holding tank to the centrifuge for separation. The pump will be designed to accommodate 100 ft of head loss and deliver the liquid to the centrifuge at 1.2 bar. It will be a centrifugal pump constructed out of stainless steel. The purchase cost will be $9085 and the bare module cost will be $29981. The power requirement will be 1.65 kW with an efficiency of 0.9.

15.3.9 Centrifuge (CNT-01)

In order to remove the live cells from the cell suspension coming off of the bioreactors, a Culturefuge 400 system from Alfa Laval will be used. The Culturefuge 400 is a solids-ejecting hermetic disc stack centrifuge capable of processing high throughputs up to 20,000 L/hr, meaning that it is capable of processing the 15,000 L/hr of cell suspension produced by the two production bioreactors. The centrifuge continuously outputs separated liquid phase at the top of the bowl and intermittently discharges solids from the bottom of the bowl. In order to reduce the need for further purification steps downstream, a centrifuge was required that would not lyse the cells. Because of a hollow spindle design that gradually accelerates the liquid entering the centrifuge, the sheer force in the Culturefuge 400 is low enough that it will not lyse cells. The Culturefuge 400 is also compatible with the CIP system that will be used for cleaning across all process units. The unit requires 22 kW of power for the installed motor and 1,010 L/hr of cooling water for the frame parts, oil cooler, and seals, and it has a footprint of 2.95 m by 1.55 m. 2 units will be purchased in order to have a backup system that the flow will be routed to during maintenance on the primary system. The cost for each system will be $400,000.

15.3.10 Cell Waste (TN-04)

The cell waste and media that is removed by the centrifuge will be sent to a holding tank at a rate of 2478809 kg/campaign. It will be sent from the holding tank to the biowaste disposal process but a holdup tank of 645000 gallons will be used to help mitigate the flow downstream should there be any complications. The size of this tank is such that it is designed to hold an entire 8 day campaigns worth of waste. It is in this holding tank that the waste will be neutralized and digested before being sent to municipal waste. The tank will be made of stainless steel and have a floating roof, costing $2659866 according to the Equipment Costing Spreadsheet. It will have agitation at a rate of 962 kW.

15.3.11 Pump to Filtration 1 (P-07)

This pump is designed to deliver the liquid exiting the centrifuge to the first ultrafiltration system. The pump will be designed to accommodate 100 ft of head loss and deliver the liquid to the ultrafiltration at 1.2 bar. It will be a centrifugal pump constructed out of stainless steel. The purchase cost will be $9092 and the bare module cost will be $30005. The power requirement will be 1.47 kW with an efficiency of 0.9.

15.3.12 45kDa Ultrafiltration (TFF-01)

A tangential flow ultrafiltration system with the 45kDa membranes will be used to separate the larger extracellular proteins and any cell remnants after the centrifuge. The Prostak tangential flow filtration unit from MilliporeSigma will be used for this process. This unit allows for high product recovery and low hold up volume. The membranes that will be used are hydrophilic polyvinylidene difluoride which allows them to have lower protein binding and therefore better separation. They are also functional in the pH range 2-11, which will offer maximum functionality in this system. The cross flow rate, area, and transmembrane pressure will be optimized at lab scale using the MilliporeSigma Single Pass Tangential Flow Filtration system and scaled up to ensure 90% separation. The estimated membrane area is 30 m^2 and the estimated cross flow is 25,200 L/hr. The system will measure the pressure at on the feed, retentate and permeate because the driving force for the separation comes from the transmembrane pressure. Two units will need to be purchased because after processing 1 hour’s worth of material the system will need to undergo a CIP. The cost of the each unit will be $11M for the tank, cassette holders, and associated pumps. There will be an additional cost of $58,588 for the desired membranes.

The power needed by each unit will be 22 kW. The price of the process water in the cross flow stream is accounted for in the utilities of the plant.

15.3.13 Pump to Cell Waste (P-08)

This pump is for the purpose of delivering the waste from the first ultrafiltration to the cell waste tank. The pump will be designed to accommodate 100 ft of head loss and deliver the liquid to the cell waste bin at 1.2 bar. It will be a centrifugal pump constructed out of stainless steel. The purchase cost will be $9114 and the bare module cost will be $30077. The power requirement will be 1.34 kW with an efficiency of 0.9.

15.3.14 Pump to Filtration 2 (P-09)

This pump will deliver the extract from the first filtration step to the second filtration system. The pump will be designed to accommodate 100 ft of head loss and deliver the liquid to the cell waste bin at 1.2 bar. It will be a centrifugal pump constructed out of stainless steel. The purchase cost will be $9228 and the bare module cost will be $30454. The power requirement will be 2.48 kW with an efficiency of 0.9.

15.3.15 8kDa Tangential Flow Filtration (TFF-02)

The second tangential flow filtration step will consist of 8kDa membranes to capture the desired enzyme and the retentate from this step will be sent to the spray dryer. Biomax membranes will be used for this step because they are engineered for high flux, high yield and high reliability. They have the ability to operate with in pH of 1-14 and have low protein binding because they are hydrophilic. The MilliporeSigma systems are linearly scalable so the cross flow rate, area, and transmembrane pressure will be optimized at lab scale to reach 87% separation. It will then be scaled up to manufacturing scale. The estimated membrane area is 60 m^2 and the estimated crossflow is 25,200 L/hr. The system will measure the pressure at on the feed, retentate and permeate because the driving force for the separation comes from the transmembrane pressure. Two units will need to be purchased because after processing 1 hour's worth of material the system will need to undergo a CIP process. The cost of each unit will be $2307920 for the tank, cassette holders, and associated pumps. There will be an additional cost of $307,920 for the Biomax filters. The power needed by each unit will be 22 kW. The price of the process water in the cross flow stream is accounted for in the utilities of the plant.

15.3.16 Pump to Sterilization (P-10)

This pump will deliver the waste media from the second filtration step to the sterlization process so that the media may be recycled. The pump will be designed to accommodate 100 ft of head loss and deliver the liquid to the cell waste bin at 1.2 bar. It will be a centrifugal pump constructed out of stainless steel. The purchase cost will be $9209 and the bare module cost will be $30390. The power requirement will be 2.41 kW with an efficiency of 0.9.

15.3.17 Purge Waste Tank (TN-05)

The cell waste and media that is removed by the centrifuge will be sent to a holding tank at a rate of 1662870 kg/campaign. It will be sent from the holding tank to the biowaste disposal process but a holdup tank of 438000 gallons will be used to help mitigate the flow downstream should there be any complications. The size of this tank is such that it is designed to hold an entire 8 day campaigns worth of waste. It is in this holding tank that the waste will be neutralized before being sent to municipal waste. It will have 2 h.p./ 1000 gallons of agitation. The tank will be made of stainless steel and have a floating roof, costing

$364325 according to the Equipment Costing Spreadsheet. It will require 653 kW of agitation.

15.3.18 Economizer (HX-04)

The economizer is the first in a set of three heat exchangers designed to sterilize the media at 140 °C. This exchanger will use the media exiting the sterilizer (the second exchanger) to heat the media before it enters the sterilizer. The hot stream, i.e. the media exiting the sterilizer, will be cooled down in the economizer before entering the cooler. Cool media will be entering the tube side at a pressure of 1.2 bar and temperature of 25 °C with flow rate 21788 kg/hr. It will be leaving the exchanger at 63 °C. Hot media at 140 °C will enter the shell side at a flow rate of 21788 kg/hr and a pressure of 3.62 bar and will leave the heat exchanger at 106 °C. The pressure drop across both sides of the exchanger was negligible. The exchanger was designed with 0.0005 ft^2 hr °F/btu and the product of the overall heat transfer coefficient and the heat transfer area was 23111.4 btu hr^{-1} $°F^{-1}$. The exchanger will be constructed out of 316L stainless steel. The tubes will be 0.75 in in outer diameter and will have pitch 0.9375. The tube pattern will be 30-triangular and they will be located in the baffle windows. The baffle type will be single segmental and they will be cut horizontally. The shell inside and outside diameter respectively will be 6.407 in and 6.625 in. They will be 153.5 in long and there will be 27 tubes and only 1 pass. The baffles will be spaced 11.6 in apart center to center and there will be 12 baffles. Each exchanger will cost $12000.

15.3.19 Pump to Sterilizer (P-12)

A pump will be used to raise the pressure of the liquid stream exiting the economizer and entering the sterilizer. This pressure increase is to prevent vaporization in the sterilizer. It will be a centrifugal pump constructed out of stainless steel. The purchase cost will be $9125 and the bare module cost will be $30112. The power requirement will be 2.41 kW with an efficiency of 0.9.

15.3.20 Sterilizer (HX-05)

The sterilizer is a heat exchanger that heats up the liquid media stream to 140 °C so that any biological contaminants may be destroyed. The media, i.e. the cool stream, enters on the tube side at a pressure of 3.62 bar and a temperature of 63 °C. The flow rate of the media is 21788 kg/hr and it exits the exchanger at 140 °C. Steam at a pressure of 10.34 bar and a temperature of 185 °C enters the shell side of the exchanger at a flow rate of 158718 kg/hr. The steam exits the exchanger at 181 °C, with a vapor fraction of 0.97. The pressure drop across both ends was negligible. The exchanger was designed with 0.0005 ft^2 hr °F/btu and the product of the overall heat transfer coefficient and the heat transfer area was 54986 btu hr^{-1} $°F^{-1}$. The exchanger will be constructed out of 316L stainless steel. The tubes will be 0.5 in in outer diameter and will have pitch 0.625. The tube pattern will be 30-triangular and they will be located in the baffle windows. The baffle type will be single segmental and they will be cut horizontally. The shell inside and outside diameter respectively will be 29.53 in and 30.08 in. They will be 59.1 in long and there will be 1870 tubes and only 1 pass. The baffles will be spaced 6.5 in apart center to center and there will be 6 baffles. Each exchanger will cost $80485. Steam is available at a price of $15.30 / 1000 kg.

15.3.21 Cooler (HX-06)

The cooler will be cooling the liquid media exiting the economizer-sterilizer loop. The media, i.e. the hot stream in the cooler, enters the exchanger on the shell side at 106 °C and 3.62 bar. The flow rate of the media is is 21788 kg/hr. It then exits at 32 °C. Cooling water enters the exchanger on the tube side at 1 bar, 26.66 °C and at a flow rate of 68633 kg/hr. It exits the exchanger at 51.1 °C. The pressure drop across both ends was negligible. The exchanger was designed with 0.0005 ft^2 hr °F/btu and the product of the overall heat transfer coefficient and the heat transfer area was 173666 btu hr^{-1} $°F^{-1}$. The exchanger will be constructed out of 316L stainless steel. The tubes will be 0.75 in in outer diameter and will have pitch 0.9375. The tube pattern will be 30-triangular and they will be located in the baffle windows. The baffle type will be single segmental and they will be cut horizontally. The shell inside and outside diameter respectively will be 16.73 in and 17.13 in. They will be 230.3 in long and there will be 247 tubes and only 1 pass. The baffles will be spaced 4.2 in apart center to center and there will be 52 baffles. Each exchanger will cost $40257. Cooling water is available at $0.027 / m^3. Following the sterilizer, the piping to the media holding tank will be such that the head loss will be calibrated to ensure delivery of fluid at 1.2 bar to the holding tank.

15.3.22 Pump to Evaporator (P-11)

A pump will be used to deliver the product stream from the second filtration step to the evaporator. The pump will be designed to accommodate 100 ft of head loss and deliver the liquid to the evaporator at 1.2 bar. It will be a centrifugal pump constructed out of stainless steel. The purchase cost will be $9212 and the bare module cost will be $30399. The power requirement will be 43.57 kW with an efficiency of 0.9.

15.3.23 Air Compressor (CMP-01)

In order to pressurize the air to be used as the drying air in the spray dryer, an air compressor will be used. 1000 kg/hr of air will be compressed to 1.471 bar by the compressor. The unit will be a carbon steel centrifugal compressor powered electrically with an 18.03 hp motor. The compressor will cost $61,936.

15.3.24 Heat Exchanger for Air Heating (HX-08)

This heat exchanger will be used to heat the air before it is utilized in the spray dryer unit. The air will be the cool stream, entering the tube side at a flow rate of 1000 kg/hr, with 13 kg/hr of water vapor entering alongside due to the air having nonzero humidity. The air enters at 1.47 bar 76 °C and will be heated to 160 °C. Steam enters the shell side at a flow rate of 1923 kg/hr, with inlet temperature 186 °C and pressure 10.34 bar. The steam exits at 181 °C. The pressure drop across both ends is negligible. The exchanger was designed with 0.0005 ft^2 hr °F/btu and the product of the overall heat transfer coefficient and the heat transfer area was 839 btu hr^{-1} $°F^{-1}$. The exchanger will be constructed out of 316L stainless steel. The tubes will be 0.75 in in outer diameter and will have pitch 0.9375. The tube pattern will be 30-triangular and they will be located in the baffle windows. The baffle type will be single segmental and they will be cut horizontally. The shell inside and outside diameter respectively will be 6.407 in and 6.625 in. They will be 47.3 in long and there will be 27 tubes and only 1 pass. The baffles will be spaced 4.4 in apart center to center and there will be 6 baffles. Each exchanger will cost $10514.

15.3.25 Evaporator (EVP-01)

The evaporator will be a skid-mounted triple-effect operator which can be modeled as a heat exchanger to heat up the incoming liquid to 100 °C followed by three heat exchangers in series (denoted as evaporators). In each of the evaporators, a third of the required amount of vaporization occurs, i.e. approximately 8300 kg/hr of water is vaporized in each of the evaporators. The approximation that each of the evaporators has the same heat duty is used. The heat transfer coefficient is 150 btu hr^{-1} ft^{-2} $°F^{-1}$,

15.3.26 Spray Dryer (SD-01)

The spray dryer used in this process will be a VERSATILE-SD unit from GEA, specifically the VSD-80 model for its high water evaporation capacity of 320 kg/hr. The evaporator previous to this step will have been tuned to remove enough water for the protein slurry to contain the correct amount of water for the VSD-80 to handle, producing 762.4 kg/hr of dry product protein. The VERSATILE-SD system offers a variety of standard operating conditions that can be experimentally determined to ensure a setup that produces product at the desired quality. Compressed air heated to 160°C will be used as the inlet gas in co-current flow operation, and the air containing water vapor will be exhausted from the dryer at 80°C. The spray dryer will require 142.5 m^2 of space, 14.9 kW of power, and will be CIP compatible. The system will cost $500,000.

15.3.27 Product Bin (PB-01)

Dried product powder from the spray dryer will be fed to a 5,000L carbon steel vertical holding tank with a Wamgroup BA150 bin activator attached to the bottom of the tank. Wamgroup will provide a full assembly kit including plasma cut flange for straightforward assembly and attachment of the bin activator system. The bin activator has a 323 mm standard outlet with a carbon steel inner cone that is vibrated through electric motor-vibrators to ensure constant, smooth flow of product powder. Suspensions within the bin activator ensure that the cone is the only piece of the structure that vibrates, protecting the structural integrity of the bin. Vibration induced mass flow of the product will ensure that the required flow rate of 762.4 kg/hr of product to final packaging during continuous operation is met. The bin activator motors require 7.5 kW of power for operation, and the bin will cost $19,240 and the activator will cost $35,000.

15.4 Other Units

15.4.1 Mixers and Splitters

There are many different points in the process where mixing and splitting is required such as sending the media from the holding tank to the bioreactors, sending the two bioreactor outlets to a single holding tank and mixing the ammonia and oxygen gas streams, to name a few. The easiest way for these streams to be mixed or split is by introducing a tee to the pipeline at that point. The controls and valves for these tee's are considered to be covered by the bare module cost of the other process elements and have not been costed individually.

15.4.2 Clean In Place (CIP)

The Clean-In-Place (CIP) system will be used to clean the major units of the process including the bioreactors, centrifuge, filtration systems, and spray dryer. CIP will occur during process downtime as described in Section 10.4. A Multi-Tank Detergent and Rinse Recovery/Reuse system from Sani-Matic will be used with piping to connect all process units to the system. The Multi-Tank system uses multiple tanks to all for recovery and reuse of wash solution and rinse water. Because the CIP system will be used for cleaning of all process units, reuse of solution and water will be important to reduce cost associated with cleaning. The high bioreactor operation temperature also ensures that the risk of contamination is low, facilitating reuse of solutions. Additionally, Sani-Matic CIP system controls can be automated and cycle times can be optimized to ensure efficient use of time during the windows of process downtime every 8 days. The system will cost $80,000 with a power requirement of 20 hp.

15.4.3 Steam Generation

This process will require 174,124 kg/hr of medium pressure steam for use in the sterilization loop, drying air heat exchanger and evaporator. Steam will be generated on-site using a standard steam generation process. Exhaust gas containing oxygen from the bioreactors will be sent to the steam generator as a supplementary combustion source, and condensed water from the evaporator will be sent back to the steam generator to be vaporized. The operative cost of the steam generator is captured by the cost of steam as outline in Sections 14.1 and 19.2.

15.4.4 Water Cooling Tower

20,718 kg/hr of cooling water and 23,925 kg/hr of chilled water are required for this process and will be provided be a water cooling tower. Water temperature will vary from upwards of 7°C, meaning that year round cooling will need to be available, even in winter months. The cost of operation of the water cooling tower is captured by the cost of cooling water and chilled water in Sections 14.1 and 19.2.

15.4.5 Biowaste Disposal System

The various waste streams coming from the cell waste tanks and the media purge will go through an inactivation system. This will include neutralizing the streams, since the process is basic, and adding bleach to them to kill the cells before disposal. These costs are included in the annual expenses of the plant.

15.4.6 Genetic Engineering

The *Natronomonus pharaonis* bacterial cells purchased from ATCC will need to be genetically modified to secrete the desired enzyme extracellular. This will be a onetime expense and the cells grown after this purchase will be preserved in a cell bank to ensure there are always some of the engineered cells available for inoculation.

15.4.7 Quality Control Lab

A quality control lab will be utilized to check and ensure the manufactured product meets the quality requirements desired. This includes percent purity measurements and testing for enzymatic function. Random samples will be taken at varying intervals throughout production. This lab is included in the annual costs of the plant.

15.4.8 Packaging

The final product will be packaged in 850 L Super Sacks made of polypropylene. At the production rate of 762.39 kg/hr of final product, 27 Super Sacks will be filled a day with 680 kg of product each. The Super Sacks are $15 each and are available for purchase through Bag Corp. The Super Sack will have 4 handles and a cinched close top for easy moving and filling. The total annual expense on Super Sacks will $139,955.

16. Specification Sheets

Ammonia Storage

Identification	Item	Storage Tank	Date: April 21, 2020
	Item No:	TN-01	Vendor: N/A
	No. Required	1	

Function: Deliver ammonia in liquid form to the process.

Operating: Batch/continuous   Annual Duration: 8332 hrs/yr

Materials Handled	Outlet				
Temperature (°C)	32				
Pressure (bar)	1.2				
Overall (kg/hr)	53				
Water	0				
Glucose	0				
Carbon Waste	0				
Sodium Phosphate	0				
Sulfur	0				
Sodium Chloride	0				
Potassium Hydroxide	0				
Magnesium Chloride	0				
Boric Acid	0				
Oxygen	0				
Ammonia	53				
Carbon Dioxide	0				
Cells	0				
Product Protein	0				
Other Protein	0				

Design Data:
Material of Construction: 316L
Volume: 100L
Roof Type: Cone

Utilities:
Purchase Cost: $10,573
Controls:
Comments

Media Tank

Identification	Item	Storage Tank	Date: April 21, 2020
	Item No:	TN-02	Vendor: N/A
	No. Required	1	

Function: Hold media before releasing it into the process

Operating: Batch/continuous   Annual Duration:

Materials Handled	Sterilization	Outlet			
Temperature (°C)	32	32			
Pressure (bar)	1.2	1.2			
Overall (kg/hr)	21788	21788			
Water	14958	14958			
Glucose	3508	3508			
Carbon Waste	0	0			
Sodium Phosphate	43	43			
Sulfur	20	20			
Sodium Chloride	3037	3037			
Potassium Hydroxide	137	137			
Magnesium Chloride	7	7			
Boric Acid	80	80			
Oxygen	0	0			
Ammonia	0	0			
Carbon Dioxide	0	0			
Cells	0	0			
Product Protein	0	0			
Other Protein	0	0			

Design Data:

Volume: 44480 L
Material of Construction: 316L
Diameter: 10 ft
Length: 20 ft
Elasticity: 30×10^6 psi
Corrosion Thickness: 0.125 in
Density: 490 lb/ft^3

Utilities: 8.76 kW
Purchase Cost: $102427
Controls:
Comments

Ammonia Vaporizer

Identification	Item	Vaporizer	Date: April 21, 2020
	Item No:	B-01	Vendor: Algas SDI
	No. Required	1	

Function: Ammonia will be stored in an anhydrous, liquid form, so the ammonia vaporizer will be used to produce the pressurized ammonia gas required to be sparged to the bioreactors.

Operating: Batch/continuous Annual Duration: 8322 hrs/yr

Materials Handled	Holding Tank	Gas Feed			
Temperature (°C)	32	66			
Pressure (bar)	1.2	1.4			
Overall (kg/hr)	53	53			
Water	0	0			
Glucose	0	0			
Carbon Waste	0	0			
Sodium Phosphate	0	0			
Sulfur	0	0			
Sodium Chloride	0	0			
Potassium Hydroxide	0	0			
Magnesium Chloride	0	0			
Boric Acid	0	0			
Oxygen	0	0			
Ammonia	53	53			
Carbon Dioxide	0	0			
Cells	0	0			
Product Protein	0	0			
Other Protein	0	0			

Design Data:

Model = PowerXP120AA
Vaporization Capacity = 54 kg.hr
Heatx Surface Area = 0.40 m^3
Design Pressure = 17.6 kg/cm^2
Liquid Capacity = 6.0 L
Operating Temp Range = 71-79°C
Thermal Efficiency = 98%
Footprint = 0.09 m^2

Utilities: 20 kW
Purchase Cost: $20,000
Controls:
Comments

Oxygen Generator

Identification	Item	Generator	Date: April 21, 2020
	Item No:	GEN-01	Vendor: Air Products
	No. Required	1	

Function: The oxygen generator is used to produce pure oxygen gas by absorbing the nitrogen from air and releasing it, allowing pure oxygen to continue on to the bioreactor. Pure oxygen is required due to the high oxygen uptake rate by the cells and the desire to minimize VVM.

Operating: Batch/continuous Annual Duration: 8322 hrs/yr

Materials Handled	Gas Out				
Temperature (°C)	66				
Pressure (bar)	1.4				
Overall (kg/hr)	324				
Water	0				
Glucose	0				
Carbon Waste	0				
Sodium Phosphate	0				
Sulfur	0				
Sodium Chloride	0				
Potassium Hydroxide	0				
Magnesium Chloride	0				
Boric Acid	0				
Oxygen	324				
Ammonia	0				
Carbon Dioxide	0				
Cells	0				
Product Protein	0				
Other Protein	0				

Design Data:
Model = PRISM VSA T Series
Generation Capacity = 25 MT/day
Oxygen Purity = 90-93%

Utilities: 74.57 kW
Purchase Cost: $1.3MM
Controls:
Comments

1 mL Test Tubes

Identification	Item	Aliquot tubes	Date: April 21, 2020
	Item No:	SBR-01, SBR-03, SBR-05	Vendor: Fisherbrand
	No. Required	3 per batch	

Function: These tubes will be used to store *N. pharaonis* cells at -80°C for inoculation of 2L seed train reactors at the start of campaigns.

Operating: Batch

Materials Handled	Inoculum				
Temperature (°C)	32				
Pressure (bar)	1				
Overall (g/batch)	1.03				
Water	1				
Glucose	0				
Carbon Waste	0				
Sodium Phosphate	0				
Sulfur	0				
Sodium Chloride	0				
Potassium Hydroxide	0				
Magnesium Chloride	0				
Boric Acid	0				
Oxygen	0				
Ammonia	0				
Carbon Dioxide	0				
Cells	0.03				
Product Protein	0				
Other Protein	0				

Design Data:

Volume = 1 mL
Length = 125 mm
Diameter = 16 mm
Material = Polystyrene
Cap Type = Screw
Sterility = Sterile

Utilities:

Purchase Cost: $521.50 per 1,000

Controls:

Comments

2 L Bioreactor

Identification	Item	Seed Reactor	Date: April 21, 2020
	Item No:	SBR-02, SBR-04, SBR-06	Vendor: Sartorius Stedim Biotech
	No. Required	3	

Function: This is the first seed reactor to grow the cells up to a maximum cell density of 30 g/L before input into the 2,000 L seed reactor. A 1 mL aliquot of cell suspension will inoculate the reactor, and the required media components and gases will be pumped into the reactor. Three 2L reactors will be run simultaneously in case of contamination, and so that the best batch of cells can be used for the rest of the process.

Operating: Batch Annual Duration: 1344 hrs/yr

Materials Handled	Inoculum	Gas Feed	Gas Vent	Liquid Product
Temperature (°C)	32	66	60	60
Pressure (bar)	1	1.4	1.2	1.2
Overall (g/batch)	2137	264	160	1755
Water	1618	0	0	1619
Glucose	151	0	0	0
Carbon Waste	0	0	0	9
Sodium Phosphate	5	0	0	1
Sulfur	0.04	0	0	0
Sodium Chloride	329	0	0	66
Potassium Hydroxide	15	0	0	3
Magnesium Chloride	0.7	0	0	0
Boric Acid	0.9	0	0	9
Oxygen	0	0	52	0
Ammonia	0	259	1	0
Carbon Dioxide	0	5	111	0
Cells	0.03	0	0	49
Product Protein	0	0	0	0
Other Protein	0	0	0	0

Design Data:

Total Volume = 3 L
Working Volume = 2 L
Material = Borosilicate glass, AISI 316L SS
Integrated Pumps = 3

External Pumps = 1
Probes = Temp, DO, pH, Foam
Cooling = Chiller w/ automatic cooling water valves
Heating = Electric heating jacket

Utilities: 0.15 kW for agitation, 0.15 kW for cooling

Purchase Cost: $25,000

Controls:

Comments

2,000 L Bioreactor

Identification	Item	Seed Reactor	Date: April 21, 2020
	Item No:	SBR-07	Vendor: Sartorius Stedim Biotech
	No. Required	1	

Function: This is a second seed reactor to grow the cells up to a maximum cell density of 30 g/L before input into the production scale bioreactor. The 2000 L seed reactor will take the entire contents of one 2 L reactor as well as the required media components and gases. The reactor uses single use Flexsafe STR Bags that will be replaced after every batch for ease of cleaning and maintenance.

Operating: Batch Annual Duration: 1848 hrs/yr

Materials Handled	Liquid Feed	Gas Feed	Gas Vent	Liquid Product
Temperature (°C)	60	52	60	60
Pressure (bar)	1.2	1.4	1.2	1.2
Overall (kg/batch)	2126	247	160	1755
Water	1617	0	0	1619
Glucose	151	0	0	0
Carbon Waste	0	0	0	9
Sodium Phosphate	5	0	0	1
Sulfur	0	0	0	0
Sodium Chloride	329	0	0	66
Potassium Hydroxide	15	0	0	3
Magnesium Chloride	1	0	0	0
Boric Acid	9	0	0	9
Oxygen	0	242	48	0
Ammonia	0	5	1	0
Carbon Dioxide	0	0	111	0
Cells	0	0	0	49
Product Protein	0	0	0	0
Other Protein	0	0	0	0

Design Data:	Footprint = 9.35 m^2	Working Volume = 2000 L
	Weight = 2,045 kg	Turndown Ratio = 1:4
	Material = AISI 304 SS	Bag Diameter = 1,295 mm
	Max Stirrer Speed = 70 rpm	Bag Height = 2,330 mm
		Liquid Height = 1,670 mm
	Flexsafe STR Bag Data	Impeller Diameter = 492 mm
	Total Volume = 2800 L	Distance Btw Impellers = 640 mm

Utilities: 0.55 kW for agitation

Purchase Cost: $563,000

Controls: Control loop on cooling loop

Comments

Seed Train Cooling Loop Heat Exchanger

Identification	Item	Heat Exchanger	Date: April 21, 2020
	Item No:	HX-01	Vendor: N/A
	No. Required	1	

Function: This heat exchanger will cool the fluid in the 2000 L fermentation reactors before recycling the fluid back into the reactor.

Operating: Batch   Annual Duration:

Materials Handled	Hot In	Hot Out	Cold In	Cold Out
Temperature (°C)	60	32	26.66	48.88
Pressure (bar)	1.2	1.2	1	1
Overall (kg/batch)	6347	6347	536	536
Water	5855	5855	536	536
Glucose	0	0	0	0
Carbon Waste	33	33	0	0
Sodium Phosphate	4	4	0	0
Sulfur	0	0	0	0
Sodium Chloride	238	238	0	0
Potassium Hydroxide	11	11	0	0
Magnesium Chloride	0	0	0	0
Boric Acid	31	31	0	0
Oxygen	0	0	0	0
Ammonia	0	0	0	0
Carbon Dioxide	0	0	0	0
Cells	176	176	0	0
Product Protein	0	0	0	0
Other Protein	0	0	0	0

Design Data:
Heat Exchanger Type: Double pipe
Heat Transfer Area: 2.02 ft^2
Heat Transfer Coefficient: 150 btu hr^{-1} ft^{-2} F^{-1}

Utilities: 536 kg/hr of cooling water
Purchase Cost: $5113
Controls:
Comments

20,000 L Bioreactor

Identification	Item	Bioreactor	Date: April 21, 2020
	Item No:	BR-01, BR-02	Vendor: Paul Mueller Company
	No. Required	2	

Function: This is a large fermentation vessel where the product enzyme is produced. This vessel will have as input an inoculum (batch), growth media and gas flow. It will have as output the suspension with cells and enzyme in it as well as a waste gas flow. It will also contain an agitator.

Operating: Batch/Continuous   Annual Duration: 7243 hrs/yr

Materials Handled	Liquid Feed	Gas Feed	Gas Vent	Liquid Product
Temperature (°C)	32	66	60	60
Pressure (bar)	1.2	1.43	1.2	1.2
Overall (kg/hr)	10894	189	1522	8547
Water	7479	0	0	7479
Glucose	1754	0	0	0
Carbon Waste	0	0	0	57
Sodium Phosphate	21	0	0	4
Sulfur	10	0	0	2
Sodium Chloride	1518	0	0	304
Potassium Hydroxide	68	0	0	14
Magnesium Chloride	3	0	0	1
Boric Acid	40	0	0	40
Oxygen	0	162	32	0
Ammonia	0	27	27	0
Carbon Dioxide	0	0	1463	0
Cells	0	0	0	224
Product Protein	0	0	0	411
Other Protein	0	0	0	11

Design Data:

Tank Inside Diameter = 2100 mm
Bottom Head Type = ASME D&F
Material Thickness = 2.67 mm
Inside Knuckle Radius = 127 mm
Straight Flange Length = 25.4 mm
Inside Dish Radius = 2105.03 mm
Shell Material Thickness = 2.67 mm
Shell Short Side Height = 5300 mm
Top Head Type = ASME D&F

Material Thickness = 2.67 mm
Inside Knuckle Radius = 127 mm
Straight Flange Length = 25.4 mm
Inside Dish Radius = 2105.03 mm
Bottom Head Volume = 839.03 liters
Shell Volume = 18357.11 liters
Top Head Volume = 839.03 liters
Total Volume = 20035.18 liters

Utilities: 12.6 kW for agitation

Controls: Proportional Integral control loop on cooling loop

Purchase Cost: $410,000

Comments

Cooling Loop Heat Exchanger

Identification	Item	Heat Exchanger	Date: April 21, 2020
	Item No:	HX-02, HX-03	Vendor: N/A
	No. Required	1	

Function: This heat exchanger will cool the fluid in the 20000 L fermentation reactors before recycling the fluid back into the reactor.

Operating: Batch/Continuous   Annual Duration: 7243 hrs/yr

Materials Handled	Hot In	Hot Out	Cold In	Cold Out
Temperature (°C)	60	32	26.66	48.88
Pressure (bar)	1.2	1.2	1	1
Overall (kg/hr)	7821	7820	9854	9854
Water	6844	6844	9854	9854
Glucose	0	0	0	0
Carbon Waste	52	52	0	0
Sodium Phosphate	4	4	0	0
Sulfur	2	2	0	0
Sodium Chloride	278	278	0	0
Potassium Hydroxide	13	13	0	0
Magnesium Chloride	1	1	0	0
Boric Acid	37	37	0	0
Oxygen	0	0	0	0
Ammonia	0	0	0	0
Carbon Dioxide	0	0	0	0
Cells	205	205	0	0
Product Protein	376	376	0	0
Other Protein	10	10	0	0

Design Data:

Material of Construction: 316L
UA: 62299 btu hr^{-1} $°F^{-1}$
Tube OD: 0.75 in
Pitch: 0.9375
Tube Pattern: 30 Triangular
Baffle Type: Single Segmental
Baffle Cut: Horizontal
Shell ID: 8.407 in
Shell OD: 8.625 in

Tube Length: 183.1 in
Number of Tubes: 44
Number of Passes: 2
Baffle Spacing: 7.67 in
Number of Baffles: 22
Shells in Series: 3

Utilities: 9854 kg/hr of cooling water
Controls:
Purchase Cost: $47,769
Comments

Waste Gas Tank

Identification	Item	Gas tank	Date: April 21, 2020
	Item No:	GNT-01	Vendor: N/A
	No. Required	2	

Function: Store waste gas before releasing it to the steam generation.

Operating: Batch/Continuous Annual Duration:

Materials Handled	Inlet	Outlet			
Temperature (C)	60	60			
Pressure (bar)	1.2	1.2			
Overall (kg/hr)	1522	3044			
Water	0	0			
Glucose	0	0			
Carbon Waste	0	0			
Sodium Phosphate	0	0			
Sulfur	0	0			
Sodium Chloride	0	0			
Potassium Hydroxide	0	0			
Magnesium Chloride	0	0			
Boric Acid	0	0			
Oxygen	32	65			
Ammonia	27	53			
Carbon Dioxide	1463	2926			
Cells	0	0			
Product Protein	0	0			
Other Protein	0	0			

Design Data: Volume: 422000 gallons
Roof Type: Floating

Utilities:
Purchase Cost: $357516
Controls:
Comments

Holding Tank Heat Exchanger

Identification	Item	Heat Exchanger	Date: April 21, 2020
	Item No:	HX-07	Vendor: N/A
	No. Required	1	

Function: This heat exchanger will cool the effluent from the 20000 L fermentation reactors before storing the effluent in a holding tank.

Operating: Continuous   Annual Duration: 6782 hrs/yr

Materials Handled	Hot In	Hot Out	Cold In	Cold Out
Temperature (°C)	60	25	7.22	32.22
Pressure (bar)	1.2	1.2	1	1
Overall (kg/hr)	17093	17093	23925	23925
Water	14958	14958	23925	23925
Glucose	0	0	0	0
Carbon Waste	113	113	0	0
Sodium Phosphate	9	9	0	0
Sulfur	4	4	0	0
Sodium Chloride	607	607	0	0
Potassium Hydroxide	27	27	0	0
Magnesium Chloride	1	1	0	0
Boric Acid	80	80	0	0
Oxygen	0	0	0	0
Ammonia	0	0	0	0
Carbon Dioxide	0	0	0	0
Cells	449	449	0	0
Product Protein	822	822	0	0
Other Protein	22	22	0	0

Design Data:

Material of Construction: 316L
UA: 591611 btu hr^{-1} $°F^{-1}$
Tube OD: 0.5 in
Pitch: 0.625
Tube Pattern: 30 Triangular
Baffle Type: Single Segmental
Baffle Cut: Horizontal
Shell ID: 8.407 in
Shell OD: 8.625 in

Tube Length: 189.0 in
Number of Tubes: 118
Number of Passes: 1
Baffle Spacing: 7.67 in
Number of Baffles: 22
Shells in Series: 1

Utilities: 23925 kg/hr of chilled water
Controls:
Purchase Cost: $16,940
Comments

Holding Tank

Identification	Item	Horizontal Pressure vessel	Date: April 21, 2020 Vendor: N/A
	Item No:	TN-03	
	No. Required	1	

Function: This holding tank will contain the effluent from both bioreactors before sending the fluid down

Operating: Continuous   Annual Duration: 6782 hrs/yr

Materials Handled	Liquid In	Liquid Out			
Temperature (°C)	25	25			
Pressure (bar)	1.2	1.2			
Overall (kg/hr)	17093	17093			
Water	14958	14958			
Glucose	0	0			
Carbon Waste	113	113			
Sodium Phosphate	9	9			
Sulfur	4	4			
Sodium Chloride	607	607			
Potassium Hydroxide	27	27			
Magnesium Chloride	1	1			
Boric Acid	80	80			
Oxygen	0	0			
Ammonia	0	0			
Carbon Dioxide	0	0			
Cells	449	449			
Product Protein	822	822			
Other Protein	22	22			

Design Data:

Volume: 44480 L
Material of Construction: 316L
Diameter: 10 ft
Length: 20 ft
Elasticity: 30×10^6 psi
Corrosion Thickness: 0.125 in
Density: 490 lb/ft^3

Utilities: 8.76 kW of agitation
Controls:
Purchase Cost: $102,427
Comments

Centrifuge

Identification	Item	Centrifuge	Date: April 21, 2020
	Item No:	CNT-01	By: Alfa Lavall
	No. Required	2	

Function: A high capacity continuous disc stack centrifuge for separation of solid cell mass from liquid supernatant containing the extracellular product enzyme. The cell mass with residual liquid will be sent to waste, while the remaining supernatant will continue on for further downstream purification.

Operation: Continuous Annual Duration: 6782 hrs/yr

Materials Handled	Liquid Feed	Solid Waste	Liquid Out		
Temperature (°C)	32	32	32		
Pressure (bar)	1.2	1.2	1.2		
Overall (kg/hr)	17093	1333	15760		
Water	14958	799	14160		
Glucose	0	0	0		
Carbon Waste	113	6	107		
Sodium Phosphate	9	0	8		
Sulfur	4	0	4		
Sodium Chloride	607	32	575		
Potassium Hydroxide	27	1	26		
Magnesium Chloride	1	0	1		
Boric Acid	80	4	76		
Oxygen	0	0	0		
Ammonia	0	0	0		
Carbon Dioxide	0	0	0		
Cells	449	444	4		
Product Protein	823	44	779		
Other Protein	22	1	21		

Design Data:

Height = 2,300 mm
Width = 2,950 mm
Depth = 1,550 mm
Contact Part Material = High-grade stainless steel
Throughput Capacity = max 20 m^3/h
Solids Handling Capacity = max 600 L/h
Bowl Volume = 30 L
Sludge Space Volume = 10 L

Discharge Volume = 3-30 L
Bowl Speed = max 5,119 rpm
G-force = max. 7,425
Motor speed (60Hz) = 1,800 rpm
Starting Time Min/Max = 8/10 min
Stopping Time = 10 mins
Feed Temperature Range = 0-100°C
Sound Pressure = 73 dB(A)
Overhead Hoist Capacity = min 1,000 kg

Utilities: 1,010 L/h of cooling water, 22 kW for operation

Controls

Purchase Cost: $400,000

Comments

Cell Waste

Identification	Item	Storage Tank	Date: April 21, 2020
	Item No:	TN-04	Vendor: N/A
	No. Required	1	

Function: This storage tank will hold cell waste from the centrifuge. The fluid will be neutralized before moving to waste disposal.

Operating: Continuous Annual Duration: 6782 hrs/yr

Materials Handled	Liquid In				
Temperature (°C)	25				
Pressure (bar)	1.2				
Overall (kg/hr)	1333				
Water	799				
Glucose	0				
Carbon Waste	6				
Sodium Phosphate	1				
Sulfur	1				
Sodium Chloride	32				
Potassium Hydroxide	2				
Magnesium Chloride	0				
Boric Acid	4				
Oxygen	0				
Ammonia	0				
Carbon Dioxide	0				
Cells	444				
Product Protein	44				
Other Protein	1				

Design Data:
Volume: 300000 L
Material of Construction: 316L
Roof Type: Cone

Utilities:
Controls:
Purchase Cost: $443,311
Comments

45 kDa Ultrafiltration

Identification	Item	Tangential Flow Filtration	Date: April 21, 2020
	Item No:	TFF-01	Vendor: N/A
	No. Required	1	

Function: This ultrafiltration membrane will remove all components of the incoming stream that have a molecular weight greater than 45 kDa. This leaves as the permeate and becomes the product stream.

Operating: Continuous   Annual Duration: 6782 hrs/yr

Materials Handled	Liquid In	Permeate	Waste Stream		
Temperature (°C)	25	25	25		
Pressure (bar)	1.2	1.2	1.2		
Overall (kg/hr)	15760	26628	14332		
Water	14160	25200	14160		
Glucose	0	0	0		
Carbon Waste	107	96	11		
Sodium Phosphate	8	7	1		
Sulfur	4	3	0		
Sodium Chloride	575	517	58		
Potassium Hydroxide	26	23	3		
Magnesium Chloride	1	1	0		
Boric Acid	76	68	8		
Oxygen	0	0	0		
Ammonia	0	0	0		
Carbon Dioxide	0	0	0		
Cells	5	0	5		
Product Protein	779	701	78		
Other Protein	21	11	10		

Design Data:

Membrane Type: Hydrophilic Polyvinylidene Difluoride

pH range: 2-11

Separation: 90%

Membrane Area: 30 m^2

Utilities: 25200 kg/hr of process water, 22 kW for operation

Controls:

Purchase Cost: $2,117,180

Comments

8 kDa Ultrafiltration

Identification	Item	Tangential Flow Filtration	Date: April 21, 2020
	Item No:	TFF-02	Vendor: N/A
	No. Required	1	

Function: This ultrafiltration membrane will remove all components of the incoming stream that have a molecular weight greater than 8 kDa. This leaves as the retentate and becomes the product stream.

Operating: Continuous   Annual Duration: 6782 hrs/yr

Materials Handled	Liquid In	Permeate	Retentate		
Temperature (°C)	25	25	25		
Pressure (bar)	1.2	1.2	1.2		
Overall (kg/hr)	15760	25856	25962		
Water	14160	25200	25200		
Glucose	0	0	0		
Carbon Waste	107	82	15		
Sodium Phosphate	8	6	1		
Sulfur	4	3	1		
Sodium Chloride	575	440	78		
Potassium Hydroxide	26	20	4		
Magnesium Chloride	1	1	0		
Boric Acid	76	58	0		
Oxygen	0	0	0		
Ammonia	0	0	0		
Carbon Dioxide	0	0	0		
Cells	5	0	0		
Product Protein	779	37	664		
Other Protein	21	9	2		

Design Data:

Membrane Type: Biomax

pH range: 1-14

Separation: 87%

Membrane Area: 60 m^2

Utilities: 25200 kg/hr of process water, 22 kW for operation

Controls:

Purchase Cost: $2,615,840

Comments

Purge Waste

Identification	Item	Storage Tank	Date: April 21, 2020
	Item No:	TN-05	Vendor: N/A
	No. Required	1	

Function: Hold waste media for neutralization before releasing it to municipal waste.

Operating: Continuous Annual Duration: 8322 hrs/yr

Materials Handled	Liquid In				
Temperature (°C)	32				
Pressure (bar)	1.2				
Overall (kg/hr)	10508				
Water	10242				
Glucose	0				
Carbon Waste	33				
Sodium Phosphate	3				
Sulfur	1				
Sodium Chloride	179				
Potassium Hydroxide	8				
Magnesium Chloride	0				
Boric Acid	24				
Oxygen	0				
Ammonia	0				
Carbon Dioxide	0				
Cells	0				
Product Protein	15				
Other Protein	4				

Design Data: Material of Construction: 316L
Volume: 438000 gallons
Roof Type: Floating Roof

Utilities: 653 kW
Controls:
Purchase Cost: $364,325
Comments

Economizer

Identification	Item	Heat Exchanger	Date: April 21, 2020
	Item No:	HX-04	Vendor: N/A
	No. Required	1	

Function: This heat exchanger will heat up the media to be recycled before sending it to the sterilizer where it will be further heated to remove contaminants.

Operating: Batch/Continuous Annual Duration: 7243 hrs/yr

Materials Handled	Hot In	Hot Out	Cold In	Cold Out
Temperature (°C)	140	106	25	63
Pressure (bar)	3.62	3.62	1.2	1.2
Overall (kg/hr)	21788	21788	21788	21788
Water	14958	14958	14958	14958
Glucose	3508	3508	3508	3508
Carbon Waste	0	0	0	0
Sodium Phosphate	43	43	43	43
Sulfur	20	20	20	20
Sodium Chloride	3037	3037	3037	3037
Potassium Hydroxide	137	137	137	137
Magnesium Chloride	7	7	7	7
Boric Acid	80	80	80	80
Oxygen	0	0	0	0
Ammonia	0	0	0	0
Carbon Dioxide	0	0	0	0
Cells	0	0	0	0
Product Protein	0	0	0	0
Other Protein	0	0	0	0

Design Data:		
	Material of Construction: 316L	Tube Length: 153.5 in
	UA: 23111 btu hr^{-1} $°F^{-1}$	Number of Tubes: 27
	Tube OD: 0.75 in	Number of Passes: 1
	Pitch: 0.9375	Baffle Spacing: 11.6 in
	Tube Pattern: 30 Triangular	Number of Baffles: 12
	Baffle Type: Single Segmental	Shells in Series: 1
	Baffle Cut: Horizontal	
	Shell ID: 6.407 in	
	Shell OD: 6.625 in	

Utilities:

Controls:

Purchase Cost: $12,000

Comments

Sterilizer

Identification	Item	Heat Exchanger	Date: April 21, 2020	
	Item No:	HX-05	Vendor: N/A	
	No. Required	1		

Function: This heat exchanger will heat up the media to be recycled to a high enough temperature to destroy contaminants.

Operating: Batch/Continuous Annual Duration: 7243 hrs/yr

Materials Handled	Hot In	Hot Out	Cold In	Cold Out
Temperature (°C)	186	181	63	140
Pressure (bar)	10.34	10.34	3.62	3.62
Overall (kg/hr)	158718	158718	21788	21788
Water	158718	158718	14958	14958
Glucose	0	0	3508	3508
Carbon Waste	0	0	0	0
Sodium Phosphate	0	0	43	43
Sulfur	0	0	20	20
Sodium Chloride	0	0	3037	3037
Potassium Hydroxide	0	0	137	137
Magnesium Chloride	0	0	7	7
Boric Acid	0	0	80	80
Oxygen	0	0	0	0
Ammonia	0	0	0	0
Carbon Dioxide	0	0	0	0
Cells	0	0	0	0
Product Protein	0	0	0	0
Other Protein	0	0	0	0

Design Data:

Material of Construction: 316L
UA: 54986 btu hr^{-1} $°F^{-1}$
Tube OD: 0.5 in
Pitch: 0.625
Tube Pattern: 60 Rotated Triangular
Baffle Type: Single Segmental
Baffle Cut: Horizontal
Shell ID: 29.53 in
Shell OD: 30.08 in

Tube Length: 59.05 in
Number of Tubes: 1870
Number of Passes: 1
Baffle Spacing: 6.5 in
Number of Baffles: 6
Shells in Series: 1

Utilities: 158718 kg/hr of 150 psig steam
Controls:
Purchase Cost: $80,485
Comments

Cooler

Identification	Item	Heat Exchanger	Date: April 21, 2020
	Item No:	HX-06	Vendor: N/A
	No. Required	1	

Function: This heat exchanger will cool down the media coming from the sterlizer before sending it to the media storage tank.

Operating: Batch/Continuous Annual Duration: 7243 hrs/yr

Materials Handled	Hot In	Hot Out	Cold In	Cold Out
Temperature (°C)	106	32	25	63
Pressure (bar)	3.62	3.62	1.2	1.2
Overall (kg/hr)	21788	21788	68633	68633
Water	14958	14958	68633	68633
Glucose	3508	3508	0	0
Carbon Waste	0	0	0	0
Sodium Phosphate	43	43	0	0
Sulfur	20	20	0	0
Sodium Chloride	3037	3037	0	0
Potassium Hydroxide	137	137	0	0
Magnesium Chloride	7	7	0	0
Boric Acid	80	80	0	0
Oxygen	0	0	0	0
Ammonia	0	0	0	0
Carbon Dioxide	0	0	0	0
Cells	0	0	0	0
Product Protein	0	0	0	0
Other Protein	0	0	0	0

Design Data:

Material of Construction: 316L
UA: 173666 btu hr^{-1} $°F^{-1}$
Tube OD: 0.75 in
Pitch: 0.9375
Tube Pattern: 30 Triangular
Baffle Type: Single Segmental
Baffle Cut: Horizontal
Shell ID: 16.73 in
Shell OD: 17.13 in

Tube Length: 230.32 in
Number of Tubes: 247
Number of Passes: 1
Baffle Spacing: 4.13 in
Number of Baffles: 52
Shells in Series: 1

Utilities: 68633 kg/hr of cooling water
Controls:
Purchase Cost: $40,257
Comments

Air Heater

Identification	Item	Heat Exchanger	Date: April 21, 2020
	Item No:	HX-08	Vendor: N/A
	No. Required	1	

Function: This heat exchanger will heat up air before it is sent to the spray dryer.

Operating: Continuous Annual Duration: 6782 hrs/yr

Materials Handled	Hot In	Hot Out	Cold In	Cold Out
Temperature (°C)	186	181	76	160
Pressure (bar)	10.34	10.34	1.2	1.2
Overall (kg/hr)	1923	1923	1013	1013
Water	1923	1923	13	13
Air	0	0	1000	1000

Design Data:

Material of Construction: 316L	Tube Length: 47.24 in
UA: 839 btu hr^{-1} $°F^{-1}$	Number of Tubes: 27
Tube OD: 0.75 in	Number of Passes: 1
Pitch: 0.9375	Baffle Spacing: 4.33 in
Tube Pattern: 30 Triangular	Number of Baffles: 6
Baffle Type: Single Segmental	Shells in Series: 1
Baffle Cut: Horizontal	
Shell ID: 6.407 in	
Shell OD: 6.625 in	

Utilities: 1923 kg/hr of 150 psig steam

Controls:

Purchase Cost: $10,514

Comments

Air Compressor

Identification	Item	Heat Exchanger	Date: April 21, 2020
	Item No:	CMP-01	Vendor: N/A
	No. Required	1	

Function: This air compressor will compress the air to be used as drying air coming into the spray dryer.

Operating: Continuous Annual Duration: 6782 hrs/yr

Materials Handled	Gas In	Gas Out			
Temperature (°C)	28.9	76			
Pressure (bar)	1	1.47			
Overall (kg/hr)	1013	1013			
Water	13	13			
Air	1000	1000			

Design Data:
Compressor Type = Centrifugal
Material = Cast Iron
Power Type = Electric

Utilities: 13.4 kW for power
Controls:
Purchase Cost: $61,936
Comments

Evaporator

Identification	Item	Multi-effect Evaporator	Date: April 21, 2020
			Vendor:
	Item No:	EVP-01	
	No. Required	1	

Function: Evaporate the water from the product stream before sending the stream to the spray dryer.

Operating: Continuous   Annual Duration: 6782 hrs/yr

Materials Handled	Inlet	Outlet			
Temperature (°C)	32	32			
Pressure (bar)	1.2	1.2			
Overall (kg/hr)	25962	513			
Water	25200	320			
Glucose	0	0			
Carbon Waste	15	15			
Sodium Phosphate	1	1			
Sulfur	0	0			
Sodium Chloride	78	78			
Potassium Hydroxide	4	4			
Magnesium Chloride	0	0			
Boric Acid	0	0			
Air	0	58			
Oxygen	0	0			
Ammonia	0	0			
Carbon Dioxide	0	0			
Cells	0	0			
Product Protein	664	0			
Other Protein	2	37			

Design Data:

Utilities: 13483 kg/hr of 150 psig steam, 37.3 kW

Controls:

Purchase Cost: $2,800,000

Comments

Spray Dryer

Identification	**Item**	Spray Dryer	Date: April 21, 2020
	Item No:	SD-01	Vendor: GEA
	No. Required	1	

Function: This spray dryer will remove all water still present in the product protein slurry after the multistage evaporator. The spray dryer atomizes the slurry feed, and heated drying air is used to vaporize water leaving solid protein particles.

Operating: = Continuous Annual Duration: 6782 hrs/yr

Materials Handled	Liquid In	Drying Air	Exhaust Air	Solids Out
Temperature (°C)	32	160	80	32
Pressure (bar)	1.2	1.47	1.47	1.35
Overall (kg/hr)	1083	1013	1333	763
Water	320	13	333	0
Glucose	0	0	0	0
Carbon Waste	14	0	0	14
Sodium Phosphate	1	0	0	1
Sulfur	1	0	0	1
Sodium Chloride	78	0	0	78
Potassium Hydroxide	3	0	0	3
Magnesium Chloride	0	0	0	0
Boric Acid	0	0	0	0
Air	0	1000	1000	0
Oxygen	0	0	0	0
Ammonia	0	0	0	0
Carbon Dioxide	0	0	0	0
Cells	0	0	0	0
Product Protein	663	0	0	663
Other Protein	2	0	0	2

Design Data:

Model = VSD-80

Material = Stainless steel

Water Evaporation Capacity = 320 kg/hr

Typical Mean Particle Size = 100 µm

Footprint = 142.5 m^2

Utilities: 5 kW for operation

Controls:

Purchase Cost: $500,000

Comments

Product Bin

Identification	Item	Live Bottom Bin	Date: April 21, 2020
	Item No:	PB-01	Vendor: Wamgroup
	No. Required	1	

Function: The product bin is a live bottom bin consisting of a 5,000 L holding bin with a bin activator that uses vibrations to ensure a steady continuous flow of product powder to fill super sacks for final packaging.

Operating: = Continuous   Annual Duration: 6782 hrs/yr

Materials Handled	Product In	Product Out			
Temperature (°C)	32	32			
Pressure (bar)	1.35	1			
Overall (kg/hr)	762	762			
Water	0	0			
Glucose	0	0			
Carbon Waste	14	14			
Sodium Phosphate	1	1			
Sulfur	1	1			
Sodium Chloride	78	78			
Potassium Hydroxide	3	3			
Magnesium Chloride	0	0			
Boric Acid	0	0			
Air	0	0			
Oxygen	0	0			
Ammonia	0	0			
Carbon Dioxide	0	0			
Cells	0	0			
Product Protein	663	663			
Other Protein	2	2			

Design Data:	**Holding Bin**	**Bin Activator**
	Material = Carbon Steel	Model = BA150
	Diameter = 5.31 ft	Material = 316L SS
	Length = 7.97 ft	Motors = 1
	Design Pressure = 5 psig	Attachment Diameter = 1,480 mm
		Opening Diameter = 323 mm

Utilities: 7.46 kW

Purchase Cost: Bin Activator = $35,000, Holding Bin = $19,240

Controls:

Comments

Clean In Place System

Identification	**Item**	CIP	Date: April 21, 2020
	Item No:	N/A	Vendor: Sani-Matic
	No. Required	1	

Function: A multi-tank CIP system for cleaning of process units.

Operating: = Batch/Continuous

Materials Handled	General				
Temperature (°C)	N/A				
Pressure (bar)	N/A				
Overall (kg/hr)	N/A				
Water	Cleaning				
Glucose	Residual				
Carbon Waste	Residual				
Sodium Phosphate	Residual				
Sulfur	Residual				
Sodium Chloride	Residual				
Potassium Hydroxide	Residual				
Magnesium Chloride	Residual				
Boric Acid	Residual				
Air	0				
Oxygen	0				
Ammonia	0				
Carbon Dioxide	0				
Cells	Residual				
Product Protein	Residual				
Other Protein	Residual				

Design Data: Model = Multi-Tank Detergent and Rinse Recovery/Reuse

Material = Stainless Steel

Utilities: 14.9 kW for operation

Controls:

Purchase Cost: $80,000

Comments

Super Sacks

Identification	**Item**	Super Sacks	Date: April 21, 2020
	Item No:	N/A	Vendor: Bag Corp.
	No. Required	27 per day	

Function: 850 L super sacks will be used to package final product protein powder for sale and distribution

Operating: = Batch/Continuous

Materials Handled	Product				
Temperature (°C)	32				
Pressure (bar)	1,0				
Overall (kg/hr)	762				
Water	0				
Glucose	0				
Carbon Waste	15				
Sodium Phosphate	1				
Sulfur	1				
Sodium Chloride	78				
Potassium Hydroxide	3				
Magnesium Chloride	0				
Boric Acid	0				
Air	0				
Oxygen	0				
Ammonia	0				
Carbon Dioxide	0				
Cells	0				
Product Protein	664				
Other Protein	2				

Design Data:
Volume = 850 L
Material = Polypropylene
Handles = 4
Features = Clinched close top

Utilities:
Controls:
Purchase Price: $15 per bag
Comments

17. Equipment Cost Summary

Table 17.1 shows the estimated purchase cost of every equipment unit in the process flow diagram. The cost index used in the calculations was 600. The cost table shown below includes the costs of spares which were added as the purchase cost on top of the bare module cost of a single unit. The downstream equipment was significantly expensive, namely the two ultrafiltration steps and the evaporator. Use of such expensive equipment was justified because the high degree of separation ensured a reduced need for raw materials and throughput in the upstream process. The evaporator was expensive because it was comprised of many different constituent parts.

Table 17.1 Equipment Costs for all units used in this process

Equipment ID	Equipment Name	Vendor	Price Source	Purchase Cost (USD)	Bare Module Factor	Spare?	Bare Module Cost
TN-01	Ammonia Storage		ECS*	2643	4	No	10572
TN-02	Media Tank		ECS	102427	4.16	No	426097
	Media Tank Agitation		ECS	6240	1	No	6240
B-01	Ammonia Vaporizer	Algas-SDI	Vendor	20000	2	No	40000
GEN-01	Oxygen Generator	Air Products	Vendor	1300000	2	No	2600000
SBR-01	1 mL Test Tube	Fisherbrand	Vendor	521.5	1	No	522
SBR-03	1 mL Test Tube	Fisherbrand	Vendor	521.5	1	No	522
SBR-05	1 mL Test Tube	Fisherbrand	Vendor	521.5	1	No	522
SBR-02	2 L Reactor	Sartorius Sedim	Vendor	25000	4.16	No	104000
SBR-04	2 L Reactor	Sartorius Sedim	Vendor	25000	4.16	No	104000
SBR-06	2 L Reactor	Sartorius Sedim	Vendor	25000	4.16	No	104000
SBR-07	2000 L Reactor	Sartorius Sedim	Vendor	563000	4.16	No	2342080

HX-01	Seed Train HeatX		ECS	5113	1.8	No	9203
CMP-01	Gas Compressor		ECS	61936	2.15	No	133162
BR-01	20000 L Reactor	Paul Mueller	Vendor	285000	4.16	No	1185600
BR-02	20000 L Reactor	Paul Mueller	Vendor	285000	4.16	No	1185600
	Bioreactor Agitation	Paul Mueller	Vendor	125000	4.16	No	520000
	Bioreactor Agitation	Paul Mueller	Vendor	125000	4.16	No	520000
P-02	Cooling Loop Pump		ECS	15641	3.3	Yes	67256
P-03	Cooling Loop Pump		ECS	15641	3.3	Yes	67256
HX-02	Cooling Loop HeatX		ECS	47769	3.17	Yes	167069
HX-03	Cooling Loop HeatX		ECS	47769	3.17	Yes	199197
GTN-01	Waste Gas Tank		ECS	357516	4	No	1430064
P-05	Pump to Holding Tank		ECS	9085	3.3	Yes	39066
HX-07	Holding Tank HeatX		Aspen	16940	3.17	Yes	70640
TN-03	Holding Tank		ECS	102427	4.16	No	426096
P-06	Pump to Centrifuge		ECS	9085	3.3	Yes	39066
CNT-01	Centrifuge	Alfa Laval	Vendor	400000	2.03	Yes	1212000
SD-01	Spray Dryer	GEA	Consultant	500000	2	No	1000000
TN-04	Cell Waste Tank		ECS	443311	8	No	3546488
P-07	Pump to Filtration		ECS	9092	3.3	Yes	39096
TFF-01	45 kDa Ultrafiltration	MilliporeSigma	Consultant	2117180	2.32	No	4911858
P-08	Pump to Cell Waste		ECS	9114	3.3	Yes	39190
P-09	Pump to Filtration 2		ECS	9228	3.3	Yes	39680
TFF-02	8 kDa Ultrafiltration		Consultant	2615840	2.32	no	6068749
P-10	Pump to Sterilization		ECS	9209	3.3	Yes	39599
HX-04	Economizer		Aspen	12000	3.17	No	38040
P-12	Pump to Sterilizer		ECS	9125	3.3	Yes	39238
HX-05	Sterilizer		Aspen	80485	3.17	No	255137

HX-06	Cooler		Aspen	40257	3.17	No	127615
P-11	Pump to Evaporator		ECS	9212	3.3	Yes	39612
PB-01	Bin activator		Consultant	35000	4.16	No	145600
	Product Bin		Consultant	19240	4.16	No	29723
HX-08	Spray Dryer HeatX		Aspen	10514	3.17	No	33329
	CIP System	Sani-Matic	Consultant	80000	2	No	160000
EVP-01	Multiple Effect Evaporator		Consultant	2800000	2	No	5600000
TN-05	Purge Waste Storage		ECS	364325	8	No	2914600

ECS* is the Equipment Costing Spreadsheet

Each equipment cost was either calculated using the Equipment Costing Sheet provided by Bruce Vrana and Warren Seider or with direct quotes from various manufacturers with the exception of all heat exchangers but HX-01. Heat exchangers were designed and priced in Aspen. The equations used to tabulate the costing spreadsheet can be found in Product and Process Design Principles Fourth Edition. The total equipment cost is **$38,159,824**.

18. Fixed Capital Investment Summary

The Profitability Analysis 4.0 spreadsheet was used to develop all financials models involving the construction and operation of the plant. The capital summary factors in all capital costs the plant incurs at the outset including equipment purchase costs, purchase costs, and initial construction. Financial models were estimated for ten years of plant operation.

18.1 Total Permanent Investment

The total bare module cost including spares, storage, catalysts, computers, etc. was $38,159,824. To calculate the total depreciable capital, the default values for calculating the cost of site preparation and cost of service facilities were used from the profitability analysis spreadsheet, namely 5% of the total bare module cost. No costs were allocated for utility plants as there are existing chemical plants in the area and so utilities can be used from these plants. The total direct permanent investment was then calculated to be $41,975,807. From here, the cost of contingencies, which were estimated as 18% of the direct permanent investment, was added to give the total depreciable capital. This was $49,531,453. The cost of land and plant startup were estimated using the default values given in the equipment costing spreadsheet. Royalties were neglected. This resulted in the total permanent investment coming to $55,475,227. Finally, the site factor for the American Midwest was 1.15, giving the corrected total permanent investment as $63,796,511. A summary of the calculations done to yield the total permanent investment are given in table 18.1 and the investment summary is given in table 18.2.

Table 18.1: Calculation Parameters for Total Permanent Investment

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**

Table 18.2: Investment Summary

Investment Summary

Installed Equipment Costs:				
Total Direct Materials and Labor Costs	$	38,159,824		
Miscellaneous Installation Costs	$	-		
Material and Labor G&A Overhead and Contractor Fees	$	-		
Contractor Engineering Costs	$	-		
Indirect Costs	$	-		
Total:			**$**	**38,159,824**
Direct Permanent Investment				
Cost of Site Preparations:	$	1,907,991		
Cost of Service Facilities:	$	1,907,991		
Allocated Costs for utility plants and related facilities:	$	-		
Direct Permanent Investment			**$**	**41,975,806**
Total Depreciable Capital				
Cost of Contingencies & Contractor Fees	$	7,555,645		
Total Depreciable Capital			**$**	**49,531,452**
Total Permanent Investment				
Cost of Land:	$	990,629		
Cost of Royalties:	$	-		
Cost of Plant Start-Up:	$	4,953,145		
Total Permanent Investment - Unadjusted			$	55,475,226
Site Factor				1.15
Total Permanent Investment			**$**	**63,796,510**

19. Operating Cost-Cost of Manufacture

19.1 Raw Materials

The raw materials used in this process are given in table 19.1. They consist of glucose as the nutritional source, ammonia as the nitrogen source and a variety of salts for the media. Prices were sourced from Thermo-Fisher. Total amount of each of the raw materials was calculated based on the annual hours of runtime for each feed.

Table 19.1 Raw material usage and cost for process.

Raw Material:	**Unit:**	**Required Ratio:**		**Cost of Raw Material:**		**Annual Cost**	**Cost per Kg of Product**
Glucose	kg glucose	4.602	kg glucose per kg of Extremozyme	$0.88	per kg glucose	$25,768,281.17	$4.06
Sodium Phosphate	kg Na Phosphate	0.051	kg Na Phosphate per kg of Extremozyme	$1.00	per kg Na Phosphate	$324,739.49	$0.05
Sulfur	kg sulfur	0.024	kg sulfur per kg of Extremozyme	$0.05	per kg sulfur	$7,417.33	$0.00
Sodium Chloride	kg NaCl	3.642	kg NaCl per kg of Extremozyme	$0.07	per kg NaCl	$1,550,866.03	$0.24
Potassium Hydroxide	kg KOH	0.164	kg KOH per kg of Extremozyme	$0.90	per kg KOH	$936,586.64	$0.15
Magnesium Chloride	kg MgCl	0.008	kg MgCl per kg of Extremozyme	$0.30	per kg MgCl	$15,261.71	$0.00
Boric Acid	kg boric acid	0.06	kg boric acid per kg of Extremozyme	$0.70	per kg boric acid	$266,625.25	$0.04
Ammonia	kg ammonia	0.14	kg ammonia per kg of Extremozyme	$0.47	per kg ammonia	$420,822.69	$0.07

19.2 Utilities

The utilities used in this process were medium pressure (150 psig) steam, cooling water, chilled water and electricity. The breakdown per equipment is given in section 14.2. The costs of the utilities were calculated using values given in *Product and Process Design Principles.* The annual breakdown and cost per kg of product is given in table 19.2.

Table 19.2 Utilities usage and costs of process.

Utility:	Unit:	Required Ratio		Utility Cost		Annual Cost	Cost per kg of Product
Electricity	kWh	2.08	kWh per kg of Extremozyme	$0.07	per kWh	$924,729	$0.15
Medium Pressure Steam	kg	197.65	kg per kg of Extremozyme	$0.02	per kg	$19,186,810	$3.02
Process Water	m3	0.054	m3 per kg of Extremozyme	$0.27	per m3	$92,572	$0.01
Cooling Water	m3	0.023	m3 per kg of Extremozyme	$0.03	per m3	$4,051	$0.001
Chilled Water	kg	2.56E+01	kg per kg of Extremozyme	$0.00	per kg	$81,134	$0.01

19.3 Variable Costs and Working Capital

The variable costs and working capital of this process were calculated according to the guidelines on the equipment costing spreadsheet with default values being used for all items except for research. It was decided to increase the direct research expense to 10% of sales due to the complicated nature of the extremozyme and the challenges with growing the organism at scale in a reactor. For the other variable costs, namely selling/transfer expenses, allocated research, administrative expenses, and management incentive compensation, the default values were used. The details are given in table 19.3.2. The working capital information is given in table 19.3.3, with values calculated using the default values given in the equipment costing spreadsheet.

Table 19.3.1 Variable Costs and Working Capital Input

Other Variable Costs		
General Expenses		
Selling / Transfer Expenses:	3.00%	of Sales
Direct Research:	10.00%	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
Extremozyme Inventory	⇨	4	Days
Raw Materials	⇨	2	Days

Table 19.3.2 Summary of Variable Costs

Variable Cost Summary			
Variable Costs at 100% Capacity:			
General Expenses			
Selling / Transfer Expenses:		$	4,488,607
Direct Research:		$	14,962,025
Allocated Research:		$	748,101
Administrative Expense:		$	2,992,405
Management Incentive Compensation:		$	1,870,253
Total General Expenses		$	25,061,391
Raw Materials	$4.614700 per kg of Extremozyme		$20,765,490
Byproducts	$0.000000 per kg of Extremozyme		$0
Utilities	$3.197626 per kg of Extremozyme		$14,388,860
Total Variable Costs		**$**	**60,215,741**

Table 19.3.3 Summary of Working Capital

Working Capital							
			2021		2022		2023
	Accounts Receivable	$	5,533,899	$	2,766,950	$	2,766,950
	Cash Reserves	$	2,841,503	$	1,420,751	$	1,420,751
	Accounts Payable	$	(1,300,229)	$	(650,115)	$	(650,115)
	Extremozyme Inventory	$	737,853	$	368,927	$	368,927
	Raw Materials	$	51,203	$	25,601	$	25,601
	Total	**$**	**7,864,229**	**$**	**3,932,114**	**$**	**3,932,114**
	Present Value at 15%	*$*	*6,838,460*	*$*	*2,973,243*	*$*	*2,585,429*
Total Capital Investment				**$**	**76,193,642**		

19.4 Fixed Costs

Fixed costs were calculated according to the default values on the equipment costing spreadsheet. It was decided that 45 operators, 9 per shift, were needed for operation. The continuous section of the process was divided into six sections: 1) sterilization loop,, 2) Gas input, 3) bioreactor system, 4) central holding tank and centrifuge, 5) ultrafiltration, 6) spray dryer. One operator is needed for each section. The seed train was divided into 3 sections, 1) gas generation, 2) 2L reactor triplicate and 3) the 2000L reactor. One operator is needed for each section. Thus 9 total operators per shift were required. All other aspects of the fixed costs portion of the spreadsheet were left to the default values.

Table 19.4.1 Fixed Costs Input

Fixed Costs				
Operations				
	Operators per Shift:	45	(assuming	5 shifts)
	Direct Wages and Benefits:	$40	/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:	$60,000.00	per year, for each Operator per Shift	
	Control Laboratory:	$65,000.00	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		

Table 19.4.2 Fixed Costs Summary

Operations		
	Direct Wages and Benefits	$18,720,000
	Direct Salaries and Benefits	$2,808,000
	Operating Supplies and Services	$1,123,200
	Technical Assistance to Manufacturing	$13,500,000
	Control Laboratory	$14,625,000
	Total Operations	**$50,776,200**
Maintenance		
	Wages and Benefits	$2,228,915
	Salaries and Benefits	$557,229
	Materials and Services	$2,228,915
	Maintenance Overhead	$111,446
	Total Maintenance	**$5,126,505**
Operating Overhead		
	General Plant Overhead:	$1,726,304
	Mechanical Department Services:	$583,539
	Employee Relations Department:	$1,434,535
	Business Services:	$1,799,247
	Total Operating Overhead	**$5,543,625**
Property Taxes and Insurance		
	Property Taxes and Insurance:	$990,629
Other Annual Expenses		
	Rental Fees (Office and Laboratory Space):	$ -
	Licensing Fees:	$ -
	Miscellaneous:	$ -
	Total Other Annual Expenses	**$ -**
Total Fixed Costs		**$62,436,959**
Fixed Cost /kg of Product		**$9.84**

20. Other Important Considerations

20.1 Health and Safety

The biggest safety risk in this process is the high pH of the process stream. Much of the process involves streams at a pH between 9 and 10 and so precautions will need to be taken. First, all equipment is built using 316L stainless steel which is stronger than carbon steel and should be able to handle the higher pHs. Furthermore, all onsite personnel will be required to wear the necessary personal protective equipment. Otherwise for all equipment standard safety protocols will be followed. There are not any particularly dangerous flammable chemicals in use for this process so standard protocols should suffice.

21.2 Environmental Considerations

21.2.1 Carbon Dioxide Emissions

Since 2009, the United States Environmental Agency has instated the Greenhouse Gas Reporting Program that requires major greenhouse gas producers to report carbon dioxide production from industrial processes. The cutoff for reporting requirement is 25,000 MT/yr of carbon dioxide. The amount of carbon dioxide produced during the bioreactor operation in this process is approximately 20,500 MT/yr, thus excluding this process from needing to report carbon dioxide emissions to the EPA. That being said, steps will be taken to implement measures to reduce carbon dioxide emission to the atmosphere in order to ensure a more sustainable process overall.

21.2.2 Biological Contaminants

Steps will be taken in order to reduce the possibility of live cells leaving the process. All waste gas released from the bioreactors will be sent to the steam generator for the process where the oxygen will be combusted. During combustion, the temperature will be high enough that it will kill any live cells that are present in the waste gas. Live cells that are removed during centrifugation and the first filtration step will be treated as outlined in Section 21.3.

21.3 Municipal Waste Treatment

Due to the biological components present and high pH of the waste streams produced by this process, proper adherence to waste treatment guidelines and cooperation with municipal waste treatment centers will be paramount. Due to the structure of the campaign schedule, the downstream process will not be operating for 36 hours of every 8 day campaign. During this downtime, process waste produced by the downstream process will be pretreated and sent to municipal waste treatment. Additionally, while the continuous process is running, CIP waste water will be collected, pretreated, and sent to waste treatment as well. With all waste, first, the high pH will be neutralized using an acidic buffer. Following neutralization, the cell waste will be sterilized in order to kill all living cells. After these pretreatment steps, the waste streams will be sent to a municipal waste treatment plant.

Dayton, Ohio has one primary municipal waste treatment plant known simply as the City of Dayton Wastewater Treatment Plant (WWTP) (Treatment Process, n.d.). The WWTP has a regulation where all Significant Industrial Users need to apply for a permit to discharge wastewater to the WWTP, and the process plant described in this report would qualify as an SIU as it produces more than 25,000 gallons of waste per day. Furthermore, there is an extra-strength surcharge that the City of Dayton charges to any business producing wastewater with a significant Biological Oxygen Demand or Total Suspended Solids concentration. This process will qualify for the extra-strength surcharge due to the high cell waste concentration. These regulations and surcharges will be strictly followed and paid as to maintain access to proper waste water treatment.

21. Profitability Analysis

Profitability analysis was conducted using the Profitability Analysis spreadsheet. Profitability in response to changes in a number of variables was studied, namely: 1) effects of product price 2) total permanent investment, 3) fixed costs.

21.1 Base Case

First, profitability under the conditions listed in the above sections was studied. In the problem statement, product pricing was set to a 15% premium over the leading incumbent. However, the market for extremophilic enzymes is very limited and there were no clear market leaders. Furthermore, many enzyme manufacturers did not market their enzymes to be sold in bulk and so the prices quoted by many protease manufacturers could not be used as reasonable comparison. Therefore, the base case price was set such that an internal rate of return (IRR) of ~15% was achieved. Under these conditions, the product price was set as $36.00/kg. This resulted in an IRR of 15.21% and the net present value (NPV) of the project was given as $880,900. The return on investment in this case was 17.36% and the details are shown in table 21.1.1. Additionally the cash flows for this project are shown in table 21.1.2.

Table 21.1.1 Return on Investment for Base Case in Third Production Year

ROI Analysis (Third Production Year)

Annual Sales	145,795,367
Annual Costs	(118,496,598)
Depreciation	(5,103,721)
Income Tax	(8,212,168)
Net Earnings	13,982,880
Total Capital Investment	80,562,400
ROI	17.36%

Table 21.1.2 Cash flows for this process.

Cash Flow Summary

Year	Percentage of Design Capacity	Product Unit Price	Sales	Capital Costs	Working Capital	Var Costs	Fixed Costs	Depreciation	Depletion Allowance	Taxible Income	Taxes	Net Earnings	Cash Flow	Cumulative Net Present Value at 15%
2020	0%		-	-	-	-	-	-	-	-	-	-	-	-
2021	0%		-	(63,796,500)	(8,382,900)	-	-	-	-	-	-	-	(72,179,500)	(62,764,700)
2022	45%	$36.00	72,897,700	-	(4,191,500)	(28,029,800)	(62,437,000)	(9,906,300)	-	(27,475,400)	10,165,900	(17,309,500)	(11,594,700)	(71,532,000)
2023	68%	$36.00	109,346,500	-	(4,191,500)	(42,044,700)	(62,437,000)	(15,850,100)	-	(10,985,200)	4,064,500	(6,920,700)	4,737,900	(68,416,700)
2024	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	(9,510,000)	-	17,788,700	(6,581,800)	11,206,900	20,716,900	(56,571,800)
2025	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	(5,706,000)	-	21,592,700	(7,989,300)	13,603,400	19,309,500	(46,971,600)
2026	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	(5,706,000)	-	21,592,700	(7,989,300)	13,603,400	19,309,500	(38,623,500)
2027	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	(2,853,000)	-	24,445,800	(9,044,900)	15,400,800	18,253,800	(31,761,200)
2028	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	-	-	27,298,800	(10,100,500)	17,198,200	17,198,200	(26,139,100)
2029	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	-	-	27,298,800	(10,100,500)	17,198,200	17,198,200	(21,250,300)
2030	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	-	-	27,298,800	(10,100,500)	17,198,200	17,198,200	(16,999,200)
2031	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	-	-	27,298,800	(10,100,500)	17,198,200	17,198,200	(13,302,500)
2032	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	-	-	27,298,800	(10,100,500)	17,198,200	17,198,200	(10,088,100)
2033	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	-	-	27,298,800	(10,100,500)	17,198,200	17,198,200	(7,292,900)
2034	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	-	-	27,298,800	(10,100,500)	17,198,200	17,198,200	(4,862,300)
2035	90%	$36.00	145,795,400	-	-	(56,059,600)	(62,437,000)	-	-	27,298,800	(10,100,500)	17,198,200	17,198,200	(2,748,700)
2036	90%	$36.00	145,795,400	-	16,765,900	(56,059,600)	(62,437,000)	-	-	27,298,800	(10,100,500)	17,198,200	33,964,100	880,900

Though at the base case the project is profitable, it is troubling that the project only yields a positive NPV in 2036. If demand were to drop for the product, this process would become unprofitable and given how far out in the future the process is projected to become profitable, this is cause for concern.

21.2 Sensitivity to Product Price

Tergazyme was found to be a reasonable competitor as they sell detergents containing protease. However, only prices for their detergents could be found and not the prices for the proteases they used. Depending on the bulk size, the price per kg of detergent of their products came to ~$20. This is significantly less than the price of the base case that is being used in this process even after the 15% premium is applied. The sensitivity of the ROI to the price of the extremozyme is shown in figure 21.1.

Figure 21.1: ROI change in response to changes in product price.

From the figure, it can be seen that the ROI is extremely sensitive to relatively small changes in price. The price cannot get reasonably close to the detergent price of tergazyme without yielding a highly negative return on investment. If the price of tergazyme does serve to be a metric of the price that the product of this process can be set, then demand will fall and the project will become unprofitable.

However, if further market research is conducted and it turns out that demand for the product is high within this price range, then one may consider setting the price of the product to $43.00/kg. This would make the process profitable much quicker (i.e. by 2027) and would yield an NPV of $66,617,100.

21.3 Sensitivity to Total Permanent Investment

The sensitivity to total permanent investment is shown in figure 21.2. From the figure it can be seen that the ROI is not very sensitive to the total permanent investment. To reach the ROIs that could be done by raising the product price through changes to total permanent investment alone would require significant changes in the process design, to the point of infeasibility. For example one could consider eliminating all spares but this would only lower the total equipment costs by $1,233,820, which is not nearly enough to achieve an ROI greater than 30%. Nor would this be a prudent decision. Even if other aspects like cost of site and land were reduced to unrealistic amounts, this would not be enough. Clearly one must look elsewhere to cut costs.

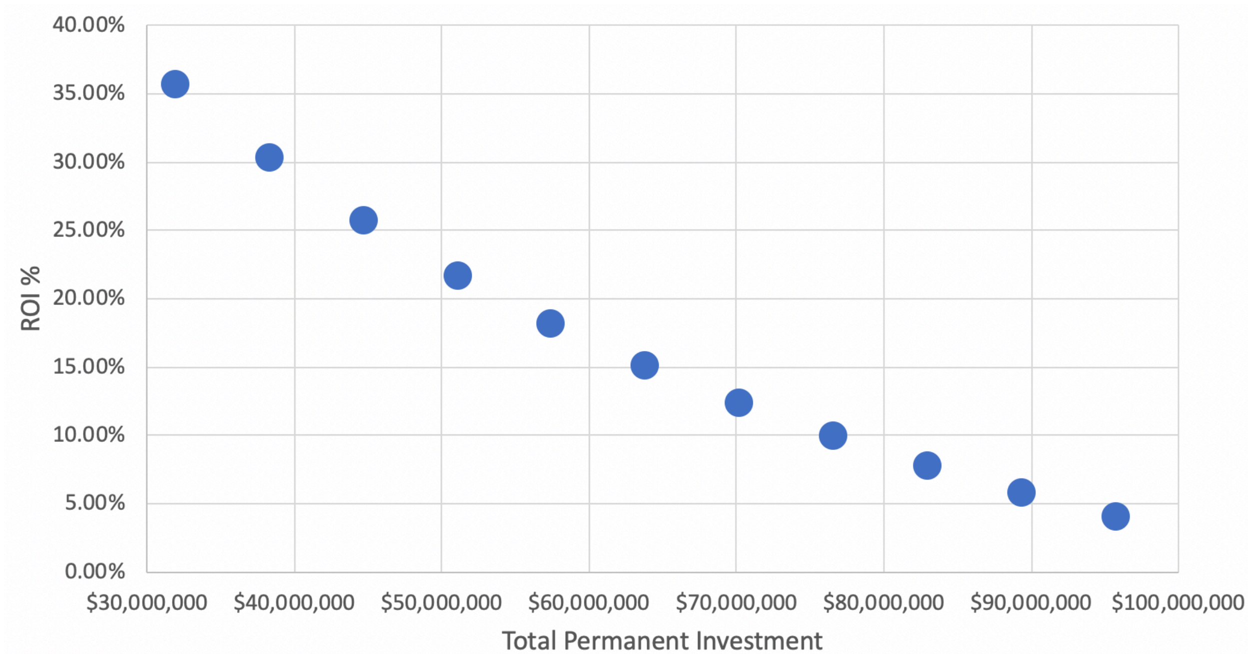

Figure 21.2: Sensitivity of ROI to Total Permanent Investment

21.4 Sensitivity to Total Fixed Costs

A final aspect that was studied was the sensitivity of the ROI to changes in fixed costs. This is shown in figure 21.3. From the figure, clearly minor changes in fixed costs do not change the ROI very much. One could accomplish lowering the fixed costs by salary cuts for example. However cutting salaries to $30/hr would only bring the ROI by ~3% and would still leave a positive NPV far out into the future (i.e. 2030). Given the drawbacks of hurting the workers, this is a very poor method of increasing the ROI. Based on these three cases, the most realistic way of increasing the profitability of the project is through raising the product price. However, only further market research can illuminate whether or not this would salvage the project.

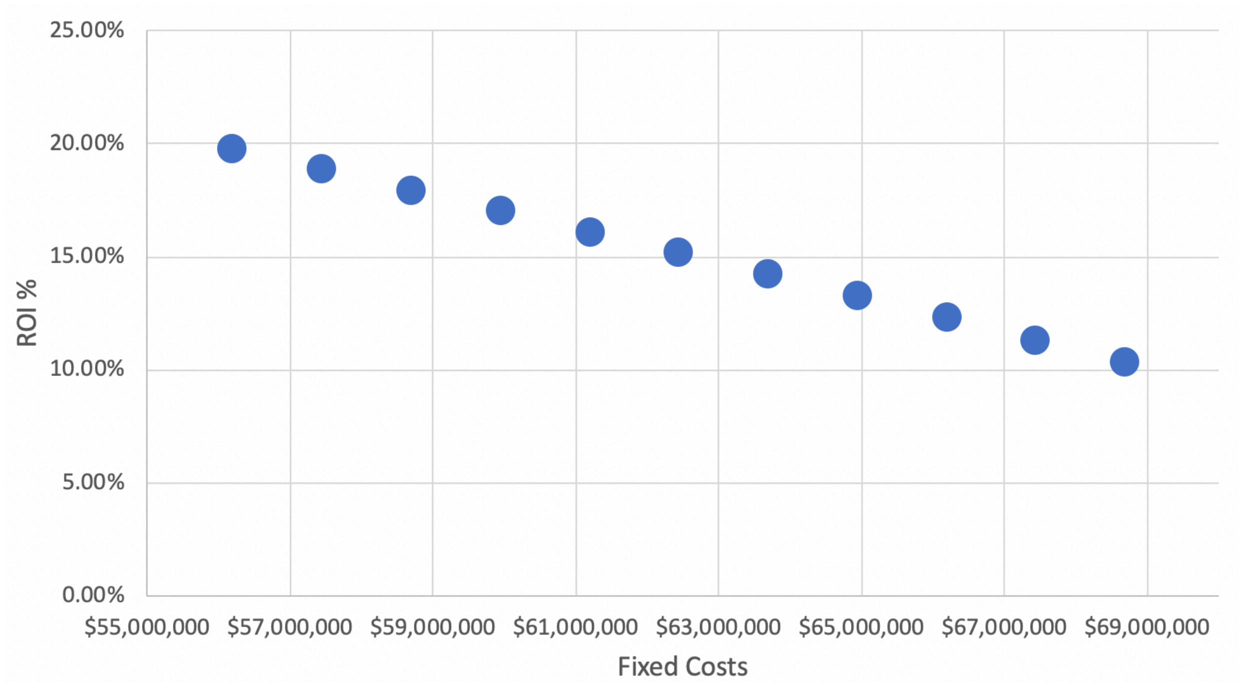

Figure 21.3: ROI Sensitivity to Fixed Costs.

22. Conclusions and Recommendations

Based on the economic analysis of this novel process for the production of a bio-detergent grade protease that is suitable for high temperatures and pH's from *N. pharaonis*, it is difficult to quantify the profitability of this process. In order to reach a generally accepted IRR for a profitable process of 15%, the bulk price for the enzyme must be $36.00/kg. Based on available literature and supplier information, it is not possible to know with a high enough confidence level whether this is a reasonable price point. Extrapolations can be made based on price points for commercially available detergents containing protease, but without knowing the price that detergent corporations are willing to pay for bulk dried enzyme, it is unwise to go forward with the production of this processing plant. As such, extensive market research outside of the scope of this report should be conducted to find bulk price points from leading enzyme suppliers such as Novozyme and Genencor. If it is found that the bulk price is similar to the price extrapolated from the product Tergazyme's selling price, the process should not be pursued. However, if the price is found to be anywhere upwards of $36.00/kg, the process has the opportunity to be extremely profitably, particularly due to the high sensitivity in ROI to price change.

If the process is found to be economically feasible and the plant is profitable far into the future, it may be worthwhile to look into the possibility of producing detergents formulations in house, instead of selling enzymes to larger detergent companies. While this change would require significant costs for both additional formulation steps as well as research and development, companies such as Novozyme have begun selling their enzymes directly to consumers in detergent formulations. Another possibility is to look into processing cell waste to be sold as animal feedstock. This would require further purification steps for the cell waste extracted during centrifugation, but may provide a boost to profitability depending on the price point set for the enzyme.

23. Acknowledgements

We would like to thank Dr. Jeffrey D. Cohen, Dr. Amish Patel, and Dr. Bruce Vrana for all of their support and through the course of the semester. We would also like to thank the other industrial consultants that met with us week to week and offered their expertise throughout this process. Special acknowledgement to Dr. Cohen for authoring this project and offering prompt and consistent support throughout the semester.

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Appendix A: Calculations

A.1 Energy Balances

Cooling requirements for the 20,000L continuous reactor are given below as an example of the energy balance calculations done throughout.

Source	Gas In	Media In	In from Heatx	Gas Out	Media Out	Out to Heatx	Generation
Temperature (C)	66	32	32	60	60	60	--
Flow Rate (kg/hr)	188.6	10894.1	7820.25754 3	-1522.2	-8546.6	- 7820.25754 3	--
Substance	Gas Mixture, assume Air	Mixture, assume Water	Mixture, Assume Water	Gas Mixture, assume CO2	Mixture, Assume Water	Mixture, Assume Water	--
Enthalpy Rxn (kJ/hr)	--	--	--	--	--	--	14400
Heat Capacity (kJ/kg K)	1.006	4.186	4.186	0.879	4.186	4.186	--
Energy (kJ/hr)	7777.08 6355	319218.7 861	229149.186 5	- 46831.2708 5	- 1252155.05 7	- 1145745.93 3	1843200
Balance							0.00000000 0

Stream Energies are given by

$$Q = \dot{m} \cdot C_p \cdot (T_{stream} - 25^\circ\ )$$

Outlet streams have negative flow rates.

Generation Term is given by

$$Q = (Oxygen\ Uptake\ Rate\ \frac{mol\ O_2}{L\ hr}) \times (Enthalpy\ of\ Fermentation\ \frac{kJ}{kg\ O_2\ consumed})$$

$$\times (0.032\ \frac{kg}{mol\ O_2}) \times (Working\ Volume\ L)$$

A.2 Process Output Calculations

Many of the parameters of the project (e.g. reactor sizes and numbers) were calculated based on the process output estimates. These calculations are outlined below.

Days Uptime: $0.95 \times 365 = 346.75\ days$

Maintenance Days per Campaign: 1 day

Number of Campaigns per Year: $\frac{Days\ Uptime}{Campaign\ Length + Maintenance\ Days} = 38$

Maximum Growth Rate: $\frac{ln2}{Doubling\ Time} = \frac{ln2}{1.5} = 0.462\ hr^{-1}$

Volume per Production Reactor: 16185 L (adjusted using trial and error)

Initial Concentration of Reactor: $Max\ Growth\ Rate \times \frac{V_{N-1}}{V_N} = 3\ g/L$

Time for Production Reactor (Reactor N) to reach Peak Cell Density:

$$ln(^{Peak\ Cell\ Density}/_{Initial\ Concentration\ of\ Reactor})\ /\ ln(2)\ \times Doubling\ Time =$$

$$4.98\ hr$$

Production Time per Campaign: $Campaign\ Period\ (hrs) \times 24\ -$

$(Time\ for\ N\ to\ reach\ Peak\ Cell\ Density\ +\ Time\ for\ N -$

$1\ to\ Reach\ peak\ cell\ density\ +\ Time\ for\ Reactor\ 1\ to\ Reach\ Peak\ Cell\ Density)\ =$

$$8 \times 24\ -\ (4.98 + 14.95 + 16.00)\ =\ 156\ hrs$$

Production time Per Reactor per Year: $Production\ Time\ per\ Campaign\ \times$

$Number\ of\ Campaigns\ per\ year\ =\ 5931\ hrs$

Flow Rate out of Production Reactor: $Max\ Growth\ Rate \times Volume\ per\ Reactor = 7480\ L/hr$

Amount of Product per Reactor Per Campaign: $(Peak\ Product\ Titer \times Flow\ Rate \times Product\ Time\ per\ Campaign) + (Peak\ Product\ Titer \times Volume\ per\ Reactor) = 65092\ kg$

Amount of product per year: $Amount\ of\ Product\ per\ reactor\ per\ campaign\ \times Number\ of\ campaigns\ per\ reactor\ per\ year\ \times Number\ of\ reactors\ =\ 4947028.7\ kg/yr$

Fraction of Product Lost in Separation (from Material Balances): 0.276

Purity of Final Product: 0.8

Annual Enzyme Production Rate considering Loss: $Production\ Target\ /\ (1 - Fraction\ Lost)$

Volumetric Flow Rate Required from Reactors: $Annual\ Enzyme\ Production\ Rate\ /\ Product\ Titer\ =\ 112986333.8\ L/yr$

Oxygen Required: $Peak\ Oxgyen\ Uptake\ Right\ \times\ Volume\ per\ Reactor \times Oxygen\ Molar\ Mass = 129.5\ kg/hr$

Fraction of Protein that is Extracellular (from references): 0.062

Total Biomass Excluding Protein: $Peak\ Cell\ Density\ /\ (1 - Fraction\ Extracellular \times Fraction\ of\ Biomass\ that\ is\ Protein) = 31.47\ g/L$

Extracellular Proteins Excluding Product: $Total\ Biomass\ Excluding\ Protein - Peak\ Cell\ Density = 1.47\ g/L$

A.3 Material Balance Calculations

Multiple calculations would be made throughout the process of constructing the seed train/batch/continuous material balances, which are collected below.

Elemental Composition of Biomass

In order to calculate the amount of media components required for cell growth, the fraction of biomass that is made up of each of a selection of key elements was collected. The fraction of cell mass and protease mass that is each element is shown below.

	Cell Mass	**Protease**
Fraction C	0.436	0.349
Fraction N	0.073	0.170
Fraction O	0.073	0.384
Fraction P	0.019	0.000
Fraction S	0.007	0.015
Fraction Na	0.024	0.000
Fraction K	0.169	0.000
Fraction Mg	0.005	0.000
Fraction Cl	0.194	0.000

Required Glucose

$$Fraction\ of\ Uptaken\ C\ Incorporated\ in\ Biomass = 0.35$$

Required Uptake C in Bioreactor

$$= \frac{Cells\ Out * Fraction\ C\ in\ Cell + Protein\ Out * Fraction\ C\ in\ Protein}{Fraction\ Uptaken\ C\ Incorporated\ in\ Biomass}$$

$$= 700.97\ kg/hr$$

$$Moles\ Glucose\ Required = \frac{Required\ Uptake\ C}{MW\ of\ C} * \frac{1\ mol\ Glucose}{6\ mol\ C}$$

$$Carbon\ in\ Glucose = Mol\ Glucose * \frac{6\ mol\ C}{mol\ Glucose} * MW\ C$$

$$Oxygen\ in\ Glucose = Mol\ Glucose * \frac{6\ mol\ O}{mol\ Glucose} * MW\ O$$

Estimated Carbon in Glucose Converted to CO2

$$= (1 - Fraction\ of\ Uptaken\ C\ Incorporated) * Carbon\ in\ Glucose$$

$$Estimated\ Mol\ CO2 = \frac{Estimated\ C\ in\ Glucose\ Converted\ to\ CO2}{MW\ C}$$

$$Estimated\ Mass\ CO2 = Mol\ CO2 * MW\ CO2$$

$$Mol\ O\ from\ Glucose = Oxygen\ in\ Glucose * MW\ O$$

$$Mol\ O\ in\ CO2 = 2 * Estimated\ Mol\ CO2$$

$$Mass\ O\ in\ CO2 = Mol\ O * MW\ O$$

$$Estimated\ Mass\ of\ O\ Out = Mass\ O\ in\ CO2 + Mass\ in\ O2\ Gass\ Out$$

$$Mass\ of\ O\ In = Mass\ O\ from\ O2\ Gas\ In + Oxygen\ in\ Glucose$$

$$Differential = Mass\ of\ O\ In - Estimated\ Mass\ of\ O\ Out$$

$$Mass\ CO2\ Actual = \frac{Mass\ O\ in\ CO2 - Differential}{MW\ of\ O} * \frac{2\ mol\ O}{mol\ CO2} * MW\ CO2 = 1463.2\ \text{kg/hr}$$

$$Mass\ Carbon\ Waste$$

$$= Estimated\ Carbon\ in\ Glucose\ Converted\ to\ CO2 - \frac{Mass\ CO2\ Actual}{MW\ CO2}$$

$$* MW\ C = 56.59\ kg/hr$$

Drying Air Required for Spray Dryer

$$Mass\ Water\ Per\ Mass\ Air\ at\ 50\%\ Rel. Humidity\ @\ 28.9C = 0.0128\ (from\ tables)$$

$$\frac{Max\ Mass\ Water}{Volume\ of\ Exhaust\ Air} = 0.530 \frac{kg}{m^3} (from\ tables)$$

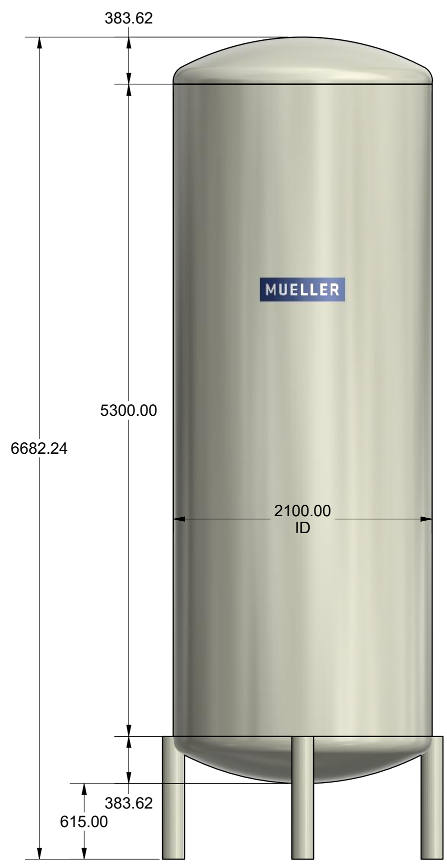
$$Volume\ Air\ Required = \frac{Mass\ Water\ Evaporated}{\frac{Max\ Mass\ Water}{Volume\ of\ Exhaust\ Air}} = 627.7\ m^3$$

$$Density\ of\ 100\%\ Saturated\ Air\ at\ 80C\ and\ 1.47\ bar = 1.273 \frac{kg}{m^3} (from\ table)$$

$$Air\ Required = 1.25 * Volume\ Air\ Required * Desnity = 1000\ kg/hr$$

Appendix B: Equipment and Resource Vendor Sheets

MUELLER



TANK VOLUME CALCULATOR RESULTS

Tank Inside Diameter = 2100 mm

Bottom Head Type = ASME D&F
Material Thickness = 2.67 mm
Inside Knuckle Radius = 127 mm
Straight Flange Length = 25.4 mm
Inside Dish Radius = 2105.03 mm

Shell Material Thickness = 2.67 mm
Shell Short Side Height = 5300 mm

Top Head Type = ASME D&F
Material Thickness = 2.67 mm
Inside Knuckle Radius = 127 mm
Straight Flange Length = 25.4 mm
Inside Dish Radius = 2105.03 mm

Bottom Head Volume = 839.03 liters
Shell Volume = 18357.11 liters
Top Head Volume = 839.03 liters
Total Volume = 20035.18 liters

Your next tank project is one step closer to being done! Contact us at contact@paulmueller.com and see how we can make this new equipment a reality on your timeline and budget.

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MUELLER
BIOPHARM SYSTEMS

BUDGETARY VESSEL SPECIFICATIONS

Customer:	**U Penn Budget**	**Proposal Number**	Budget
Vessel Volume (Liters):	20,000	**Material:**	316L
Diameter (inches):	86	**Height (inches):**	263
Finish:	#7 (15 Ra) Internal	#4 (35 Ra) External	
ASME Stamped:	Yes	**Design Pressure:**	45 Psig and Full Vacuum
		Design Temperature:	300 °F
Corrosion Allowance:	0"	**Specific Gravity;**	1.0
Dimpled Full Shell:	Yes	**Insulation/Sheathing:**	Yes
Dimpled Bottom Head:	yes	**Passivation:**	Yes
Electropolished:	no	**Leg Supports:**	Yes
H.T. Protective Paint	Yes	***Completed By:***	Bill Hons
Outer Top Head:	No	***Date:***	3/12/2020
Agitator:	No		
Documentation:	Yes		

VESSEL BUDGETARY PRICE: ***$285,000***

Nozzle Schedule and Misc. Items

Item	Qty	Description
1	1	24" MC-56 5-Lug Manway with Re-Pad
2	1	Spring Assist (#9829805)
3	4	2" NA Connect in side shell, sloped at 15 degree angle
4	2	4" TC Tank Connection with 2" TC No-Foam
5	3	4" x 2" Sprayball Assembly
6	1	6" L.J. Star Sanitary Flush Mounted MetaClamp Sight Glass
7	1	Thermowell "Burns" x 10" standard length
8	2	Lifting Lugs
9	1	Grounding Lugs
10	1	Rosemount Level spud x 2" ext.
11	6	2", 150# Blind Flange, Gasket and Bolting
12	1	8" - 150 lb Pad Flange for Agitator mount
13	1	Mixers (Top Entering or Magnetic)
14	1	Outlet Valves
15	1	Light Assemblies
16	1	Sparger Tube with Supports
17	3	Baffles
18		
19		
20		

PAUL MUELLER COMPANY
1600 West Phelps St. - PO Box 828 Springfield, MO 65801-0828
Phone: 1-800-MUELLER

ATCC

Product Sheet

Natronomonas pharaonis
(ATCC® 35678™)

Please read this FIRST

Biosafety Level
1

Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Natronomonas pharaonis* (ATCC® 35678™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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PowerXP-AA

Anhydrous Ammonia Vaporizer Series - Models XP19AA through XP120AA

- *Safely and efficiently vaporizes anhydrous ammonia for commercial and industrial applications.*
- *Two safety devices to ensure dry, superheated vapor.*
- *100% Turndown - can operate in dry condition.*
- *Available with feed-forward or feed-back design.*
- *Requires less than one minute to reach operating temperature from a cold start.*
- *Designed to meet the precise control requirements of the industrial process industry.*
- *The standard for applications such as thermal DE-NO_x systems.*
- *Class I, Division 1, Group D.*
- *Flanged connections - standard.*

Revised 04.16.01

Factory Mutual System Approved · CSA · CE · ECLIPSE

Specifications

	MODEL	XP19AA	XP38AA	XP75AA	XP120AA
Vaporization Capacity *	Lb/hr kg/hr	19 9	38 17	75 34	120 54
Heat Exchanger Surface Area	Ft^2 m^2	2.9 .27	2.9 .27	2.9 .27	4.3 .40
Pressure Vessel Design Pressure	Psig kg/cm^2	250 17.6	250 17.6	250 17.6	250 17.6
Pressure Vessel Test Pressure	Psig kg/cm^2	375 26.3	375 26.3	375 26.3	375 26.3
Pressure Vessel Liquid Capacity	U.S. Gallons liters	.95 3.61	.95 3.61	.95 3.61	1.6 6.0
Operating Temp. Range	°F °C	160-175 71-79	160-175 71-79	160-175 71-79	160-175 71-79
Shipping Weight	lbs kg	100 45	100 45	100 45	130 59

*Vaporization capacity ratings at 15 psig (1.0 kg/cm^2) and 0°F (-17.8°C) at liquid inlet with full rated voltage. Higher pressures or lower temperatures will decrease the capacity accordingly. Vaporization capacity ratings vary relative to input and rated voltage.

For more information, contact your local ASDI distributor or factory Customer Service (USA) representative.

Electrical

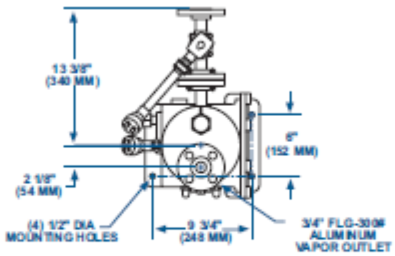
	MODEL	XP19AA		XP38AA		XP75AA		XP120AA	
VOLTAGE	PHASE	KW	AMPS	KW	AMPS	KW	AMPS	KW	AMPS
120	1	3.9	32.5						
208	1	2.9	14.1	5.9	28.1	11.7	56.2		
	3				16.3		32.5	17.8	49.6
220	1	3.3	14.9	6.5	29.7	13.1	59.5		
	3				17.2		34.4	20.0	52.4
240	1	3.9	16.2	7.8	32.4	13.0	54.0		
	3				18.8	15.6	37.5	23.8	57.3
380	3			6.5	9.9	13.1	19.9	20.0	30.3
415	3			7.7	10.8	15.6	21.7	23.8	33.1
440	3					13.1	17.2	20.0	26.3
480	3					15.6	18.8	23.8	28.7

All models are 50/60 Hz.

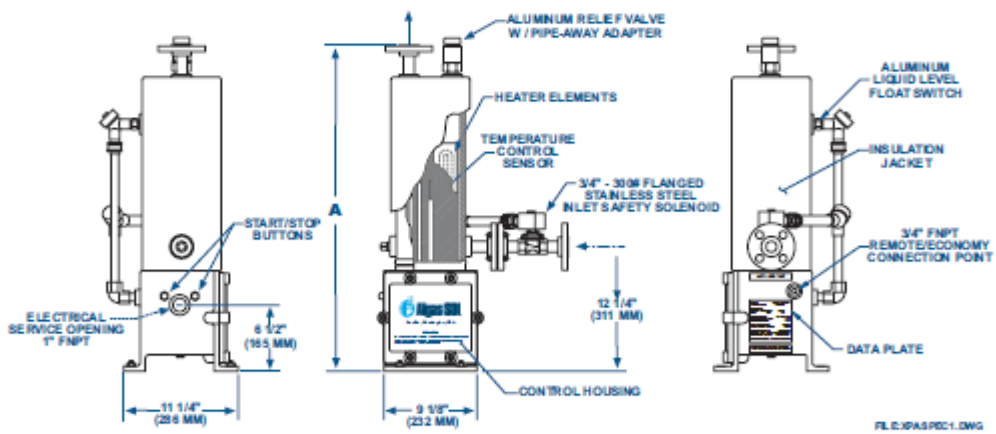
Properties of Ammonia

	Anhydrous Ammonia
Chemical Formula	NH_3
Molecular Weight	17.031
Latent Heat of Vap @ Boiling Point & 1 ATM, BTU/lb	589.3
Ignition Temperature, °F	1562
Flamability Limits (Percent In Air, By Volume)	16-25%
Boiling Point, °F (1 ATM)	-29
Specific Gravity of Gas (Air=1.00)	.588
Specific Gravity of Liquid (Water=1.00)	.618
Lbs. Per Gallon of Liquid at 60°F	5.15
Cubic Ft. of Vapor at 60°F/Gallon of Liquid at 60°F	114.9
Cubic Ft. of Vapor at 60°F/lb. of Liquid at 60°F	22.3

Dimensions



MODEL	XP19AA	XP38AA	XP75AA	XP120AA
A	32-1/4" 820 mm			39" 991 mm



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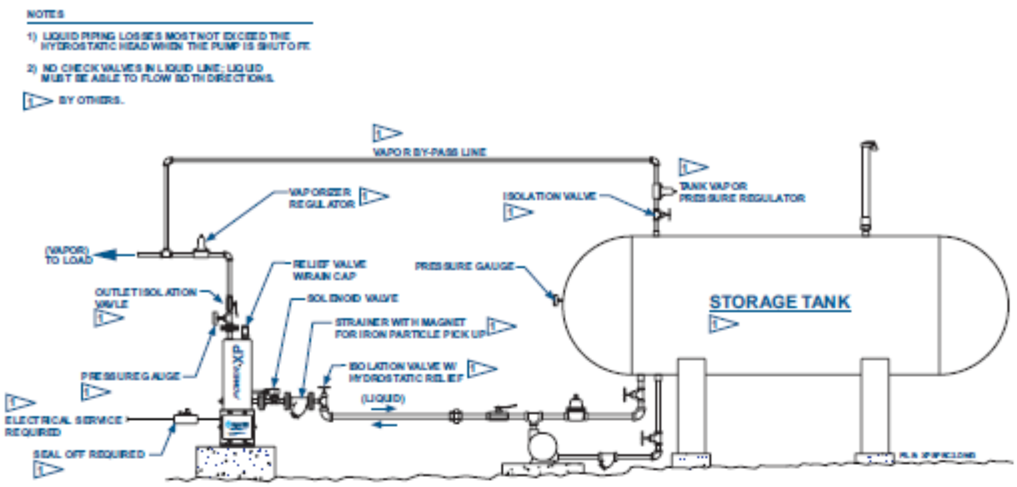
Features

- *Explosion Proof configuration meets Class I, Division 1, Group D as defined by NFPA pamphlet 70.*
- *Pressure vessel designed to ASME specifications.*
- *NEMA 4 enclosure.*
- *ASME and UL stamped aluminum relief valve: 250 psig (17.6 kg/cm²).*
- *Meets ANSI K61.1, CGA pamphlet G-2.1.*
- *Thermal efficiency exceeds 98%.*
- *FM & CSA approved.*
- *100% turndown capability.*
- *"Slide-in" PC board with quick connect fittings.*
- *Easily accessible control housing allows simple maintenance.*
- *Direct monitoring high temperature RTD sensors for operation and over temperature protection.*
- *Float activated high liquid level safety.*
- *Flanged stainless steel inlet safety solenoid valve.*
- *Vinyl coated cross-linked polyethylene insulation jacket.*
- *Pressure balanced NH_3 supply/delivery operation.*
- *Compact footprint size of approximately 1ft² [.09m²].*
- *Worldwide voltages available in 50/60 Hz.*
- *Complete with all operating and safety controls.*
- *Ready to connect to plant facilities.*
- *Factory tested.*

Options

- *Auto Restart.*
- *Economy Operation.*
- *Remote Control Panel.*
- *Spare Parts Kit.*



NOTE: No check valves in liquid line; liquid must must be able to flow in both directions.

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Form: XPAA0401

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Richmond, Virginia 23231
T: 804-236-3800
F: 804-236-3882
www.peconet.com
sales@peconet.com

AIR PRODUCTS

PRISM® VSA Oxygen Generators—T Series

Reliable on-site gas supply

Features/benefits

Low Capital Cost

- Outdoor installation on a concrete plinth
- Highly skidded and modular design

High Reliability

- Fully automatic controls
- Liquid oxygen backup available
- Oxygen analyzer as standard

Low operating costs and flexibility

- Power-efficient oxygen supply with turndown built-in
- Designed for unmanned operation

Proven, flexible solutions

Air Products supplies PRISM Oxygen VSA plants for capacities as large as 300 tons per day of oxygen. Building on its experience with state-of-the-art vacuum swing adsorption (VSA) technology, Air Products has added a compact VSA system, the T-Series, to its portfolio for requirements as large as 25 metric tons per day of oxygen.

Due to the modular design, multiple units can be supplied to customers to provide the required capacity. Together with liquid oxygen for backup, T-Series VSAs provide a reliable supply of oxygen for many applications.

Applications

Air Products' Prism VSAs are available to meet the needs of a variety of industries and applications, including:

- Environmental and wastewater treatment
- Glass
- Ferrous and nonferrous metals production
- Cement and lime production
- Pulp and paper

Customizing a solution for you

At Air Products, we don't have a "one size fits all" approach. Our PRISM T-Series VSA plant design is flexible, so the final system meets your specific requirements with the scope of supply tailored based on your interests.

Process description

The PRISM Oxygen VSA process utilized by these plants is a method of separating air into its two principal components—nitrogen and oxygen—using adsorption technology. The oxygen at 90%–93% purity is provided to the customer as product, and the impure nitrogen combined with any water and carbon dioxide is vented.

PRISM Oxygen VSA systems use a patented high-efficiency molecular sieve to selectively recover oxygen from air. This high-performance sieve significantly reduces the energy required to generate oxygen.

Oxygen VSAs operate in a batch process. During a cycle, the adsorber vessel(s) are alternately pressurized with air to produce oxygen, then evacuated to regenerate the adsorbent and remove nitrogen. Oxygen for customer use needed during the portion of the cycle when oxygen is not being produced is supplied from the product buffer tank, maintaining an uninterrupted, consistent oxygen flow.

Environmental commitment

Additionally, on-site gas generation helps sustainability-minded customers reduce their carbon footprint. It reduces the transportation of delivered products and makes the molecule directly as a gas thereby avoiding the energy needed for liquefaction. And our technology and engineering teams are constantly working to provide even higher levels of energy efficiency inour on-site gas generators.

Worldwide expertise at your doorstep

With local expertise around the world, Air Products can deliver reliable, cost-effective gas supply solutions by working with you to understand your needs. You can explore your gas supply options at: www.airproducts.com/gasgeneration or contact your local office to see how we can help you.

We welcome the opportunity to have a discussion with you. Please contact us at any of these locations:

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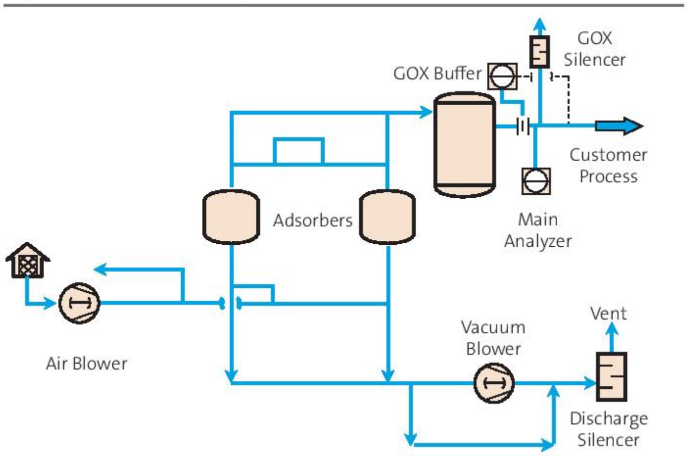
asiagg@airproducts.com

Figure 1: Process Flow Diagram



AIR PRODUCTS

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BIOSTAT® A
Your Professional Start in Cell Cultivation and Microbial Fermentation

turning science into solutions


sartorius stedim
BIOSTAT® A
3
2 L
1

BIOSTAT® A

BIOSTAT® A is an entry-level bioreactor | fermenter designed for easy control of cell growth and fermentation. It is therefore ideal for educational purposes.

- Simple and automatic aeration system
- Intuitive operation for easy control by beginners and experienced users alike
- Integrated recirculating chiller for fermentation in any lab

GERMAN DESIGN AWARD SPECIAL 2016

BIOSTAT® A at a Glance

The control tower of the BIOSTAT® A combines all features and functions for measurement and control: Fast Load pumps, aeration module and conveniently located connections for utilities and sensors. Featuring a compact footprint, the BIOSTAT® A saves valuable space in your laboratory.

Simple and Automatic Aeration Control

The aeration system provides continuous automatic flow control over the full range of each gas used. As a result, BIOSTAT® A does not require any manual adjustment of flow meters and therefore eliminates problems with pulsed aeration. Setting up the bioreactor for use is straightforward: Just connect the aeration tubing, configure the aeration profile, enter the DO setpoint – that's it!

For cell culture applications, interfaces for four gases (air, O_2, CO_2 and N_2) are available for DO and pH control. The microbial version features two gas lines (air and O_2) for DO control.

Integrated Chiller for Microbial Fermentation

The chiller allows fermentation in any laboratory and minimizes water usage. You won't need to worry about finding a suitable cooling water source for your bioreactor. Each BIOSTAT® A for microbial fermentation is equipped with a chiller that effectively removes heat from your culture. The only utility connections you need are electricity and gas supply to operate the BIOSTAT® A.

Easy Status Checks for your pH and DO probes

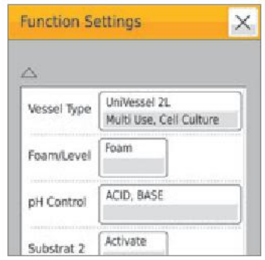
BIOSTAT® A is equipped with digital pH and DO probes. These help you immediately recognize whether you can use a probe for the next cultivation batch. Moreover, the moisture-resistant plug connector ensures secure data transmission to the BIOSTAT® A at all times.

Intuitive Operation of BIOSTAT® A – Even Outside a Laboratory

Thanks to the intuitive user interface, operating the BIOSTAT® A is ideal for beginners, reduces operating errors and speeds up training. Operation using a tablet or smartphone is a mode offered in the Advanced package and enables you to monitor and control the BIOSTAT® A on the move – in the laboratory, in the office or even at home.

BIOSTAT® A – It's the Little Things ...

... That Make Your Life Easier ...



Fast Load Peristaltic Pumps

Install tubing in three seconds: Open, insert tubing, close – done! No more pinched fingers, torn gloves or tubing slippage in the peristaltic pump head.

Convenient Data Storage and Analysis

Save your process data directly on the BIOSTAT® A; then easily transfer this data to your PC or laptop spreadsheet application for convenient analysis.

Flexibility Made Easy

Select the type and size of your culture vessel on the BIOSTAT® A menu, and all settings including PID parameters will be automatically adjusted.

... and Enable You to Take the Next Step.

Higher Cell Densities with Fed-batch Processes

The Advanced package version of the BIOSTAT® A with fed-batch control lets you achieve higher cell densities than with standard batch control. Easily implement substrate profiles in the menu to control an external variable-speed pump.

Glass and Single-use for More Flexibility – Every Day

The BIOSTAT® A is available with single-wall glass vessels in a choice of working volumes of 1, 2 or 5 liters maximum. Whether you select our reusable UniVessel® Glass version or our disposable UniVessel® SU unit with a maximum 2 liter working volume, you can use the same system controller.

Both the UniVessel® Glass and the UniVessel® SU feature the classic stirred-tank design to give you comparable results. The advantage: The same motor drives the stirrer of both vessels so you can easily change over from reusable to single-use or vice versa.

UniVessel® Glass

- Classic stirred-tank design for easy scaling to larger volumes with all characterization data available up to 2,000 L
- Proven sterile design and robustness
- Also fits in small autoclaves, saving you additional investment costs

UniVessel® SU

- Easy handling as the culture vessel is fully pre-assembled and sterilized: no parts to mount, no cleaning – just connect to get started
- No room for an autoclave to sterilize your culture vessel? No problem! The UniVessel® SU is single-use, from the vessel to the sensors.
- Eliminates preliminary setup and post-cleaning procedures so you can better meet your tight experimental deadlines and utilize your time for more important tasks

BIOSTAT® A – Technical Specifications

Base Unit

Control Tower	
Weight	13.5 kg | 29.7 lbs.
Footprint bench surface (W × H × D)	200 × 495 × 300 mm | 7.9 × 19.5 × 11.8 in.
Required space incl. connectors, pump heads etc. (W × H × D)	320 × 495 × 475 mm | 12.6 × 19.5 × 18.7 in.
Power supply	100 to 240 VAC, 50/60Hz, 8 A
Housing	Metal housing, coated
Operating Temperature	0 °C – 40 °C
Maximum relative humidity	80% for temperatures up to 31 °C decreasing linearly to 50% relative humidity at 40 °C
Motor drive	– Maintenance-free – Quiet direct drive – 150 W
Motor rpm, direct coupling	1 L Glass: 30 – 1,400 rpm | 2 L Glass: 30 – 1,100 rpm | 5 L Glass: 30 – 800 rpm | 2 L SU: 30 – 400 rpm
Interfaces	– 2 × external signal inputs 4 – 20 mA – Ethernet – Potential-free alarm contact – USB – Interface to chiller – Interface to external pump
Regulatory compliance	CE, NRTL recognized by the U.S. OSHA (Occupational Safety and Health Administration)
System requirements for operation by tablet and smartphone	iOS 7 or higher; Android 4 or higher

Pump Module

Integrated Pumps	
Quantity	3
Controller	Fixed speed (regulated on|off)
Speed	43 rpm
Pump head	Watson Marlow 114, Fast Load pump head
Flow rates (tube wall thickness 1.6 mm)	ID 0.5 mm: 0.8 mL/min | ID 0.8 mm: 1.7 mL/min | ID 1.6 mm: 6.0 mL/min | ID 2.4 mm: 12.5 mL/min | ID 3.2 mm: 20.2 mL/min
External Pumps	
Quantity	1 (only in the Advanced package)
Controller	Regulated rpms
Speed	Max. 200 rpm
Pump head	Watson Marlow 120, Fast Load pump head

Process Control and Measurements

Probes and Controllers		UniVessel® Glass	UniVessel® SU
Temperature, separate sensor	Temperature control 0 – 40°C Display resolution 0.1°C		•
Temperature integrated in pH probe	Temperature control 0 – 60°C Display resolution 0.1°C	•	
DO probe, reusable	Polarographic probe Digital communication with Control Tower Range: 0 – 100% Display resolution: 0.1%	•	
DO sensor, single-use	DO sensor patch Range: 0 – 100% air saturation Display resolution: 0.1% air saturation		•
pH probe, reusable	Combination electrode, digital communication with Control Tower Range: 2 – 12 pH Display resolution: 0.01 pH	•	
pH sensor, single-use	pH sensor patch Range: 6 – 8 pH Display resolution: 0.1 pH		•
Foam, alternative level	Electrical conductivity sensor, stainless steel, ceramic insulated	•	

Aeration Module

Gas inlet	– Gas pressure 1.5 barg (21.76 psig) ±10% – Gases: dry, oil and dust-free – Gas inlet connections with hose barb for 6 × 3 mm (0.24 × 0.12 in.) reinforced tubing
Gas outlet	– Gas outlet pressure: max. 0.8 barg (11.6 psig) – Outlets to the culture vessel with hose barb for 3.2 × 1.6 mm (0.12 × 0.06 in.) silicone tubing
Two-gas System for Microbial Fermentations	
Continuous and automatic aeration control for air and O_2	
Gas flow control units	Total of two; one per gas segment
Flow rates of the gas flow control units (all vessel sizes)	Air and O_2: 100 – 7,500 ccm
Measuring and control accuracy of the gas flow control units	± 5% full scale
Four-gas System for Cell Culture Application	
Continuous and automatic aeration control for air, O_2, N_2 and CO_2	
Gas flow control units	Total of four; one per gas segment
Flow rates of the gas flow control units (all vessel sizes)	Air and N_2: 10 – 500 ccm | O_2 and CO_2: 5 – 250 ccm
Measuring and control accuracy of the gas flow control units	± 5% full scale

Temperature Control Module

Cooling (only included in the BIOSTAT® A for microbial fermentation)	
Chiller with automatic cooling water valves for recirculated cooling of the culture vessel and exhaust cooler via cooling finger	
Weight	19.5 kg | 43 lbs.
Dimensions (W × H × D)	220 × 495 × 325 mm | 8.6 × 19.49 × 12.8 in.
Volume of cooling water tank	1.5 L
Power supply	BB-8822002: 230 VAC, 50 | 60 Hz, T2.5 A BB-8822003: 115 VAC, 60 Hz, T4 A
Supply temperature	8°C ±2°C at an ambient temperature of 21°C
Cooling capacity	150 W at an ambient temperature of 21°C
International protection rating	IP21 (protected from solid objects > 0.5 inch and vertically dripping water)
Cooling water hardness	Max. 12° dH
Connection with cooling finger via quick-connect coupling	
Heating	
Temperature control with electric heating jacket for culture vessel	
Electrical filter heating for exhaust air filter (only included in the BIOSTAT® A for cell culture applications)	
Culture vessel temperature control range	See above
Heating power	– 1 L Glass: 66 W – 2 L Glass: 112 W – 5 L Glass: 264 W – 2 L Single-use: 132 W

UniVessel® Culture Vessels – Technical Specifications

UniVessel® Glass Culture Vessel

Autoclavable, single-wall glass vessel	1 L	2 L	5 L
Material	Borosilicate glass, stainless steel AISI 316L, EPDM		
Total volume [L]	1.6	3	6.6
Working volume [L]	0.4–1	0.6–2	0.6–5
Cover ports 19 mm | 12 mm | 6 mm	3 | 2 | 6	3 | 2 | 9	3 | 3 | 8
Weight[1] kg (lbs.)	4.4 (9.4)	5.9 (13.0)	10.5 (23.1)
Space requirements [W × H × D in mm] [W × H × D in inches]	 156 × 558 × 196 6.1 × 22 × 7.7	 176 × 619 × 217 6.9 × 24.4 × 8.5	 231 × 757 × 266 9.1 × 29.8 × 10.5
Space requirements, reduced[2] [W × H × D in mm] [W × H × D in inches]		 490 × 391 × 217 18.9 × 16.1 × 11.8	 530 × 510 × 410 20.9 × 20.1 × 16.1

[1] With stand and cover, without medium
[2] Exhaust air cooler adapters for height reduction in the autoclave are available as accessories

UniVessel® SU Culture Vessel

Disposable pre-sterilized polycarbonate culture vessels for cell culture applications	
Material	Polycarbonate
Sizes [L]	2
Total volume [L]	2.6
Working volume [L]	0.6–2

Equipment Packages

BIOSTAT® A is available in pre-defined product packages. The standard equipment packages include everything you need for cultivation. With the advanced package, you can conveniently control your BIOSTAT® A using your table or smartphone and perform fed-batch processes.

BIOSTAT® A package for microbial fermentation	BIOSTAT® A package for cell culture applications
Standard equipment package with UniVessel® Glass	**Standard equipment package with UniVessel® Glass**
BIOSTAT® A Control Tower incl. motor	BIOSTAT® A Control Tower incl. motor
Two-gas aeration (air, O_2)	Four-gas aeration (air, O_2, N_2, CO_2)
Three internal pumps, fixed speed	Three internal pumps, fixed speed
Digital probes for pH and DO	Digital probes for pH and DO
Foam | Level probe	Foam | Level probe
Two external analog signal inputs, 4 – 20 mA	Two external analog signal inputs, 4 – 20 mA
pH control: acid, base	pH control: acid | CO_2, base
DO control	DO control
Combined foam | level control	Combined foam | level control
Chiller for culture vessel and exhaust cooler	Filter heater
Exhaust cooler	UniVessel® Glass 1 L, 2 L or 5 L, single-wall
UniVessel® Glass 1 L, 2 L or 5 L, single-wall	Motor coupling
Motor coupling	Three-blade segment impeller
Six-blade disk impeller	Agitator shaft
Agitator shaft	Heating blanket
Cooling finger	Blind plugs for all ports
Heating blanket	Micro-sparger
Blind plugs for all ports	Four-way addition port
Ring sparger	Manual sampler
Four-way addition port	Universal adapter
Manual sampler	Harvest pipe, adjustable height
Baffles	Inoculation port
Harvest pipe, adjustable height	Air filter
Inoculation port	Tool and accessory set
Air filter	Laptop
Tool and accessory set	
Laptop	
	Standard equipment package with UniVessel® SU
	BIOSTAT® A Control Tower incl. motor
	Four-gas aeration (air, O_2, N_2, CO_2)
	Three internal pumps, fixed speed
	Two external analog signal inputs, 4 – 20 mA
	pH control: acid | CO_2, base
	DO control
	Filter heater
	Two UniVessel® SU 2 L
	Heating blanket
	UniVessel® SU motor adapter
	UniVessel® SU holder
	UniVessel® SU holder adapter ring, 2 L
	Temperature sensor, Pt100
	Safety valve station
Advanced equipment package	**Advanced equipment package**
Operation using tablet or smartphone*	Operation using tablet or smartphone*
Substrate controller in the operator software for fed-batch process management	Substrate controller in the operator software for fed-batch process management
External, variable speed pump for substrate	External, variable speed pump for substrate

* Tablet | smartphone not included

Sales and Service Contacts

For further contacts, visit www.sartorius-stedim.com

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Robert-Bosch-Strasse 5 – 7
34302 Guxhagen

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BIOSTAT STR® Generation 3 and BIOBRAIN Automation Platform

Commercial Manufacturing

Simplifying Progress

SARTORIUS

Engineered for Quality and Precision

The BIOSTAT STR® Generation 3 single-use bioreactor system is now powered by BIOBRAIN, the new automation platform with data-driven software and a comprehensive suite of analytical tools. The system easily integrates into your existing process infrastructure and consistently delivers outstanding speed, quality, and productivity up to 2,000 L. The BIOSTAT STR® system supports the intensification of your seed train process. Start working at lower volumes, skip one or two steps in your seed train while still generating an adequate number of cells.

- ✓ Optimize capital costs
- ✓ Reduce operating expenses
- ✓ Enhanced safety and optimized space
- ✓ Improve productivity and ease of use
- ✓ Increase facility throughput

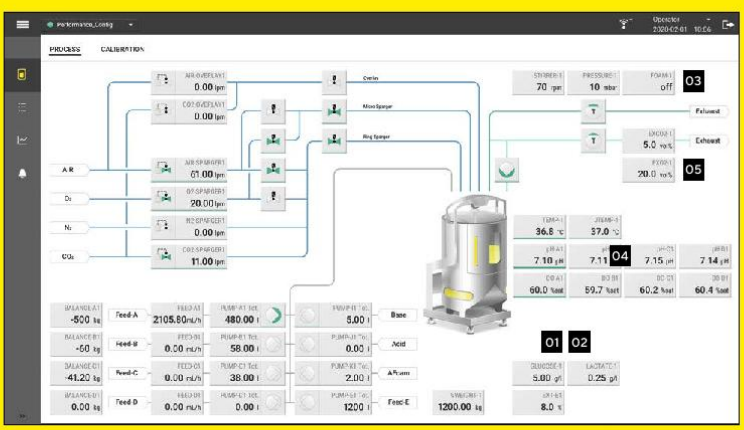
Maximize Quality and Consistency

BIOSTAT STR® system simplifies technology transfer between production scales and ensures consistent quality without surprises and re-optimization.

The Flexsafe STR® bags with optimized resin formulation and minimized additive package lead to cell viability and higher cell densities. The complete control of the raw materials, the extrusion process and the bag assembly guarantee consistent lot-to-lot cell growth performance. The thickness, strength and flexibility of the polyethylene film enhances the mechanical robustness of Flexsafe® bags, making it ideal for all biopro-cessing applications.

The Sartorius scaling tool accurately predicts conditions required to scale your process by considering volumes, process parameters, and any constraints to identify those process parameters required to achieve your goals. Our technical support team can assist you with standard operating procedure (SOP) conversion from your existing scale or technology.

The BioPAT® toolbox helps you realize the full potential of intensified processing by improving output through automated feed and bleed, better process visibility, and by reducing risks associated with manual sampling and control.



01 BioPAT® ViaMass
In-line monitoring of viable biomass and automated cell bleed control

02 BioPAT® Trace
On-line monitoring of glucose and lactate and automated feed control

03 BioPAT® Foam
Monitoring of foam level with alarming and interlock functionalities

04 Electrochemical single-use pH sensors

05 BioPAT® Xgas
Oxygen-carbon dioxide sensor featuring automatic compensation of humidity and pressure

BioPAT® Spectro
Unlock the full potential of Raman spectroscopy

Flex Your Production

The BIOSTAT STR® system with a working volume from 12.5 L to 2000 L and BIOBRAIN automation platform provides you the flexibility and power to adapt on-demand.

The fully configurable control tower can be used interchangeably with any bag holder size. The advanced user interface and improved connectivity make bioreactor operation more streamlined. Furthermore, the organizational racks for pumps and balances together with cable and tubing organizers plus flexible document tray fit in perfectly.

Each bioreactor has a fully scalable, stirred-tank design and offers high performance across a range of processes including high-cell density, micro carrier and perfusion. The Flexsafe STR® bags are designed with multiple sparger and impeller options on a magnetically coupled center-line shaft that deliver homogenous mixtures quickly, even at high volumes and low mixing speeds.

On top of that, BIOSTAT STR® supports connection to Repligen's XCell™ ATF cell retention devices to increase the cell density and productivity and enables process intensification.

Attain Operational Excellence

The BIOSTAT STR® system provides different control options to let you choose the best integration into your existing process infrastructure.

- Local management via control tower including BIOBRAIN automation platform with connection to data historians
- Full process management via distributed control systems e.g. Emerson, Siemens or Rockwell

Are you an Emerson DeltaV™ user?
Benefit from the native DeltaV™ solution.

Technical advantages:
- Complete mapping of your BIOSTAT® system
- Full access to all process values and controller parameters
- Sartorius DeltaV™ library to manage sensors, actors and advanced analytics

The integration for native DeltaV™ comes with local support of an experienced project team which provides support from initial design to final installation.

Find out more
For more information, please visit sartorius.com/biostat-str

BIOSTAT STR® Generation 3 Configurations

Our commerical manufacturing solutions have been predesigned into three configurations with flexible options and easy upgrades. Select the configuration that best fits your current needs and can conveniently adapt to any future requirements.

	Essential	Performance	Performance Plus
Perfusion ready		■	■
Process Analytical Technologies (PAT) Toolbox			
BioPAT® Foam	Optional	Optional	■
BioPAT® Trace		Optional	Optional
BioPAT® ViaMass		Optional	Optional
BioPAT® Xgas		Optional	Optional
BioPAT® Spectro		Optional	Optional
Electrochemical pH sensor	■	■	■
Peripherals			
No. of external pumps	2	4	4
Load cells	■	■	■
Mass flow controller	6	6	6
Flexsafe STR® bags	■	■	■
Sartocheck® bag tester	Optional	Optional	■
Integration Capabilities			
Recipe editing and execution	■	■	■
Local data storage	■	■	■
Native Emerson DeltaV™		■	■
Siemens SIMATIC PCS 7		■	■
Process design support and services		■	■

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Data Sheet

Cogent® Process-Scale Tangential Flow Filtration System

A fully-automated, configurable, TFF system suited for manufacturing of biopharmaceuticals and cGMP process-scale applications

The fully automated Cogent® TFF system is designed to separate and purify monoclonal antibodies, vaccines, plasma, and therapeutic proteins. It is ideally suited for both pilot and production scale applications, thereby supporting rapid scale up from small to large scale operations.

Benefiting from our leading bioprocess knowledge and engineering expertise, the Cogent® Process Scale System is the culmination of 25 years of custom system design and incorporates many unique, innovative and intelligent design features. This system has a very low hold-up volume for maximum volume concentration and optimal product recovery, thus enhancing process performance.

Benefits:

- Modular standard options allow the unique system configuration that best matches process requirements while minimizingupfront investment.
- Full process automation eases the consistent production of preclinical and clinical scale quantities of high-value drug products to cGMP standard.
- Optimized design and component integration of NovAseptic® valves and TFF cassette holders result in a low minimum working volume and ensure maximum product recovery.
- Designed to maximize TFF performances in fed-batch, concentration, total recycle or single pass mode.
- Comprehensive services ensure rapid implementation and optimized performance.

EMD Millipore is a division of Merck KGaA, Darmstadt, Germany

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Culturefuge 400

High-capacity solids-ejecting centrifuge for the biotechnological industries

Culturefuge 400 complete with motor.

Many new biological products are derived from fragile organisms. Although relatively easy to separate the trick is accomplishing the separation in a gentle manner without destroying the shear sensitive cell wall membranes that isolate the complex intracellular proteins from the extracellular target proteins. If this can be avoided, downstream purification of the target proteins becomes much easier.

The Culturefuge 400 is a steam-sterilizable hermetic machine designed for separation of mammalian and microbial cells as well as cell debris and suspended proteins at capacities up to 20 m^3/h. The unique hermetic design with bottom feed gives not only a gentle acceleration of shear-sensitive particles; it also avoids pick-up of oxygen. The hermetic inlet together with the special geometry of the separator leads to maximum separation efficiency. Special attention has been paid to a hygienic, CIP-able design which is a pre-requisite for successful sterilization.

Applications

The machine is designed for clarification duty, separating particles from one liquid, especially shear sensitive particles. Applications that require low oxygen pick-up can also take advantage of the hermetic features offered by this machine. The sterilizability makes the machine suitable for most bio-technological separation duties.

Standard design

The machine consists of a frame that has a horizontal drive shaft, worm gear, lubricating oil bath and a hollow vertical bowl spindle in the lower part. The bowl is mounted on top of the spindle, inside the space (bowl casing) formed by the upper part of the frame, the solids collecting cover and the frame hood. The bowl casing is double-walled for cooling and noise reduction. The bowl is sealed off from this space by mechanical seals. There is also a mechanical seal at the bottom of the spindle, and a mechanical seal at the top of the spindle to make sterilization with steam possible.

All metal parts in contact with the process liquid are made of stainless steel. The bowl is of the solids-ejecting disc type with an automated hydraulic operating system for discharging. It is a so-called timer triggered discharge system, meaning that the bowl content is emptied at pre-set discharge intervals. In production normally only part of the bowl content is emptied, whereas during CIP total discharges are possible. The partial discharge takes place at full speed without any interruption of the feed. The hydraulic/pneumatic system for discharge is mounted on the lower part of the frame.

The centrifuge is available with main connections as sanitary clamps and all other utility connections of clamp type. The electric motor is suitable for variable frequency drive, which makes it possible to have bowl speeds down to 80 % of the maximum bowl speed. The design conforms to a number of EC directives, and the machine is made in accordance with the general directives for machinery. The machine is equipped with nozzles for flushing of the bowl top, the bowl bottom and the solids collecting chute.

Design features

- Designed for easy cleaning-in-place (CIP)
- Fully hermetic design for minimal shear stress and absence of oxygen.
- Design pressure of the bowl casing 300 kPa.
- Design pressure for the cooling jacket 300 kPa for connection to centralized cooling circuit.
- Bowl casing and cooling jacket designed according to ASME or PED.
- Sterilizable (SIP) with 210 kPa steam in a 30 min cycle, including discharge system.
- Most parts in contact with the process liquids available with two alternative surface finishes.
- Product wetted parts passivated (optional).
- All product wetted polymers and seal rings compliant with FDA regulation.

Available models

These different surface finish executions are available:

	Ra 0,8	Ra 0,5 El.-polished
Inlet device	X	X
Bowl spindle	X	X
Separator bowl inside	X	X
Discharge housing	X	X
Outlet device	X	X
Frame top part inside incl. solids collecting ring	X	
Frame hood inside	X	
Separator bowl outside	X	

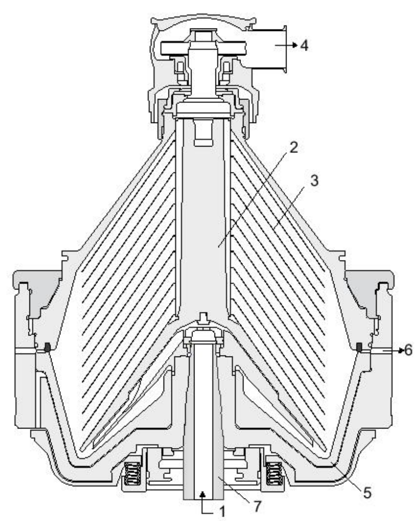
Operating principles

The feed is introduced to the rotating centrifuge bowl from the bottom via the hollow bowl spindle (1), and is accelerated in a distributor (2) before entering the disc stack (3). It is between the discs that the separation takes place.

The liquid phase moves towards the centre of the bowl, from where it is pumped out under pressure by means of a built-in pump disc (4).

The heavier solids phase is collected at the bowl periphery, from where it is discharged intermittently. The solids discharge is achieved by a hydraulic system below the separation space in the bowl, which at certain intervals forces the sliding bowl bottom (5) to drop down, thus opening the solids ports (6) at the bowl periphery.

The bowl is mounted on a vertical spindle (7).



Typical bowl drawing for a solids-ejecting hermetic centrifuge. Drawing details do not necessarily correspond to the centrifuge described.

Utilities consumption

Electric power	max. 20 kW [1]
Flushing liquid, per discharge	0–16 l
Operating liquid	2.5 l per discharge
Steam at 210 kPa pressures	20 kg per sterilization
Cooling liquid for frame parts [2]	max. 550 l/h
Cooling liquid for oil cooler [2]	max. 180 l/h
Cooling water for seals	min. 280 l/h

[1] At 20 m^3/h. Power consumption increases with the flow rate.
[2] The oil cooling and the frame cooling may be connected in series.

Material data

Bowl body, hood and lock ring	s.s. 1.4462 UNS S 31803
Frame top part (ASME)	s.s. ASME S 31603
Frame top part (PED)	Stainless steel 1.4404 (for pressurised equipment)
Frame bottom part	Cast iron Clad with s.s. 1.4301 UNS S 30400
Outlet parts	s.s. 1.4462 UNS S 31803
Gaskets and O-rings product-wetted	EPDM rubber and PTFE acc. to FDA [1]
Bowl seal ring	Amid polymer 66 acc. to FDA [2]
Seal rings in- and outlet	Resin impregn. Carbon Graphite acc. to FDA [3]
Wear ring in- and outlet	FDA approved self-sintered Silicon Carbide

[1] CFR 21§177.2600/1550 USP Class VI.
[2] CFR 21§177.1500 USP Class VI.
[3] CFR 21§177.2410.

Connections

Process (inlet and two outlets)	Clamp type, 63.5 mm [1]
Utilities	Clamp type, various dimensions [1]

[1] According to ISO 2852.

Technical specifications

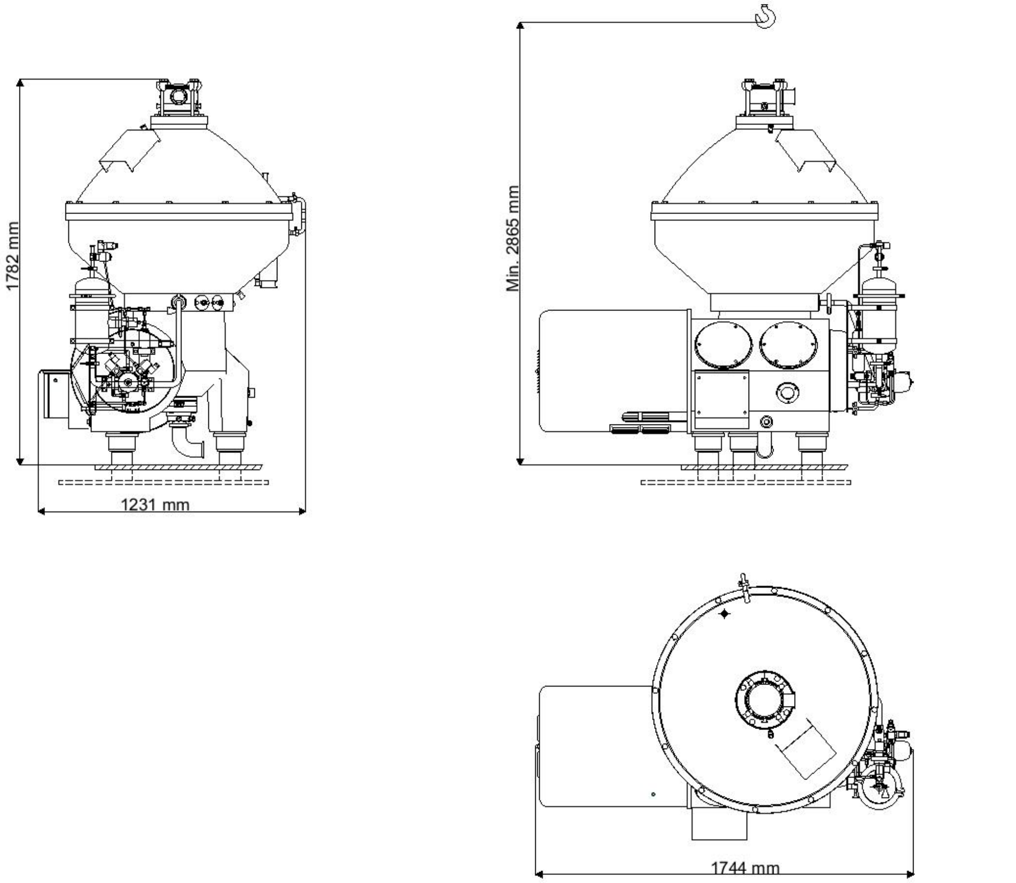
Throughput capacity	max. 20 m^3/h [1]
Solids handling capacity	max. 600 l/h [2]
Bowl volume	30 l
Sludge space volume	10 l
Discharge volume	3–30 l
Bowl speed, separation	max. 5,119 rpm
Bowl speed, sterilization	max. 120 rpm
G-force	max. 7,425
Motor speed synchronous 60Hz	1,800 rpm
Installed motor power	22 kW (30 HP)
Starting time min/max	8/10 mins
Stopping time with motor brake	10 mins
Feed temperature range	0–100°C
Feed inlet pressure required at inlet flange	max. 200 kPa
Liquid outlet pressure at outlet flange	200 kPa [3]
Sound pressure	73 dB(A) [4]
Overhead hoist lifting capacity	min. 1,000 kg

[1] Actual throughput depends on amount and type of solids in the feed, viscosity and required degree of clarification..
[2] Wet solids. Actual volume depends on discharge volume and application
[3] At 20 m^3/h and with medium sized outlet pump. Increasing with decreasing flow rate.
[4] According to ISO 3744.

Shipping data (approximate)

Centrifuge incl. bowl and motor	1,850 kg
Bowl	600 kg
Gross weight	2,150 kg
Volume	5.0 m^3

Dimensions



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EMD MILLIPORE

Data Sheet

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- Designed to maximize TFF performances in fed-batch, concentration, total recycle or single pass mode.
- Comprehensive services ensure rapid implementation and optimized performance.

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Configure your system according to your process needs...

Option 1: Tank (50, 100, 200L)

Jacketed for temperature regulation

Option 7: Filtrate conductivity

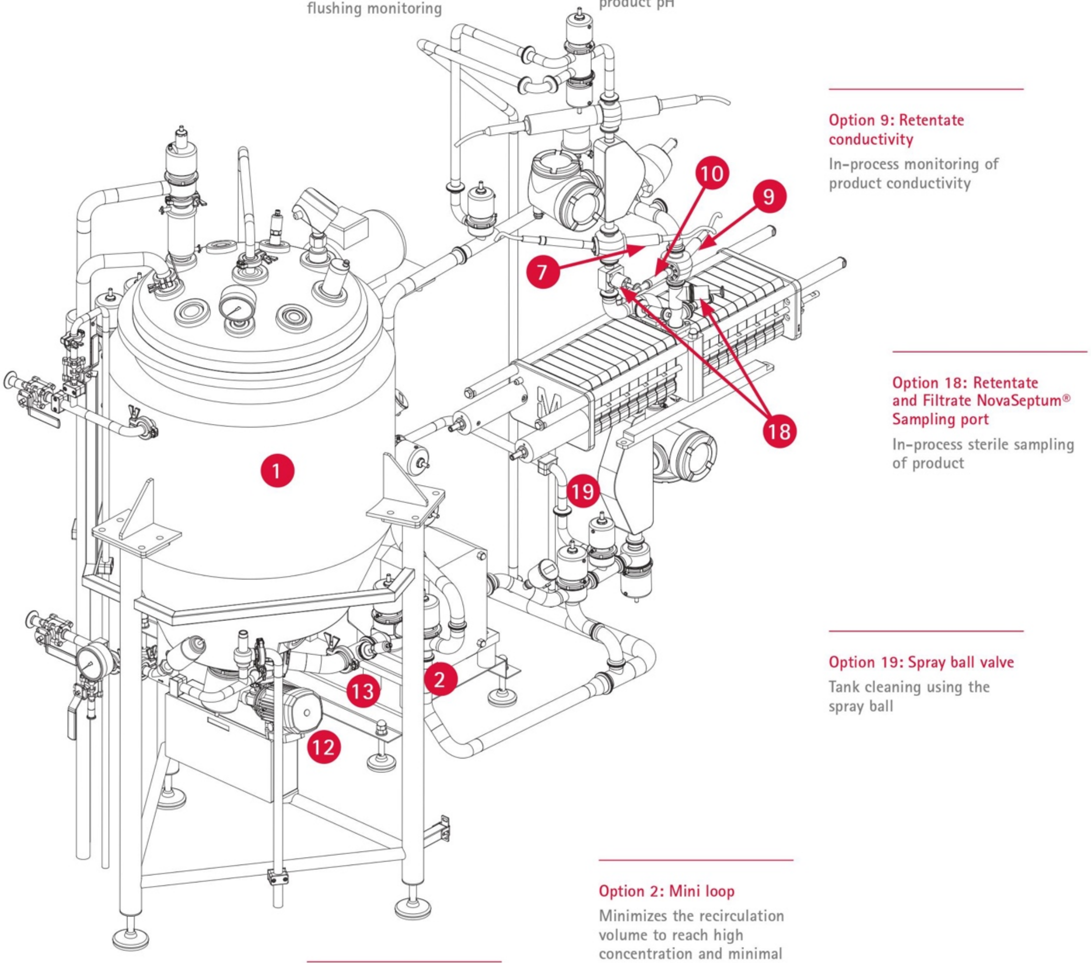
Measurement of a wide range of products (WFI, buffer solutions, protein solutions) or post CIP flushing monitoring

Option 10: Retentate pH

In-process monitoring of product pH

Option 9: Retentate conductivity

In-process monitoring of product conductivity



Option 18: Retentate and Filtrate NovaSeptum® Sampling port

In-process sterile sampling of product

Option 19: Spray ball valve

Tank cleaning using the spray ball

Option 2: Mini loop

Minimizes the recirculation volume to reach high concentration and minimal product volume

Option 13: Tank Outlet Level Switch

Allows to stop the feed pump when air reaches this sensor. E.g: In Mini loop concentration mode, detects the end of the step (tank fully empty).

Option 12: Tank NovAseptic® GMP mixer

Ensures product homogeneity, specially important during diafiltration step. Aseptic design, minimized shearing

Option 3: Transfer Pump

Transfer of product / buffers into the feed tank from any other tank. Allows fed-batch mode, and diafiltration.

Option 8: Filtrate pH

pH monitoring during cleaning and sanitization procedures

Option 11: Filtrate UV

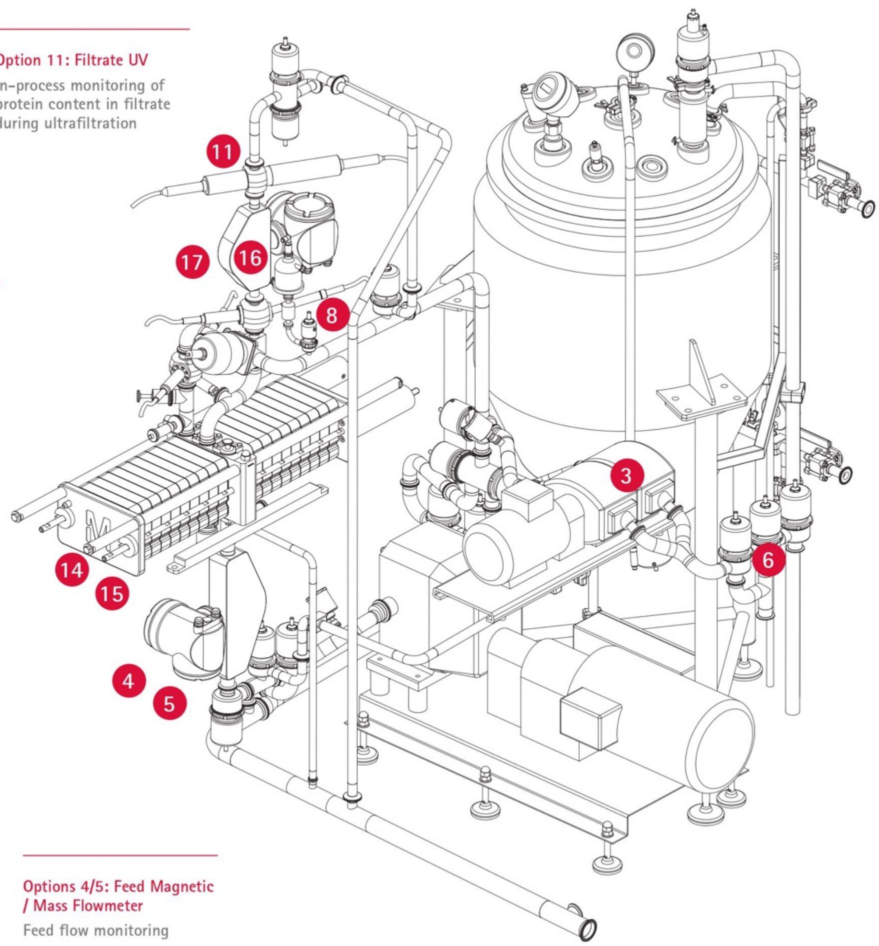
In-process monitoring of protein content in filtrate during ultrafiltration

Options 16/17: Filtrate Mass / Magnetic flowmeter

Filtrate flow and total volume monitoring in diafiltration mode

Options 14/15: Process Scale Holder

Can be configured either with manual or hydraulic closure. Hydraulic closure can be done with a hand pump or with an automated hydraulic box.



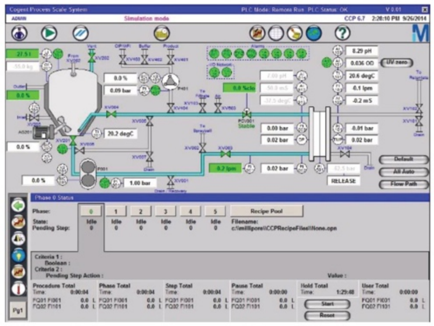
Options 4/5: Feed Magnetic / Mass Flowmeter

Feed flow monitoring

Option 6: Transfer Inlet Manifold

Allows connecting several inlets to the transfer pump head (product/WFI/CIP) at the same time, avoiding many connections / disconnections

...and build a consistent user experience



Total Process Control and Connectivity

The Cogent® Process Scale system is easily controlled via the Common Control Platform® (CCP®) software, a powerful, intuitive and graphical software that provides real-time monitoring and total in-depth control of your TFF process.

Using robust PCs, PLCs, and SCADA® technology, it meets the most stringent standards for connectivity, reliability and ease of use.

Benefits:

- Create process operations using the recipe editor, monitor or control the process in the home screen, and create reports for the batch using the configurable report generator
- Developed under GAMP guidelines and FDA 21 CFR Part 11 compliance-ready, including audit trails and electronic signatures for verification
- Sensor combinations can be adapted to process requirements allowing the maximum confidence in process monitoring
- Utilities to connect all of your separation unit operations to a central network or DCS (e.g. Delta V)
- Used on multiple unit operations CCP® software provides one familiar interface to simplify software management and reduce learning curves

NovAseptic® GMP mixer

Embedded NovAseptic® Valves, Mixer and Connectors

Engineered for optimal performance, reliability, durability and ease of maintenance.

The design and development of each component is based on more than 20 years' experience, focused on aseptic application. This is why we choose to call it "Aseptic by Design."

Benefits:

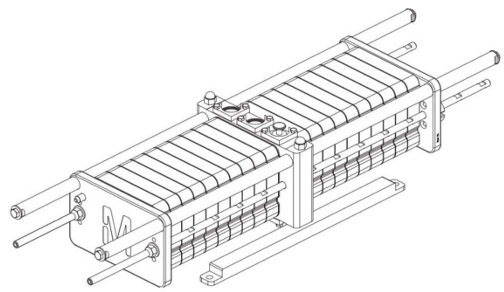
- Comply with cGMP Design Qualification criteria for aseptic processing
- NovAseptic® connector ensures no dead legs and maximum product recovery with zero hold up volume.
- Comply with the most stringent cleaning and sterilization requirements
- Mixer is clean running and is suitable for general mixing, heat transfer and shear sensitive applications.
- Reduced bioburden
- Lower cost of maintenance
- Diamond coated mixer bearings ensure long life and optimum performance.
- Ability to mix the "last drop", ensures complete product recovery

Unparalleled Ultrafiltration

Plug and Play

The Pellicon® Process-scale Holder is uniquely designed to reduce the time required to install and remove TFF cassettes at production scale while keeping the flow path unchanged.

The holder can be configured with a manual or hydraulic closure. Hydraulic closure can be done with a hand pump or with an automated hydraulic box which allows local or distant control.



Benefits:

- Compact footprint
- TFF cassettes can be installed/removed quickly
- Easy to vent and fully drainable, maximizes product recovery
- Easy retrofit from manual to hydraulic closure
- Flow path unchanged, minimizes future re-qualification and validation effort in new process applications

Air Integrity Test

In order to ensure that the cassettes have been installed properly and has not sustained any damage during storage and handling, we recommend integrity testing prior to startup and after each post use cleaning.
Air Integrity Test accessories consist of a set of air pressure regulators and fittings including assembly procedure to guarantee an easy plug and play solution.

Pellicon® 3 Ultrafiltration Cassettes

The tangential flow filtration cassette of choice for demanding filtration processes requiring unbeatable performance consistency. For use in applications including: monoclonal antibodies, recombinant and non-recombinant proteins, albumin, hormones, vaccines, and growth factors.

Biomax® membrane

Pellicon® 3 cassettes with Biomax® membranes are designed for the filtration of therapeutic proteins, albumin, hormones, vaccines and growth factors. These advanced, high-performance cassettes are ideal for today's processes that require higher operating pressures, temperatures and higher caustic cleaning regimes.

Ultracel® membrane

Pellicon® 3 Cassettes with Ultracel® membrane are the device of choice for today's higher titer therapeutic antibodies as well as the more demanding filtration processes that require low protein fouling. The new D screen is optimized for applications that require higher viscosity and concentration applications.

Benefits:

- Robust, void-free membranes for optimum product recovery and performance consistency
- All thermoplastic design, protective end cap and integrated gasket provides great process consistency and ease of use
- Predictable and fast process scalability from lab to production scale
- Robust product design ideally suited to filtration processes with higher operating pressures, temperatures and caustic cleaning regimes
- Automated manufacturing delivers unbeatable performance consistency and reliability
- Proven process expertise and technical support to partner with you from development to full scale manufacturing
- Optimized flow path for higher flux and resolution separation capability

Provantage® Bioprocess Consulting Services

Provantage® Bioprocess Consulting Services leverage our core expertise, products, services and technology in downstream production to help solve your business problem or challenge. Our commitment to your project outcomes and timelines is managed with our stage gate approach and a dedicated project manager.

Application Expertise

Our Biomanufacturing Sciences Network (BSN) is a global team of over 85 engineers, scientists and technology specialists who provide expertise and peer-to-peer support in process development and manufacturing. We act as an extension of your team, helping you to minimize potential risk and streamline your operations. With over 3,000 client engagements, our toolkit of best practices will ensure your project is delivered on time and within budget.

Design and Implement

From lab-scale to pilot and manufacturing facility start-up, EMD Millipore is a partner of choice for providing consultative expertise on current best practices to integrate device, hardware and process technology, and process automation. We can provide consultative evaluations for TFF optimization and operating strategies.

Develop

With our 35+ year history manufacturing and implementing TFF technologies, EMD Millipore application specialists develop reproducible, scalable and robust TFF processes that meet your specific requirements and your required scale.

Optimize

Starting with a comprehensive technical assessment and characterization of your existing TFF step, EMD Millipore application specialists can recommend and implement TFF enhancements that use best-practice operating conditions and state of the art processes to deliver an optimized and validatable TFF process at your targeted scale, in a timely manner.

Transfer

During the lifecycle of a biopharmaceutical, technical transfers occur at various stages: from research to clinical development to commercial manufacturing, and from one manufacturing facility to another. EMD Millipore leverages experienced technical staff, strong project management, and good documentation practices on both sides throughout the course of transfer activities to ensure a robust and successful transfer.

Troubleshoot

EMD Millipore has extensive experience in troubleshooting and investigating manufacturing, method and process development issues. Our experienced team works together collectively with your technical project team to identify the root cause and to develop a robust, acceptable path forward.

Provantage® implementation services

In the biopharmaceutical industry, implementing new equipment with respect to Quality rules and guidelines can be challenging. To help you stay ahead in today's demanding and competitive production environment, our Provantage® Services group provides unparalleled support for implementation of the Cogent® Process Scale System. With a wide range of comprehensive packages to meet your unique manufacturing requirements, resulting in peace of mind and maximum operational flexibility.

	SAT and IQ/OQ	Operator training	CCP® Software Design	CCP® Software Training	Support for PQ
Qualification package GMP	•	•			•
Single Molecule cGMP package	•	•	•		•
Full cGMP package	•	•	•	•	•

Benefits:

- Qualify your system with our IQ/OQ service protocols and use our qualified Field Service Engineers with years of product experience to ensure your system functions as specified in cGMP environments
- Train your operators with an interactive, hands-on courses for either system operation, or advanced CCP® software recipe creation training by certified trainers
- Get the support of our experienced Biomanufacturing Engineers during your Process Performance Qualification
- Maintain your system with annual preventive maintenance by qualified Field Service Engineers to ensure the lifetime of the system and ultimately reduce your capital expenditures

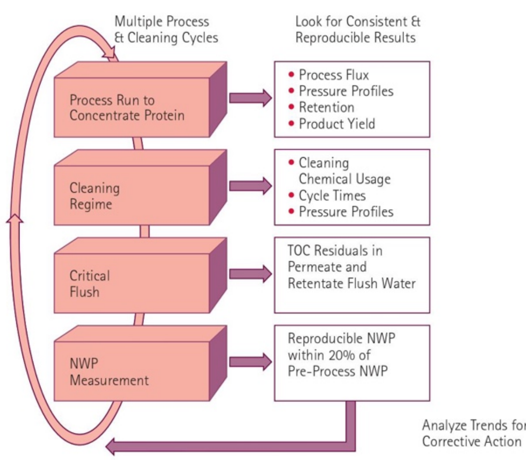
Provantage® Lab Services

Establishing an effective cleaning and sanitization plan for equipment used is a fundamental cGMP requirement necessary to assure the quality and consistency of your drug substance. Effective and consistent membrane cleaning and sanitization after each process cycle is the single most important factor in maintaining system performance.

Cleaning and sanitization after every cycle removes residual foulants and contaminants from the membrane, preventing batch-to-batch carry over, maintaining optimal performance and maximizing the useful life of the filter cassettes.

Effectiveness is measured by the ability to control and eliminate microbial contamination, and to remove process foulants to restore membrane performance such that consistent flux and separation are achieved batch after batch.

Our Provantage® TFF Cleaning Services can help you develop cleaning and sanitization procedures that assure the safety and purity of your product and maximize the useful life of your TFF cassettes.



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Overview

Biomax® Membranes

The membrane of choice for fast processing and exceptional chemical resistance

Typical Applications

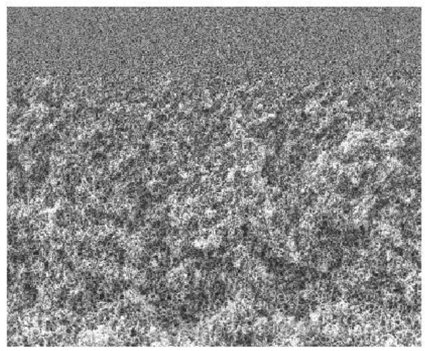
- **Concentration, buffer exchange, and depyrogenation of protein solutions containing biomolecules such as albumin, IgG, IgM, monoclonal antibodies, hormones, and growth factors**
- **Harvest, clarification, and concentration of vaccines**

The more open average pore size permitted by the void-free structure of the Biomax® membrane results in higher fluxes with maximum retention.

Conventional UF membranes cast with macrovoids have tighter average pore sizes and must operate with reduced flux to keep retention high.

The high flux and high retention properties of the Biomax® membrane result in faster processing speeds with higher yields, which means shortened processing times and a bioprocessing system that can be smaller and more compact.

Biomax® membranes are composed of polyethersulfone and are resistant to harsh chemicals used in cleaning, biological decontamination, and sanitization. The polyethersulfone Biomax® membrane has been designed to reduce non-specific protein binding compared to conventional polyethersulfone membranes.



Biomax® membrane composite polyethersulfone with void-free structure

Advantages of Choosing Biomax® Membranes

- Void-free structure results in high flux, excellent retention and higher yields
- Polyethersulfone membrane provides a stable hydraulic environment, resulting in excellent mechanical strength and integrity
- Biomax® membrane has superior resistance to harsh cleaning chemicals with no degradation of processing performance through multiple cleaning cycles
- Biomax® membranes are available in a wide range of molecular weight cut-offs to meet all of your application needs

Tighter Retention Profile

The retention profile of Biomax® 10 kDa membrane is much sharper than that of a conventional 10 kDa membrane, translating into improved protein retention in your process stream (Figure 1).

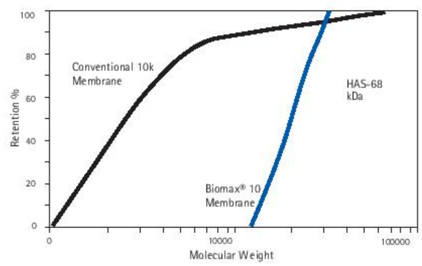


Figure 1.
Protein retention of Biomax® membrane versus conventional polyethersulfone UF membrane

Improved Integrity

The void-free structure of the Biomax® membrane significantly reduces the incidence of microdefects, resulting in improved membrane integrity (Figure 2).

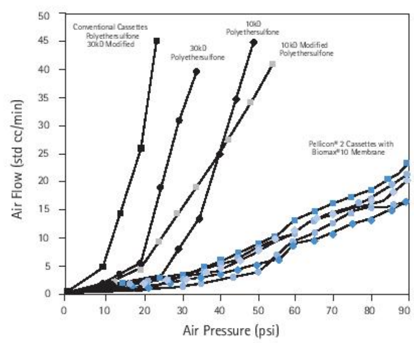


Figure 2.
Integrity testing of Biomax® membranes versus conventional polyethersulfone UF membranes

Improved Process Yields

You can decrease the size of the system and improve your yield, thereby reducing your overall processing costs (Table 1).

Table 1.

Parameter	Biomax® 10 Membrane	Conventional Polyethersulfone (10 kDa) Membrane
Retention (%)	99.95	99.9
Flux (lmh)	118.0	80.0
Recirculation rate (lpm)	4.0	6.0
Pipe diameter (inches)	1.5	2.5
Hold-up volume (liters)	8.4	20.8
Yield improvement (%)	2 – 3	–

Superior Flux

At working concentrations of protein, Biomax® membranes have higher flux for a given protein retention than conventional polyethersulfone UF membranes. In this example, Biomax® 10 membrane demonstrates a 40% improvement in process flux over a conventional 10 kDa polyethersulfone membrane using 10% BSA (Figure 3).

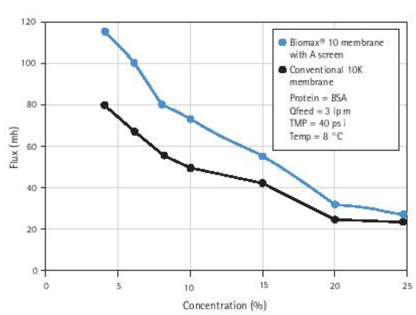


Figure 3.
High flux of Biomax® membrane versus conventional polyethersulfone UF membrane

Superior Chemical Resistance Results in Excellent Cleanability

A simple caustic cleaning regimen restores normalized water permeability (NWP) to near initial levels following sequential process runs (Figure 5).

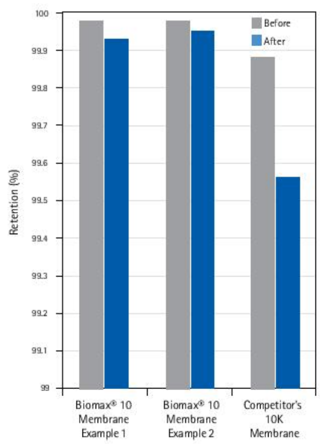


Figure 4.

Caustic resistance of Biomax® membrane versus conventional Polyethersulfone UF membrane

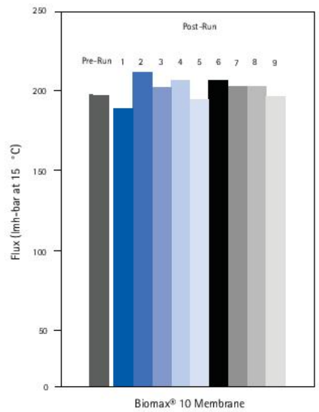


Figure 5.

Consistent return of water permeability after cleaning

Results

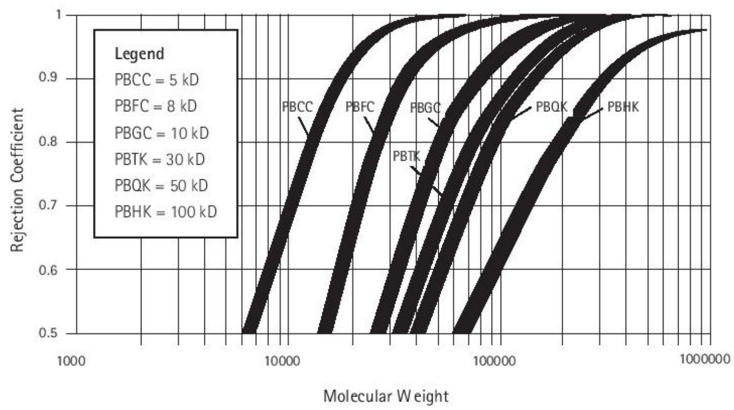
After 100 hours in 600 ppm chlorine, the Biomax® 10 membrane showed no appreciable change in air integrity or BSA retention (Table 2).

Table 2.

	Sample A	Sample B
Air integrity (sccm) prior to exposure	7	8
BSA retention % prior to exposure	99.97	99.97
Air integrity (sccm) after exposure	3	10
BSA retention % after exposure	99.97	99.97

Figure 6.
UF membrane Dextran retention profile

Biomax® Membrane Mixed Dextran Test



The need for more rejection information and for better membrane manufacturing consistency and control led us to develop the Mixed Dextran Rejection Test for UF membranes.

A large number of marker solutes has been used in the past to characterize the retention properties of ultrafiltration (UF) membranes. Traditionally, solutions of single proteins were used and a ranking system of Nominal Molecular Weight limits (NMWL) was adopted by the UF user community. For each membrane, the NMWL value gives an estimate of the molar mass of the smallest protein that is retained at an arbitrarily selected minimum level (usually 90%). This system of ranking has proved to be very useful and is still used to classify UF membranes. The NMWL method, however, offers very limited information about the properties of UF membranes (approximate rejection value for only one solute size) and therefore is no longer sufficient for the sophisticated user of state-of-the-art separation processes. Although protein processing represents the most important type of applications for UF membranes, using proteins as markers has many disadvantages, such as availability in sufficient purity, diversity of protein shape, structure and physical properties, and high cost. To satisfy the need for testing a wide variety of UF membranes, one-has to select proteins of vastly different sizes. An undesirable consequence of this selection is the potential variation of other properties, such as isoelectric point (resulting in different charge at a given pH), the nature and proportion of hydrophobic and hydrophilic groups on the surface of the molecule (resulting in different adsorption properties), solubility and size-to-molecular weight relationship. All these differences can significantly affect the measured rejection values and therefore make the interpretation more difficult.

Our rejection profile test uses dextrans as test markers. This allows an evaluation of the rejection properties of a UF membrane for a range of solute sizes spanning from solutes that are completely passed through the membrane to solutes that are completely retained, so that one test generates a complete rejection curve.

Low adsorption of dextrans to many UF membranes joins with optimized and controlled boundary conditions in the rejection profile test to assure that the measured rejection profile reflects as closely as possible the steric rejection properties of UF membranes and therefore offers useful information about the membrane pore size distribution. To take advantage of these characteristics of the rejection profile test, we adopted this test as a standard quality control method for monitoring and controlling the reproducibility of UF membranes. Rejection profile bands were specified for each membrane type. The measured rejection profile of each membrane lot has to fall within these bands. The result has been a significant improvement in lot-to-lot reproducibility of the rejection performance of our UF membranes.

Biomax® Membrane Specifications*

Materials of Construction	Polyethersulfone with void-free structure pH compatibility - 1-14 Reverse Pressure - >/= 30 psi
Relative Protein Binding	Low to moderate, for use with protein solutions containing more than 0.1 mg/mL of protein.

Biomax® Membrane Applications

Biomax® Membrane Code	NMWL* (kDa)	Typical Application
PBCC	5	Growth factors, hormones
PBFC	8	Growth factors, hormones
PBGC	10	Albumin, hemoglobin
PBTK	30	Enzymes
PBQK	50	IgGs
PBHK	100	Small viruses, antigens
PBMK	300	IgMs, large viruses
PBVK	500	Large viruses, colloids, particulates
PBXK	1000	Large viruses, cells, colloids, particulates

* Nominal Molecular Weight Limit

Device Formats

Biomax® membranes are found in Pellicon® Cassettes

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Preparation, Separation, Filtration & Monitoring Products

Data Sheet

Prostak™ Microfiltration Modules

For Convenient and Economical Perfusion and Clarification/Concentration Applications

Prostak™ modules are tangential flow stacked plate membrane devices with open feed channels, made for use in Prostak™ tangential flow filtration (TFF) systems.

Prostak™ open channel modules are available with microporous membranes and are steam sterilizable for at least 20 cycles.

Versatility

Prostak™ open-channel modules are available in four sizes: 2, 4, 10 and 20 Stack, making them applicable for bench-top, pilot and production scale systems.

Reliability

Membrane and module integrity is tested on every module during manufacturing.

Easy to Validate

No adhesives and only two materials of construction to simplify validation of extractables.

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High Product Recovery

The open channel design and the low hold-up volume allow for a high product recovery and makes the flow path gentle to process fragile cells and shear-sensitive materials.

Typical Applications

Prostak™ microfiltration modules can be used for perfusion or for clarification/harvest operations, in any of these typical applications:

- Mammalian, bacterial and mycelial cell suspensions
- Emulsions and colloidal suspensions
- Viruses, proteins and other bio-organic macromolecular solutions
- Polysaccharides and other high-viscosity solutions
- Yeast, algae and other high solids suspensions
- Protein precipitates
- Perfusion
- Vaccines

Physical Specifications

Recommended Prefiltration				
150 µm nominal				
Materials of Construction				
Polysulfone (10% glass filled) plates; Membrane: Durapore® hydrophilic or hydrophobic PVDF microporous membranes				
Channel Height				
Approximately 0.5 mm				
	2 Stack	**4 Stack**	**10 Stack**	**20 Stack**
Dry Weight (kg)	1.6	2.1	3.6	6.2
Length (cm)	38.9	38.9	38.9	38.9
Width (cm)	19.8	19.8	19.8	19.8
Height (cm)	4.6	5.6	8.1	12.4
Approximate Internal Volume				
Total Volume (ml)	500	700	1250	2500
Feed Side (ml)	300	420	750	1500
Filtrate Side (ml)	200	280	500	1000
Effective Membrane Area				
Area (m^2)	0.17	0.33	0.84	1.7

Operating Guidelines

Maximum Inlet Pressure		
at <50°C		5.5 bar (80 psi)*
at 50 – 80°C		4.1 bar (60 psi)
Maximum Transmembrane Pressure - Forward		
at <50°C		4.1 bar (60 psi)*
at 50 – 80°C		2.1 bar (30 psi)
Reverse Transmembrane Pressure		not recommended
pH Range	short duration (<1 hr cycle)	continuous
Durapore® Hydrophilic PVDF Membrane	1 – 11	2 – 10
PZHK Membrane and Hydrophobic PVDF	1 – 13	2 – 12

*Note: Durapore® 0.65 µm membrane, 3.5 bar maximum forward pressure

Sterilization Guidelines

Steam (in Place)	
Steam Pressure	20 psi (1.4 bar)
Steam Temperature	126 °C (259 °F)
Maximum Feed to Retentate Pressure Drop	0.14 bar (2 psi)
Maximum Transmembrane Pressure - Forward	0.14 bar (2 psi)
Cycle Time	1 Hour

Ordering Information

Modules

Pore Size (µm)	2 Stack	4 Stack	10 Stack	20 Stack
Microporous Membranes - Hydrophilic PVDF Durapore® Membrane				
0.1	**PSVV AG0 21**	**PSVV AG0 41**	**PSVV AG1 01**	**SK2P 127 E1**
0.22	**PSGV AG0 21**	**PSGV AG0 41**	**PSGV AG1 01**	**SK2P 484 E0**
0.45	**PSHV AG0 21**	**PSHV AG0 41**	**PSHV AG1 01**	**SK2P 242 E9**
0.65	**PSDV AG0 21**	**PSDV AG0 41**	**PSDV AG1 01**	**SK2P 446 E0**
Microporous Membranes - Hydrophobic PVDF Durapore® Membrane				
0.22	—	—	—	**SK2P 344 W2**
0.45	**SK2P 012 W6**	—	—	**SK2P 013 W4**
PZHK Membrane - Hydrophobic PVDF				
200*	—	—	—	**SK2R B30 A1**

*Nominal Molecular Weight Limit in kilodaltons
There is one module per package. Sanitary gaskets are supplied with each module to provide a leak-free connection between the module(s) and holder.

Holder and Spare Parts

Part	Description	Material of Construction	Catalog Number	Quantity/Pack
Holder	Prostak™ holder	Stainless Steel	PSKPMF001	1
Sanitary Gaskets	Short sanitary gasket kit, for use without holding plates.	Silicone	PSKP02200	12 for permeate, 12 for feed/retentate
	Tall sanitary gasket kit, for use with gasket holding plates.	Silicone	PSKP0220P	16 for permeate, 16 for feed/retentate
		EPDM	PSKP0210P	16 for permeate, 16 for feed/retentate
Gasket Support Plates (Optional)	Gasket support plate, for non-steaming applications	Polycarbonate	PSKPMFG06	6
	Gasket support plate, for steaming applications	Polysulfone	SK2P007W8	6

For information on Prostak™ holders and systems, please contact your local account representative or visit **EMDMillipore.com**

To Place an Order or Receive Technical Assistance

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Application Note

Single-Pass Tangential Flow Filtration

Introduction

Tangential flow filtration (TFF) is widely used in the biopharmaceutical industry for downstream processing applications. Typical TFF steps concentrate product through volume reduction, and buffer exchange through diafiltration, to achieve high yields. TFF typically utilizes ultrafiltration membranes ranging from 1 – 1000 kD nominal molecular weight limit (NMWL) to retain different size molecules. Traditional TFF requires multiple passes through a system, using a pump to drive feed through a filter and sending the retentate back to a tank for another pass through the system.

Single-pass TFF runs at constant operating conditions throughout the process, simplifies the required hardware, allows higher concentration factors and higher product recovery without significant dilution by reducing hold-up volume, and reduces the risk of product damage associated with recirculating TFF operations. Single-pass TFF is also a continuous process and can be run together with another step. This is convenient to reduce volumes to eliminate tank bottlenecks and reduce column sizes, especially in existing facilities where space may be limited.

Principles of single-pass TFF

Traditional TFF operates in batch mode, where the feed/retentate is recirculated through the filter assembly (**Figure 1A**). Typically, TFF cassettes operate in parallel, with multiple passes through membranes required to achieve the desired concentration.

Our single-pass TFF is a different application of an existing technology (**Figure 1B**). Single-pass TFF uses existing Pellicon® 2 or Pellicon® 3 cassettes with standard holders. The TFF step is sufficiently concentrated after a single-pass through the filter assembly such that retentate recycle is not required.

The basic single-pass TFF underlying principle is that increased residence time in the feed channel results in increased conversion. Increased residence time can be accomplished by reducing flow rate or increasing path length in a serial configuration. Configuring TFF cassettes in series can improve conversion. Cassettes in series have a higher mass transfer when compared to parallel configurations at equivalent residence times (**Figures 1C, 1D**).

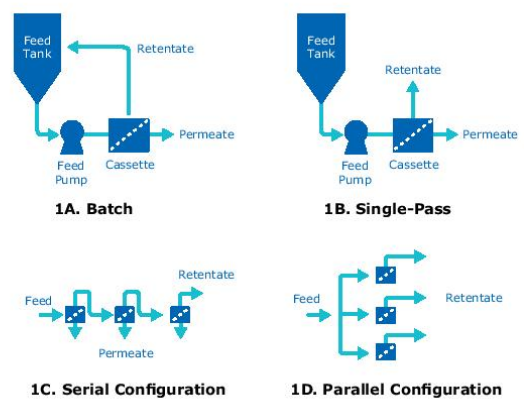

Figure 1. Compared to traditional TFF configuration (A), a single-pass TFF configuration (B) does not recycle the retentate. Conversion increases with residence time with a serial feed channel configuration (C), offering a slight advantage over a parallel configuration (D) due to higher flow velocities and mass transfer. Permeate is not shown in 1D.

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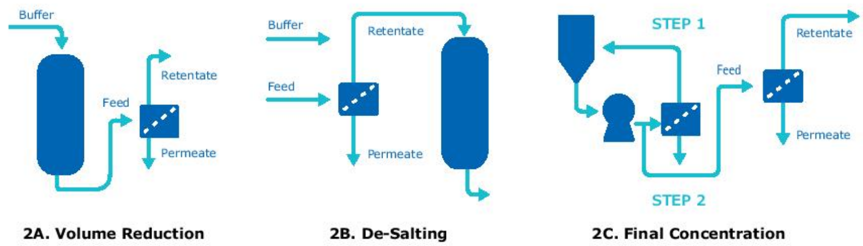
Applications of single-pass TFF

Single-pass TFF has several applications, including:

- **Product concentration/volume reduction:** single-pass TFF can be used in between other unit operations to reduce intermediate pool volumes. In turn, this can de-bottleneck a process limited by tank volumes and/or reduce column sizes and/or number of cycles required for downstream chromatography (**Figure 2A**).
- **In-line dilutions/de-salting:** single-pass TFF can be used for in-line desalting before ion exchange chromatography, membrane adsorbers or virus prefilters without expanding the pool volume by dilution (**Figure 2B**).
- **Final formulation/concentration (post-batch UF/DF):** single-pass TFF reduces working volume limitations compared to traditional TFF, allowing the process to achieve higher final concentrations and minimizing post-use recovery dilution (**Figure 2C**).

Figure 2. Applications for single-pass TFF setup include product concentration and volume reduction (A), in-line dilutions and de-salting (B), and final concentration (C).



Evaluation of single-pass TFF at small scale

Bench scale experiments using single-pass TFF are typically performed with three identical Pellicon® cassettes in conventional mini holder(s) (**Figure 3A**). Specially designed single-pass TFF diverter plates are used to allow the three cassettes to be configured in series within a single holder (**Figure 3B** and **Figure 3C**). This experimental setup allows the operator to evaluate the performance of one-, two- and three-section single-pass TFF processes at the same time. Alternatively, three Pellicon® mini holders can be configured in series.

Trials typically require 1 – 2 L of feed material and 4 – 6 hours of run time. The experiment is run in either total recycle mode or as a true single pass, depending on the volume of feed available. If the experiment is run in total recycle mode, a well-mixed tank is required to ensure proper mixing of the concentrated retentate with recycled permeate.

Single-pass TFF trials require measurement of feed and retentate pressures and retentate and permeate flow rates, at varying feed flow rates. Typically, the trial starts at feed flux rates of 1 L/min/m², and is stepped down to near 0.1 L/min/m², until the desired target conversion is reached or a gel point is reached and the product cannot be further concentrated. Each feed flow point is stabilized for 5 – 10 minutes prior to taking measurements. Longer times are required at lower flow rates to allow for displacement of channel volumes.

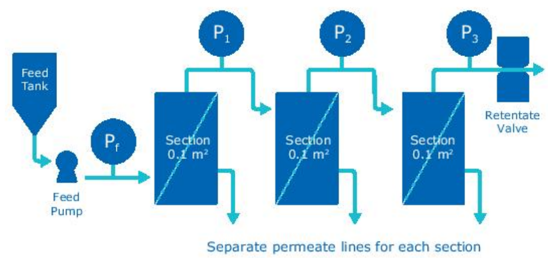


Figure 3A. Bench-scale setup for single-pass TFF system.

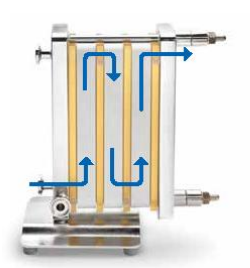


Figure 3B. Bench-scale setup for single-pass TFF system using diverter plates.

Figure 3C. A diverter plate for bench-scale single-pass TFF setup.

It is convenient to plot the experimentally obtained conversion $Y=Q_{Ptotal}/Q_F$ vs the feed flux J_F. For any number of sections in series, one uses the total permeate in calculating Y and the total area in calculating J_F.

$$Y_n = \frac{\sum_{i=1}^{n} Q_i}{Q_F}$$

Where Y_n is the conversion for an n-section SPTFF process, Q_i is the permeate flow measured for sections 1 through i, and Q_F is the total feed flow into the system measured as inlet or sum of retentate and permeate. The volume reduction factor (VRF) is defined as:

$$VRF = 1/(1-Y)$$

By conservation of mass, the steady-state retentate concentration is expressed as:

$$C_{ret} = C_{Feed} VRF^R$$

Evaluation of single-pass TFF with model feed of 20 g/L and 75 g/L demonstrates how the conversion increases with decreasing feed flow rate, and increases with the number of sections in series (**Figure 4A**). Measured retentate concentrations confirm predicted concentrations using the mass balance (**Figure 4B**).

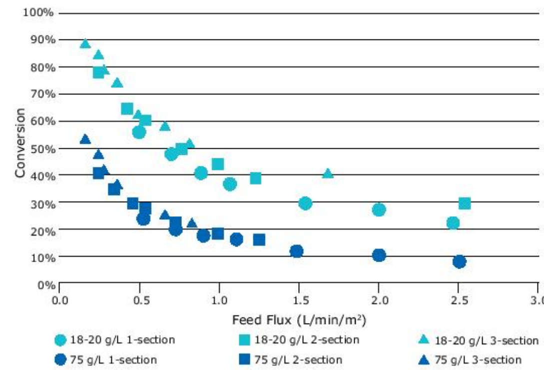


Figure 4A. Example of feed flux excursion.

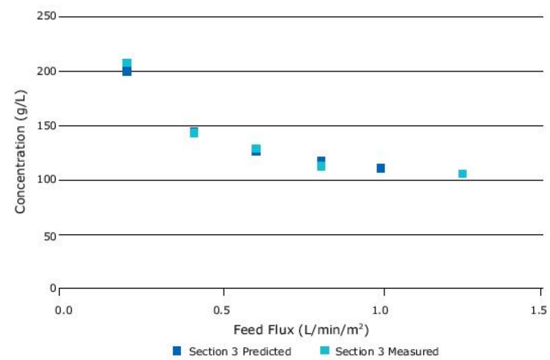


Figure 4B. Comparison of measured vs predicted retentate concentrations.

Cleaning in single-pass TFF

Cleaning of single-pass TFF systems can be performed in single-pass mode without a recirculation loop, using typical cleaning agents (**Figure 5**). Cleaning does not require different pump or piping.

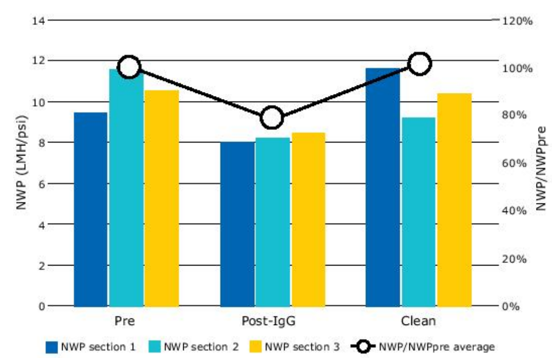


Figure 5. Cleaning of cassettes in series.

Scaling up single-pass TFF

Scale-up uses existing holders and cassettes, including Pellicon® cassettes that are commercially available, and requires no additional customized equipment.

To scale up single-pass TFF, first define the feed flow (equals batch volume divided by the desired process time) and target conversion or concentration. **Figure 6** shows an example in which conversion greater than 52% was required, with a feed flow of 25 L/min. Using the data in **Figure 6**, a 52% conversion (yellow line) gives feed flux values of 0.6 L/min/m² for one-section, 0.7 L/min/m² for two-section, and 0.8 L/min/m² for three-section single-pass TFF.

Next, define the required membrane area by dividing the desired feed flow rate by the feed flux. In the **Figure 6** example, the calculation for a two-section process is: 25 L/min ÷ 0.7 L/min/m² = 36 m² total area. This corresponds to a two-section process with 18 m² area per section.

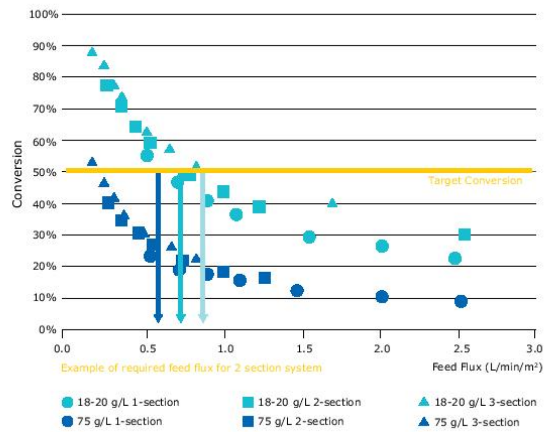


Figure 6. Using the feed flux excursion graph to determine the required feed flux for 1 section (dark blue), 2 sections (blue), and 3 sections (light blue).

Case study at manufacturing scale

An example of a single-pass TFF application for an existing manufacturing facility, where improved cell culture titers placed increased demand on downstream operations and tank capacities, was reported by Teske et. al. (Biotechnol. Prog. 26(4):1068-72). In this process, the product pool following virus filtration exceeded the available tank capacity by 14%. The use of 4 m² of cassettes in a single section met the 20 L/min flow and volume reduction requirements.

Summary

Use of Pellicon® cassettes in a single-pass tangential flow filtration mode is an effective way of concentrating biomolecules. Small-scale evaluations are simple to execute and analyze. The power of implementing single-pass TFF lies in its ability to reduce in-process volumes in a simple and easy-to-use step. Single-pass TFF enables higher concentration formulations and allows facilities to meet the demands of higher titer processes without major investments in new equipment.

MilliporeSigma
400 Summit Drive
Burlington, MA 01803

To place an order or receive technical assistance

Please visit
EMDMillipore.com/contactPS

For additional information, please visit
EMDMillipore.com

AN5572EN00 Ver. 2.0
2016-00978
03/2018

MILLIPORE

- Leading-edge void-free membranes to match virtually any separation challenge
- Short flow path for higher flux and higher resolution separation capability
- Choice of flow channel configuration providing process optimization capability
- Predictable, fast, scale-up
- True linear scalability from laboratory size modules to industrial assemblies for processing thousands of liters

DATA SHEET

Pellicon® 2 Filters and Holders

High-performance tangential flow filters for biopharmaceutical process development, scale-up/scale-down and concentration/purification/cell harvesting applications

Typical Applications

Concentration, desalting or buffer exchange of:

- Protein solutions
- Polysaccharide solutions
- Virus suspensions

Harvest, washing or clarification of:

- Cell cultures and lysates
- Colloidal suspensions
- Viral cultures

Superior TFF Performance

For research, process development, scale-up and production, Pellicon 2 filters and holders offer the following benefits:

Consistent High Flux and High Product Recovery

Millipore's Biomax® polyethersulfone and Ultracel® PLC-composite regenerated cellulose membranes have void-free structures that guard against leakage of solutes through microdefects normally associated with voids beneath the thin skins of conventional UF membranes (Figures 1 and 2).

These void-free membranes are more permeable, resulting in high-flux with equivalent or superior product retention (Figure 3). These void-free membranes provide the advantages of fast, high yield processing and smaller systems.

The long established Durapore® hydrophilic PVDF microfiltration membrane is well known for its exceptional combination of high flux, low protein binding and high product recoveries.

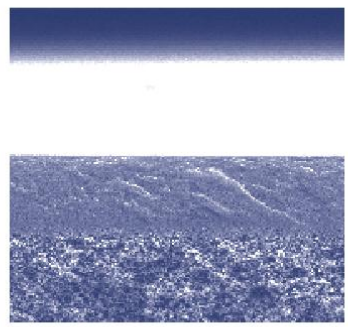


Figure 1. *Void-free Biomax 10 modified polyethersulfone membrane*

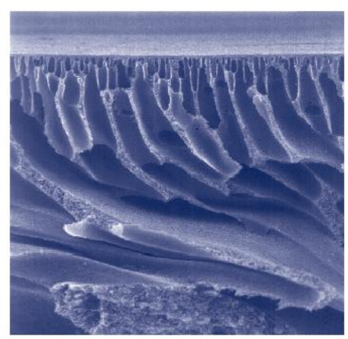


Figure 2. *Conventional 10 kD polyethersulfone membrane with sub-surface voids*

Easy, Reliable Linear Scale-Up from the Lab to the Production Plant

Pellicon 2 Mini filters scale-up easily and reliably from the laboratory to the production plant (Figures 4 and 5). By ensuring every flow channel has the same length, height and turbulence promoter as well as flow direction and materials of construction, we maintain the same ultrafilter/microfilter performance at all scales. Thus, rapid and reliable translation of processes from lab to manufacturing scale is easily achieved.

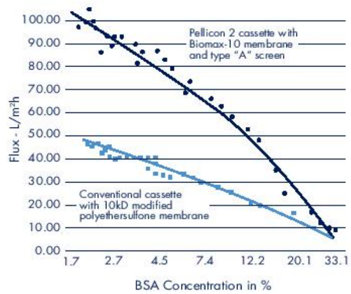
Linear Scale-Up

Mini filters (0.1 m²/1.1 ft²) and holders are designed for laboratory ultrafiltration/microfiltration of 100 mL to 10 L volumes, yet scale up linearly to Pellicon 2 Cassette (0.5 m²/5.4 ft²) and Maxi (2.5 m²/26.9 ft²) filters used in the pilot or manufacturing plant to process volumes from one liter to thousands of liters.

Thus, whether you operate 0.1 m² or 100 m² of installed area, every Pellicon 2 filter operates with the same pressure drop, flow velocity and concentration profile for true, rapid and simple linear scale-up.

Pellicon 2 Filters Proof of Performance

Improved Flux



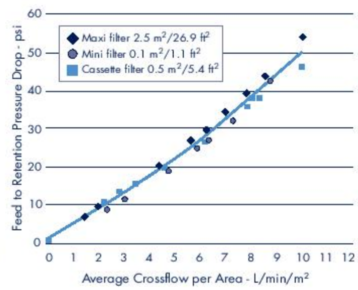
Feed pressure: 5.6 bar/80 psi
Retentate pressure: 2.1 bar/30 psi
Temperature: 10 – 13.5 °C
Initial volume 28 L
Final volume: 2 L

Conclusion

Pellicon 2 filters with Biomax membranes provide up to two-times the process flux of conventional cassettes resulting in faster processing and smaller systems.

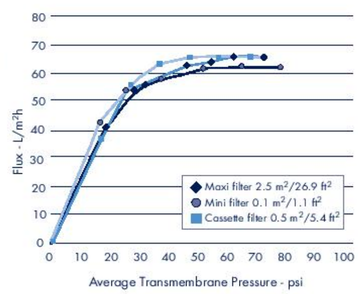
Figure 3. *Flux versus BSA concentration*

Linear Scalability



Temperature: 8 °C

Figure 4. *Feed to retentate pressure drop versus average crossflow on a 10% BSA solution*



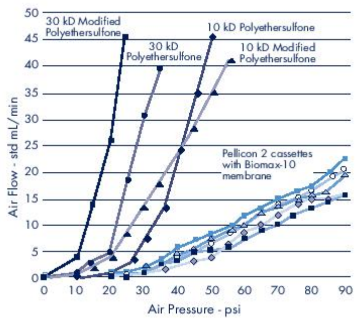
Temperature: 8 °C
Feed to retentate pressure drop: 2.8 bar/40 psi

Conclusion

(Figures 4 and 5) Pellicon 2 family of cassette filters scale linearly from 0.1 to 0.5 to 2.5 m² (1.1 to 5.4 to 26.9 ft²) sizes for rapid, accurate and safe process scale-up and transfer.

Figure 5. *Flux versus average transmembrane pressure on a 10% BSA solution.*

Improved Reliability



Conclusion

The void-free structure of Biomax membranes is demonstrated by low, linear air diffusion values. This performance ensures better process reliability and safety and better product retention for higher yields.

Figure 6. *Integrity test comparison-air flow through wetted cassettes*

Greater Process Reliability and Reproducibility

The combination of defect-free membranes with Millipore's highly reliable manufacturing processes, offers greater consistency of process parameters.

The high quality of Millipore's ultrafiltration membranes is further ensured by our pioneering multiple-solute mixed-dextran retention profile test. Unlike the single solute protein retention test, Millipore's retention profile test measures and ensures reproducible retention performance of our UF membranes over the entire range of molecular weights retained by the membrane, not just at one or two molecular weights.

Low Product Loss

Pellicon 2 filters have a low minimum working volume – as low as 175 mL of retentate volume per square meter of membrane area. This low retentate volume permits high concentration factors to be reached with low starting volumes and maximizes the recovery of small sample volumes.

To prevent product loss, Pellicon 2 filters are 100% tested in manufacturing to ensure that every filter is integral.

In addition, Biomax and Ultracel membranes are exposed to a new high-pressure integrity test that provides greater sensitivity. The integrity test procedure and specifications are supplied so users can confirm integrity at high pressure when the filter is installed (Figure 6).

Biocompatibility

All wetted parts have been tested and meet the requirements of the USP Class VI biological test for plastics.

Superior Filter Quality

Pellicon cassettes are subjected to a complete array of quality control release tests.

A Certificate of Quality is included with every cassette.

Each casette is identified with a unique serial number.

Validatable

Since 1973, Pellicon filters and systems have been successfully used for development and scale-up of processes for manufacturing injectable protein and polysaccharide drugs, in the serum fractionation, biotechnology, vaccine and pharmaceutical industries.

Pellicon 2 filters and systems were developed based upon Millipore's experience serving these applications, and are supported by an extensive Validation Support Data Package proving performance claims and demonstrating the suitability of these filters for drug manufacturing in validated processes. This package is available upon request.

Millipore can further assist your validation efforts through:

- Design and fabrication of standard and custom turnkey TFF systems for drug manufacturing facilities
- Installation and operational qualification services for these systems
- Validation support services for tangential flow filter use in drug manufacturing processes.
- Training on TFF process scale-up, optimization and development.

A Choice of Feed Channel Screens

For optimal performance in a range of applications Pellicon 2 filters incorporate three types of feed-channel screens:

- *Type A screen (tight screen)* is optimized to operate Biomax membranes with maximum flux with low-viscosity solutions.
- Type C *screen (coarse screen)* is optimized to operate PLC series membranes with maximum flux. The Type C screen is also available with Biomax-50, 100, 300, 500 and Biomax 1000 membranes for concentration and diafiltration of viscous solutions.
- *Type V screen (open channel)* is optimized for very viscous solutions or solutions with higher levels of suspended solids.

For More Detailed Information

Request literature number P17512 – User Guide for Pellicon Filters.

Normalized Recirculation Rates

Parameter	Unit	Typical ΔP		
Screen Type		A	C	V
Recirculation Rate	L/min/m^2	4/6	5/35	
Differential Pressure	bar/psi	1.4/20	0.4/6	

Screen Selection Guidelines

Solution Type	Screen Type
Dilute protein solution or low viscosity solutions (MAbs, interferons)	A screen (tight screen)
Concentrated protein solutions or high viscosity solutions (IgG, biopolymers)	C screen (course screen)
High viscosity solutions (polysaccharides, certain microfiltration or clarification applications)	V screen (loose screen)

Specifications

Temperature Range

Mini, Cassette and Maxi:
4 to 50 °C

Maximum Forward Transmembrane Pressure

Device Size (m²)	Biomax	Ultracel
0.1	6.8 bar (100 psi) Max	6.8 bar (100 psi) Max
0.5	6.8 bar (100 psi) at 30 °C	3.4 bar (50 psi) at 30 °C
2.5	6.8 bar (100 psi) at 30 °C	3.4 bar (50 psi) at 30 °C

Maximum Reverse Transmembrane Pressure

Device Size (m²)	Biomax	Ultracel
0.1	0.33 bar (5 psi)	0.33 bar (5 psi)
0.5	0.33 bar (5 psi)	0.33 bar (5 psi)
2.5	0.33 bar (5 psi)	0.33 bar (5 psi)

Prefiltration Required

Mini, Cassette and Maxi:
100 µm

Dimensions

Device	Width	Length	Thickness
Mini	5.6 cm	21 cm	1.5 cm (V screen-2.16 cm)
Cassette	17.8 cm	21 cm	1.5 cm (V screen-2.16 cm)
Maxi	17.8 cm	21 cm	7.6 cm (V screen-9.0 cm)

Membrane Selection Guideline

Membrane Type	Materials	Benefits
Biomax	Modified polyethersulfone	Highest flux ultrafiltration membrane Excellent chemical resistance Void-free structure for higher yield and reliability
Ultracel PLC	Regenerated cellulose (ideal for protein solutions < 20 g/L)	Extremely low protein binding hydrophilic membrane
	PLC membranes are composite membranes cast on a microporous substrate for defect-free membranes with superior adhesion.	Highest product recovery and improved performance with difficult to process streams (antifoams, lipids, protein transmission applications)
	Brings higher resolution, improved yields and superior back-pressure resistance	
Durapore	Hydrophilic PVDF	Very hydrophilic microporous membrane for cell harvest or clarification applications

Pellicon 2 Membrane Selection Chart

Approximate Molecular Weight (range of solutes retained >99%, kD)		Membrane	NMWL (kD) or Microns	Membrane Material	pH Range
High Flux Biomax Membranes – Void-free for Higher Yield and Reliability					
12 – 25	(growth factors, hormones)	Biomax-5	5	modified polyethersulfone	1 – 14
25 – 50	(growth factors, hormones)	Biomax-8	8	modified polyethersulfone	1 – 14
50 – 100	(albumin, hemoglobin)	Biomax-10	10	modified polyethersulfone	1 – 14
100 – 140	(enzymes)	Biomax-30	30	modified polyethersulfone	1 – 14
140 – 300	(IgG's)	Biomax-50	50	modified polyethersulfone	1 – 14
300 – 500	(small viruses and antigens)	Biomax-100	100	modified polyethersulfone	1 – 14
>500	(IgM's, large viruses)	Biomax-300	300	modified polyethersulfone	1 – 14
>0.03 μm	(large viruses, colloids, particulates)	Biomax-500	500	modified polyethersulfone	1 – 14
>0.03 μm	(large viruses, cells, colloids, particulates)	Biomax-1000	1000	modified polyethersulfone	1 – 14
Ultracel PLC Series – for High Recoveries					
8 – 18	(proinsulin, hematopoetic factors)	PLCCC	5	regenerated cellulose	2 – 13
18 – 60	(hemoglobin, enzymes)	PLCGC	10	regenerated cellulose	2 – 13
60 – 200	(monoclonal IgG's)	PLCTK	30	regenerated cellulose	2 – 13
200 – 500	(small viruses, viral antigens)	PLCHK	100	regenerated cellulose	2 – 13
>500	(large viruses, IgM's)	PLCMK	300	regenerated cellulose	2 – 13
>0.03 μm	(large viruses, cells, colloids, particulates)	PLCXK	1000	regenerated cellulose	2 – 13
Durapore Membranes – for Microporous Applications					
Clarify cell lysates and protein solutions, clarify viral cultures		VVPP	0.1 μm	hydrophilic PVDF	2 – 11
Harvest & wash colloidal suspensions, bacterial cells; clarify protein solutions and viral cultures		GVPP	0.22 μm	hydrophilic PVDF	2 – 11
Harvest & wash colloidal suspensions, cell & viral cultures, clarify protein solutions & viral cultures		HVMP	0.45 μm	hydrophilic PVDF	2 – 11
Harvest cell cultures or colloidal suspensions		DVPP	0.65 μm	hydrophilic PVDF	2 – 11

Ordering Information

Pellicon 2 Filters

	Filters with A Screens (Tight Screen)			Filters with Type C Screens (Coarse Screen)		
Membrane	**0.1 m²/1.1 ft²**	**0.5 m²/5.4 ft²**	**2.5 m²/26.9 ft²**	**0.1 m²/1.1 ft²**	**0.5 m²/5.4 ft²**	**2.5 m²/26.9 ft²**
Biomax Series – Modified Polyethersulfone						
Biomax 5	P2B0 05A 01	P2B0 05A 05	P2B0 05A 25	+	+	+
Biomax 8	P2B0 08A 01	P2B0 08A 05	P2B0 08A 25	+	+	+
Biomax 10	P2B0 10A 01	P2B0 10A 05	P2B0 10A 25	+	+	+
Biomax 30	P2B0 30A 01	P2B0 30A 05	P2B0 30A 25	+	+	+
Biomax 50	P2B0 50A 01	P2B0 50A 05	P2B0 50A 25	P2B0 50C 01	P2B0 50C 05	P2B0 50C 25
Biomax 100	P2B1 00A 01	P2B1 00A 05	P2B1 00A 25	P2B1 00C 01	P2B1 00C 05	P2B1 00C 25
Biomax 300	+	+	+	P2B3 00C 01	P2B3 00C 05	P2B3 00C 25
Biomax 500	+	+	+	P2B5 00C 01	P2B5 00C 05	P2B5 00C 25
Biomax 1000	+	+	+	P2B0 1MC 01	P2B0 1MC 05	P2B0 1MC 25
Ultracel PLC Series – Regenerated Cellulose, Composite Construction						
5 kD	NA	NA	NA	P2C0 05C 01	P2C0 05C 05	P2C0 05C 25
10 kD	NA	NA	NA	P2C0 10C 01	P2C0 10C 05	P2C0 10C 25
30 kD	NA	NA	NA	P2C0 30C 01	P2C0 30C 05	P2C0 30C 25
100 kD	NA	NA	NA	P2C1 00C 01	P2C1 00C 05	P2C1 00C 25
300 kD	NA	NA	NA	P2C3 00C 01	P2C3 00C 05	P2C3 00C 25
1000 kD	NA	NA	NA	P2C0 1MC 01	P2C0 1MC 05	P2C0 1MC 25
Durapore – Hydrophilic PVDF						
0.1 μm	+	+	+	P2VV PPC 01	P2VV PPC 05	P2VV PPC 25
0.22 μm	+	+	+	P2GV PPC 01	P2GV PPC 05	P2GV PPC 25
0.45 μm	+	+	+	P2HV MPC 01	P2HV MPC 05	P2HV MPC 25
0.65 μm	+	+	+	P2DV PPC 01	P2DV PPC 05	P2DV PPC 25

Each Pellicon filter is packed one per box and includes Operating Instructions. A Certificate of Quality is included in every box.
Silicone intercassette gaskets are required for use with Pellicon 2 filters. Two gaskets are packed in the box with every Pellicon 2 filter.
\+ = On request (custom order)
NA = not available

Filters with V Screens (Loose Screen)		
0.1 m²/1.1 ft²	0.5 m²/5.4 ft²	2.0 m²/21.5 ft²
P2B0 05V 01	P2B0 05V 05	P2B0 05V 20
P2B0 08V 01	P2B0 08V 05	P2B0 08V 20
P2B0 10V 01	P2B0 10V 05	P2B0 10V 20
P2B0 30V 01	P2B0 30V 05	P2B0 30V 20
P2B0 50V 01	P2B0 50V 05	P2B0 50V 20
P2B 100V 01	P2B1 00V 05	P2B1 00V 20
P2B3 00V 01	P2B3 00V 05	P2B3 00V 20
P2B5 00V 01	P2B5 00V 05	P2B5 00V 20
P2B0 1MV 01	P2B0 1MV 05	P2B0 1MV 20
P2C0 05V 01	P2C005V 05	P2C0 05V 20
P2C0 10V 01	P2C0 10V 05	P2C0 10V 20
P2C0 30V 01	P2C0 30V 05	P2C0 30V 20
P2C1 00V 01	P2C1 00V 05	P2C1 00V 20
P2C3 00V 01	P2C3 00V 05	P2C3 00V 20
P2C0 1MV 01	P2C0 1MV 05	P2C01MV 20
P2VV PPV 01	P2VV PPV 05	P2VV PPV 20
P2GV PPV 01	P2GV PPV 05	P2GV PPV 20
P2HV MPV 01	P2HV MPV 05	P2HV MPV 20
P2DV PPV 01	P2DV PPV 05	P2DV PPV 20

Pellicon 2 Mini Holder

Pellicon 2 Mini holder operates one to three Mini filters in parallel for total areas of 0.1 to 0.3 m^2 (1.1 – 3.3 ft^2). This sanitary holder is tightened with a small torque wrench to compress the filters between a manifold plate that conveys fluids in and out of the filters and an end plate that seals the filters together. The Mini holder is designed for process development and small volume pharmaceutical manufacturing.

Materials of Construction

Manifold and End Plates:
316 L stainless steel

Base, Tie Rods, Spacers and Washers:
304 stainless steel

Feet:
Thermoplastic rubber

Gaskets:
Silicone

Nuts:
Silicone bronze

Separator Plates

An optional separator plate allows processing simultaneously with up to three 0.1 m^2/1.1 ft^2 cassettes to determine the best molecular weight cut-off in a single study on the same feed material.

Connections

All manifold connections are standard ½-inch sanitary clamp type.

Operating Parameters

Temperature Range:
4 to 50 °C. The Mini holder can be autoclaved without filters installed. The filters themselves cannot be autoclaved.

Maximum Pressure:
6.8 bar

Dimensions

Height: 260 mm; *Width:* 114 mm

Length: 140 mm; *Weight:* 5 kg

Holder Manifold Volume:

Feed plus retentate: 5.3 mL

Permeate: 6.4 mL

Stainless Steel Pellicon Holder

XX42P0080

The stainless steel Pellicon filter holder, designed for sanitary applications, can be used alone or to expand existing cassette ultrafiltration (CUF) systems or to replace existing holders.

It requires only to be connected to an existing sanitary pump and piping for tangential flow microporous filtration or ultrafiltration.

It can accomodate up to 5 m²/55 ft² filter area as shipped with long tie rods or 0.5 to 2.5 m² (5.4 – 26.9 ft²) with accessory short tie rods.

Materials of Construction

Wetted Surfaces:
316 L stainless steel

Non-wetted Surfaces:
Silicon bronze nuts

Dimensions

Length: 28 cm; *Width:* 19 cm

Height: 25 cm

Operating Parameters

Operating Temperature Range:
4 to 50 °C. The Pellicon holder can be autoclaved without pressure gauges and filters; holder with gauges cannot be steamed. Pellicon filters cannot be steamed or autoclaved.

Connections

Sanitary ¾" TC connections;
1½" TC connections for gauges.

Shipping Weight

24 kg

Process-scale Pellicon Holder

The Pellicon Process-scale Holder is a unique innovation for production scale Pellicon systems. This holder, vertically mounted, can hold up to 80 m²/880 ft² of membrane area.

Benefits

- Extremely compact footprint
- Easy to change cassettes
- Easy to vent and fully drain
- Simple connections
- Up to 4 levels. Can be easily extended in levels for simple membrane area expansion
- Each level up to 20 m²/220 ft²
- Uses standard and Maxi Cassettes
- Can be adapted for series or parallel configurations
- Simplifies pipework connection
- Hydraulic closure systems are available for the stainless-steel Pellicon holder and the process-scale Pellicon holder. These systems are convenient, reliable and easy to use to enable rapid and repeatable loading operation and storage of Pellicon 2 cassettes.

Materials of Construction

Manifold segment, fitting blocks and end plate 316 L stainless steel; tie rods 304 and 304 L stainless steel.

Ordering Information

Pellicon 2 Filter Holders

Description	Catalogue No.
Pellicon 2 Mini filter holder	XX42 PMI NI
Pressure gauges One diaphragm-protected digital pressure gauge, 0 – 7 bar, ¾-inch fittings	XX42 PSG 01L
Pressure gauge adapters	XX42 PM0 01
Fitting kit Contains all tees, clamps, gaskets and a valve to connect tubing and pressure gauges to the Pellicon 2 Mini holder	XX42 PFK 01
Pellicon filter holder (for cassettes and Maxi filters)	XX42 P00 80
Pellicon 2 double thick gasket	PSSP 2XC 10
Pellicon Process-scale holder support and plate	XX42 SSP LT
Pellicon Process-scale holder	On request

To Place an Order or Receive Technical Assistance

For additional information call your nearest Millipore office:
In the U.S. and Canada, call toll-free **1-800-MILLIPORE (1-800-645-5476)**
In the U.S., Canada and Puerto Rico, fax orders to **1-800-MILLIFX (1-800-645-5439)**
Outside of North America contact your local office. To find the office nearest you visit **www.millipore.com/offices.**
Internet: **www.millipore.com**
Technical Service:
www.millipore.com/techservice

MILLIPORE

A Typical Pellicon Production Processing System

Millipore supplies a range of standard and custom engineered systems. These systems can contain from 1 m²/11 ft² to several hunded m² of membrane area, with Clean-in-Place (CIP) or Steam-in-Place (SIP) integrated as appropriate. Systems can also be supplied with integrated process vessels in manual or fully automatic versions.

All systems are designed, engineered and manufactured in ISO® 9001 registered facilities, and are supplied with extensive validation data support packages.

Please contact us to discuss your specific application and process requirements.

Pellicon XL Devices for Process Development

For process development of volumes from 50 mL to 1 liter, Millipore offers Pellicon XL devices. This small volume TFF filter is designed for true scalability by providing the same flow path, channel length, and channel height as the Pellicon 2 cassettes. Based on proven TFF membrane technology, Pellicon XL devices ensure reliable, consistent and predictable performance.

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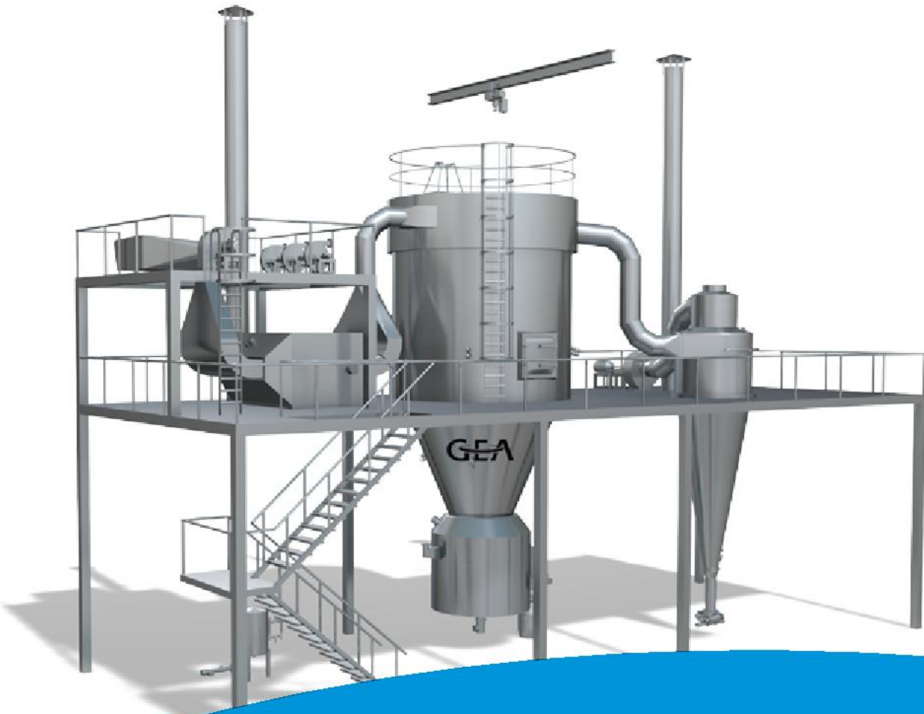


VERSATILE-SD®
Versatile Spray Dryer, FSD®

Designed for food

GEA engineering for a better world

gea.com

Expedite your time to market

GEA offers a range of spray dryers designed specifically for R&D, product development and small-volume production. As a pioneer in all aspects of spray drying, with more than 10,000 contracted and installed plants worldwide, we can help you to choose the most suitable equipment, assess each project on its individual needs, and tailor both the process and the spray dryer to match your specific requirements.

However, we also understand that not every plant needs to be custom built. That is why we have designed standard spray drying units that are flexible, compact and easy to install.

To optimize your process development projects and shorten your time to market, the VERSATILE-SD® (VSD) and the Versatile Spray Dryer, FSD® (VFSD) have been designed to fast-track product development without compromising powder quality. These units are perfect for both large and small companies that wish to launch a new food concept or produce small batches of high-value products, saving time and cost on the design, purchase and installation of the spray drying unit.

Flexible, safe and easy to clean

Not only are the VSD and VFSD cost-effective, compact and easy to install, they are also extremely versatile. Five sizes with five standard configurations and different exhaust systems provide the flexibility needed to produce different foods, flavors and other products at a variety of production rates.

Being modular, these standard units can be quickly reconfigured or expanded, offering fast turnaround times between product changeovers. As with all GEA equipment, the VSD and VFSD are equipped with the ultimate in hygiene, safety and monitoring features, including optional clean-in-place (CIP) functionality.

Consistent and reliable

With quality control being a key concern, the VSD and VFSD have been developed to be reliable and consistent in delivering products of the desired quality. The control system and HMI (interface) allow specific product and parameter settings to provide both repeatability and easy operation.

More than just a spray dryer

We help our customers to configure the right product recovery system and develop the right process for their product, assessing factors such as organoleptic properties and powder functionality. We can also advise on plant configuration, auxiliary equipment and environmental aspects.

Spray dryers can also be used for spray congealing applications.

GEA test centers are available for process development, tests and trial runs.

Versatile Spray Dryer, VERSATILE-SD®

The VSD spray dryer processes single-particle powder products. The flexible atomization system has a wide range of functions that atomize and convert a liquid feed into a fine powder according to your specifications.

Key benefits

- Ready-to-use standard units for easy product selection, delivery and installation
- Available with five different exhaust systems
- Available in five different sizes with water evaporation capacities from 6 to 320 kg/h
- Easy to operate, clean and switch between batches
- Handles food products at low air inlet temperatures, producing a fine powder
- Explosion protection system
- Optional features include CIP, electrical air heat booster, etc.

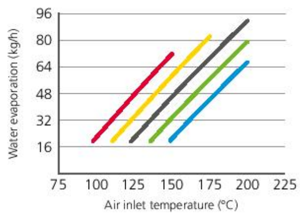
All VSD configurations include the following: an indirect gas heater, a rotary atomizer, a swan-neck chamber and an exhaust system with the following options: cyclone, bag filter, cyclone plus cyclone, cyclone plus bag filter or cyclone plus scrubber.

The VSD is available in five different sizes ranging from a VERSATILE-SD® size 6.3 to a VERSATILE-SD® size 80. We can also offer smaller-scale units such as our trusted MOBILE MINOR® and PRODUCTION MINOR®.

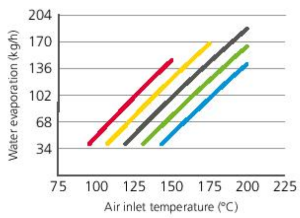
VERSATILE-SD®

Size	VSD-25	VSD-50	VSD-80
Water evaporation capacity (kg/h)	20-90	40-185	60-320
Typical mean particle size (µm)	20-100	20-100	20-100
Space requirements L x W x H (m)	13 x 6 x 11	14.5 x 6.7 x 12.5	19 x 7.5 x 14.5

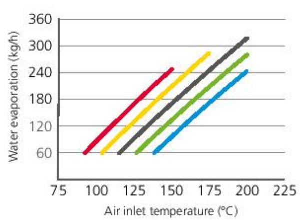
VSD-25 co-current atomization



VSD-50 co-current atomization



VSD-80 co-current atomization



Air outlet temperature

- 70 °C
- 80 °C
- 90 °C
- 100 °C
- 110 °C

*VSDs can be configured for temperatures above 200 °C

Versatile Spray Dryer, FSD®

The VFSD spray drying plant is a multistage dryer with an integrated fluid bed under the drying chamber. By combining spray drying and fluid bed technology it provides the same performance as a dryer with external fluid beds – but with a much smaller footprint.

The VFSD dries product into larger agglomerated particles. Drying and agglomeration take place in the same chamber producing coarse, dustless, free-flowing powders. This technology is particularly appropriate for sticky, hygroscopic and/or heat-sensitive food applications.

Agglomeration improves the powder's ability to flow and disperse without forming lumps. The fluid bed process can also be used to improve a powder's bulk density or particle size to make a more homogeneous product with a lower segregation tendency.

Key benefits

- Ready-to-use standard units for easy product selection, delivery and installation
- Available with five different exhaust systems
- Available in six different sizes with water evaporation capacities from 4 to 265 kg/h
- Easy to operate, clean and switch between batches
- Handles foodstuffs to produce large agglomerated powder particles
- Explosion protection system
- Optional features include CIP, electrical air heat booster, etc.

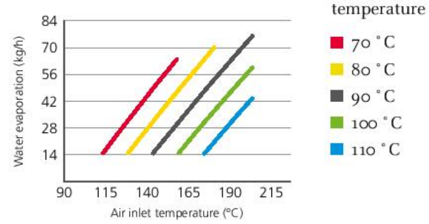
Versatile Spray Dryer, FSD®

Size	VFSD-25	VFSD-50	VFSD-80
Water evaporation capacity (kg/h)	15-77	30-165	40-265
Typical mean particle size (µm)	100-250	100-250	100-250
Space requirements L x W x H (m)	13.5 x 6 x 11	16 x 6.7 x 12	19 x 7.5 x 13.1

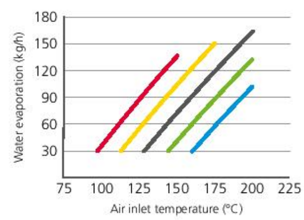
All VFSD configurations include the following: an indirect gas heater, pressure nozzle atomization, a chamber with an integrated 'wraparound' static fluid bed for gentle drying and cooling prior to powder discharge and bagging, and an exhaust system with the following options: cyclone, bag filter, cyclone plus bag filter, cyclone plus cyclone or cyclone plus scrubber.

The VFSD is available in six different sizes ranging from a versatile FSD® size 4.0 to a FSD® size 80. We also offer the smaller-scale FSD MINOR® for R&D purposes.

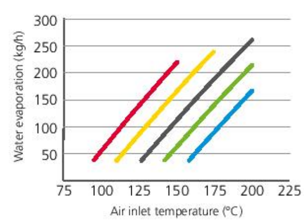
VFSD-25 co-current atomization



VFSD-50 co-current atomization



VFSD-80 co-current atomization



*VFSDs can be configured for temperatures above 200 °C

engineering for
a better world

We live our values.

Excellence • Passion • Integrity • Responsibility • GEA-versity

GEA is a global technology company with multi-billion euro sales operations in more than 50 countries. Founded in 1881 the company is one of the largest providers of innovative equipment and process technology. GEA is listed in the STOXX® Europe 600 Index. In addition, the company is included in selected MSCI Global Sustainability Indexes.

GEA Denmark

GEA Process Engineering A/S

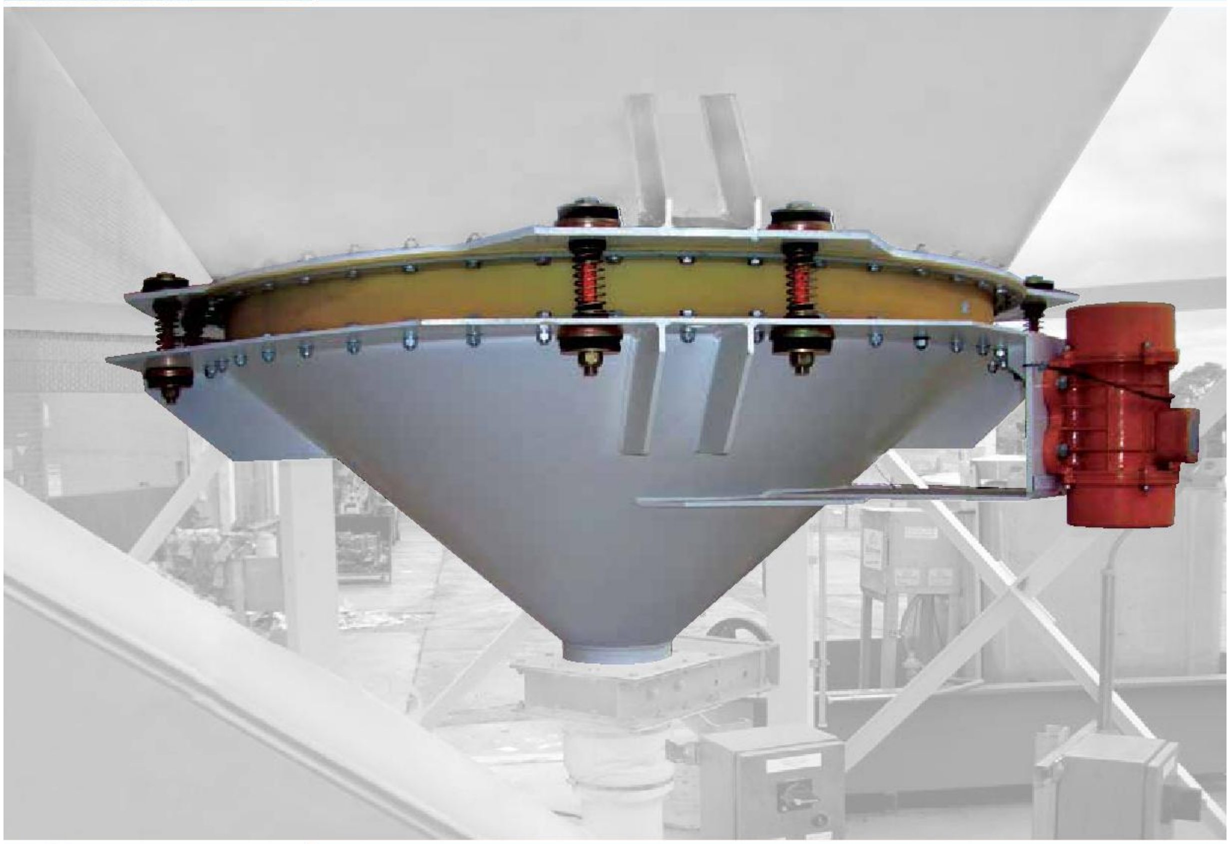
Gladsaxevej 305 | Tel +45 39 54 54 54 | gea.com/contact

DK-2860 Soeborg | Fax +45 39 54 58 00 | gea.com/food

SOLIDS DISCHARGING EQUIPMENT

BA

Bin Activators



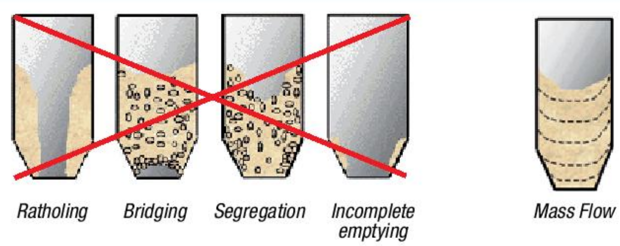
WAMGROUP

EXTRAC

BA
Bin Activators
SOLIDS DISCHARGING EQUIPMENT

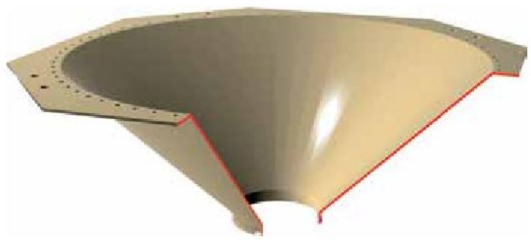
The BA Bin Activator is a discharging device which, thanks to controlled vibration, ensures continuous down-flow of the material from silos and hoppers.

Using this device ensures product extraction with optimum "Mass Flow".



- Ensures uniform descent of the product (MASS FLOW) inside the silo
- Avoids bridging in the discharge cone
- Prevents "ratholing" and segregation of the material to be discharged
- Stops dangerous flushing
- Prevents economic loss due to plant downtimes

- Turned cone constructed using industrial techniques
- Seamless discharging cone
- High flowability
- Maximum resistance to stress
- Available with food-grade painting and in 304L SS

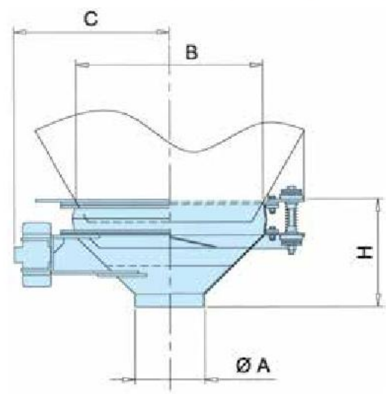


- Suspensions pivoting at 360°
- Maximum efficiency
- Minimum vibration energy consumption
- Minimum vibrations transmitted to silo

- Electric vibrator with adjustable masses, ATEX certified for zone 21

ATEX version zone 22

Negative pressure version for silos

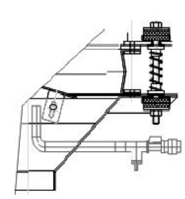


TYPE	Size	Ø A STD	B	C	H	Motors	kg
BA040	400	114	380	427	330	1	59
BA060	600	168	580	519	408	1	80
BA075	750	219	730	609	456	1	99
BA090	900	219	880	684	531	1	134
BA100	1,000	273	980	734	555	1	146
BA125	1,250	273	1,230	937	730	1	290
BA150	1,500	323	1,480	1,120	774	1	475
BA180	1,800	323	1,780	1,194	924	2	726
BA210	2,100	406	2,080	1,420	1,033	2	881
BA235	2,350	406	2,330	1,547	1,166	2	1,255
BA250	2,500	406	2,480	1,705	1,307	2	1,530
BA300	3,000	406	2,980	1,955	1,568	2	2,456

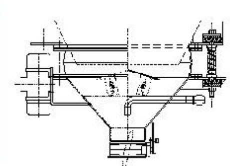
Dimensions in mm

OPTIONS & ACCESSORIES

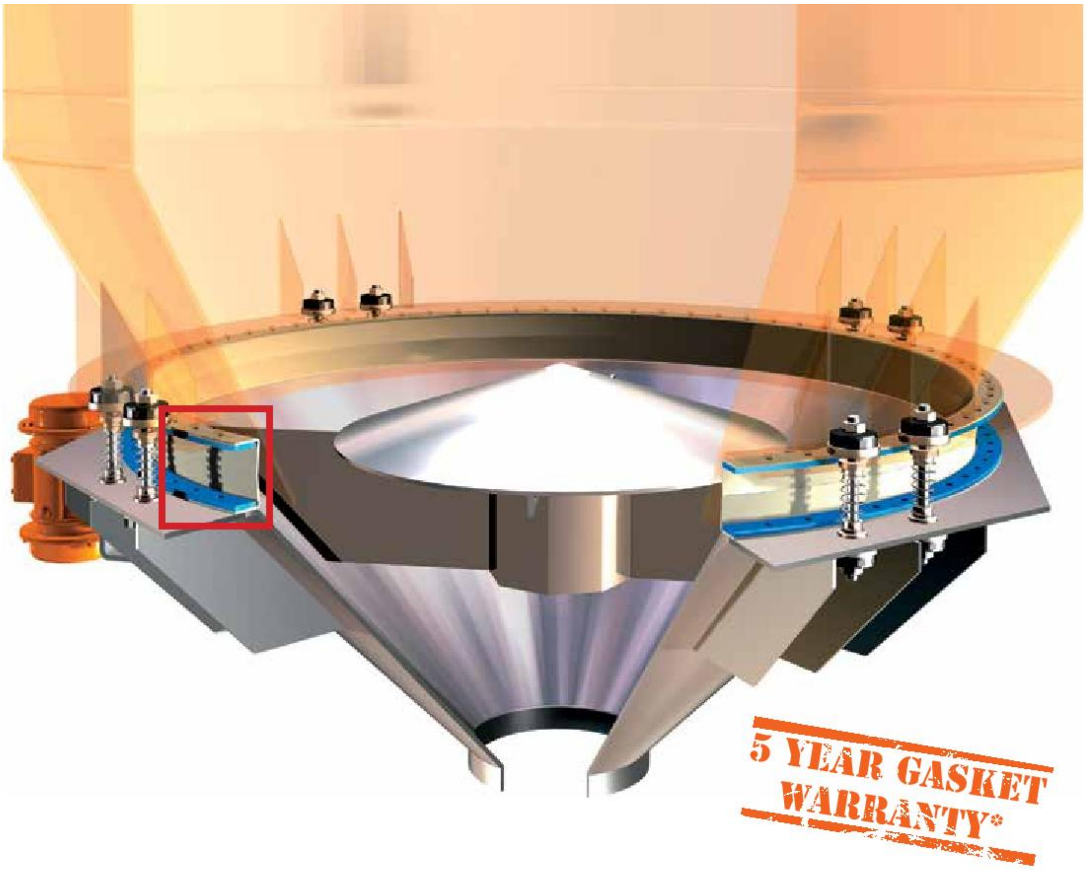
Large range of baffle plates



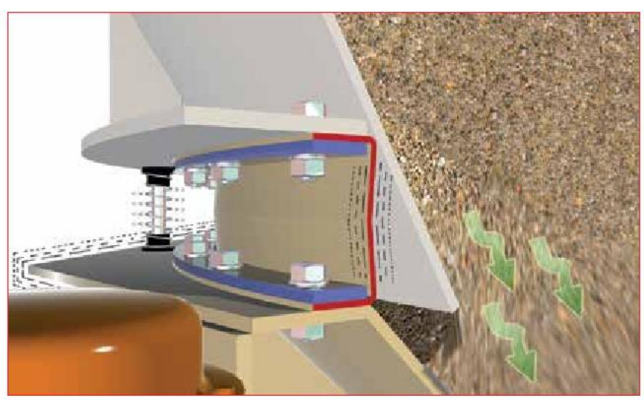
Decompressor



Pneumatic accelerator



GASKET WITH MAXIMUM RELIABILITY:



- Seamless one-piece without joints for maximum resistance to stress
- Double flange for perfect fixing and optimum sealing
- Construction in wear-resistant SINT® food-grade engineering polymer
- Design studied to prevent stagnation, ideal for food and perishable products

** The validity of the warranty is conditioned by strict respect of the conditions of use described in the Installation, Use & Maintenance Manual.*

APPLICATIONS




- Food
- Flour milling
- Animal feed milling
- Plastics
- Chemicals
- Pharmaceuticals
- Glass
- Fertilizers
- Waste water treatment
- Foundries
- Premixed adhesives for ceramics industry

063001569 September 2012

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Food & Beverage

SANI-MATIC.

Clean-In-Place (CIP) Systems:
Consistent, Sanitary Cleaning of Process Lines, Tanks and Vessels.

One-Tank Single-Use

Multi-Tank Detergent and Rinse Recovery/Reuse

Sani-Matic Clean-In-Place (CIP) Systems are engineered to your specific plant application and utility requirements for effective and efficient process equipment cleaning. Proper CIP design and sizing ensure sufficient flow and appropriate pressure to thoroughly remove residue, rinse effectively, shorten cycle times, reduce operating costs and promote worker safety. The Sani-Matic CIP Systems are sanitary and can be engineered to 3-A guidelines.

sanimatic.com

Sani-Matic Clean-In-Place (CIP) Systems can be portable or stationary, single-tank, two-tank, or multi-tank, single-use, re-use, or once-through, as well as designed for multi-circuit capabilities.

Advantages

- **Programming & Controls.** Sani-Matic controls are designed to optimize cycle times and reduce chemical and water use. Ethernet communications interface with individual process control systems for an integrated solution.
- **Craftsmanship.** Sani-Matic craftsmen have decades of experience constructing sanitary systems and weld to AWS D18 standards.
- **Documentation.** Complete documentation to comply with regulatory and customer standards is available, as is the SaniTrend data acquisition and management system.
- **Services.** Sani-Matic's dedicated service team provides full technical support during the start-up and after installation.

Industry Standard Compliance

- UL 508A
- ANSI/ISA-88 Batch Control
- Authorized to Provide Canadian Registration Numbers (CRN)
- Helps Meet FSMA and HACCP Sanitation Preventive Controls
- Can be engineered to 3-A guidelines

Features

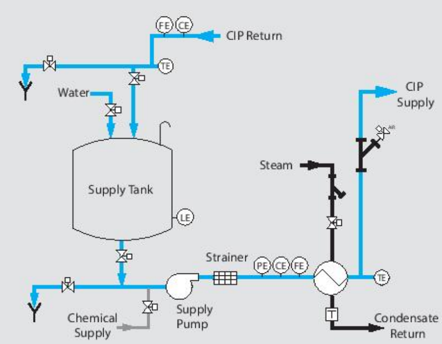
One-Tank Single-Use

- Single-use source of cleaning solution and rinse water
- Lower capital investment
- Portable or stationary design
- Once-through or recirculated
- Simple and flexible operation
- Lower space requirement

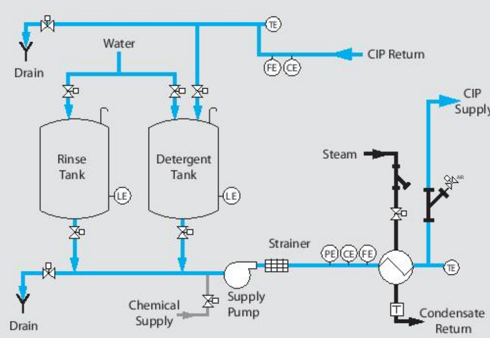
Two-Tank Detergent Recovery/Reuse

- Permits recovery and reuse of wash solution
- Used where water utilities are limited
- Supply/recirculation option
- Decreases wash cycle time

One-Tank Schematic

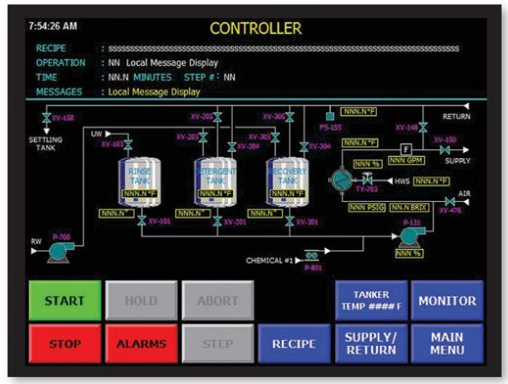


Two-Tank Schematic



Controls

Sani-Matic has designed, manufactured and supported thousands of CIP control systems with its in-house programmers and UL authorized panel shop.



Advantages of Sani-Matic Controls

- **Experts.** The Sani-Matic programming and automation team specializes in cleaning applications and provides proven expertise and customized strategies.
- **Automation Ease & Flexibility.** Easy-to-understand Recipe Editor provides access to setpoints. Defined alarms reduce troubleshooting time.
- **Water & Chemical Savings.** Recipe optimization shortens cycle time and minimizes water and chemical use.

TACT
Time • Action • Chemical • Temperature

We mix our industry-specific expertise with the perfect combination of Time, Action, Chemical and Temperature to deliver the best solution for your cleaning challenge.

Features

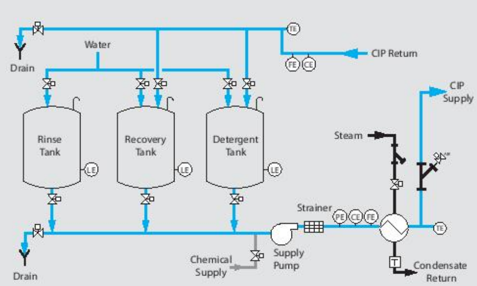
Multi-Tank Detergent and Rinse Recovery/Reuse

- Permits recovery and reuse of wash solution and rinse water
- Reduces water and waste water costs
- Fourth tank may be added for acid recovery and reuse applications (shown in photo above)

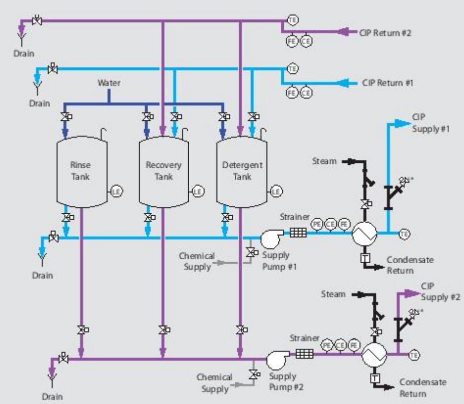
Multi-Circuit and Recovery/Reuse

- Serves multiple circuits independently and simultaneously
- Substantial cost and space-saving advantages over multiple separate units
- Centralized controls
- Reduced wash and rinse cycle times

Multi-Tank Schematic



Multi-Circuit Schematic (Dual Operating)



Cleaning Confidence.

Repeatable results you can count on every time you clean your process parts and equipment.
That's Cleaning Confidence from Sani-Matic.

SANI-MATIC.

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Sani-Matic, Inc. (p) 800-356-3300 (f) 608-222-5348

Appendix C: Material Safety Data Sheets

ATCC® THE ESSENTIALS OF LIFE SCIENCE RESEARCH GLOBALLY DELIVERED™

SAFETY DATA SHEET

SECTION 1 - SUBSTANCE IDENTITY AND COMPANY INFORMATION

Product Name: Various Microbial Cultures at Biosafety Level 1 or 2 or 3
ATCC Catalog #: Various

COMPANY INFORMATION: AMERICAN TYPE CULTURE COLLECTION
PO BOX 1549
MANASSAS, VA 20108

FOR INFORMATION CALL: 800-638-6597 or 703-365-2700
AFTER-HOURS CONTACT: 703-365-2710
CHEMTREC EMERGENCY: 800-424-9300 or 703-527-3887

SECTION 2 - COMPOSITION/INFORMATION ON INGREDIENTS

Either freeze dried, frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water). Frozen Cultures may also contain a 5%-10% solution of Dimethyl sulfoxide as a cryoprotectant.

SECTION 3 - HAZARD IDENTIFICATION

HMIS Rating: N/A
NFPA Rating: N/A

This substance is not hazardous as defined by OSHA 29CFR 1910.1200 however this product should be handled according to good lab practices, with proper personal protective equipment, proper engineering controls and within the parameters of the purchaser's safety program.

Health Hazards

ATCC recommends that all ATCC microbial cultures be handled by qualified microbiologists using appropriate safety procedures and precautions. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming et al) and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories.** This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/biosafety/publications/bmbl5/index.htm .

Information on the classification of human etiologic agents on the basis of hazard can be found as Appendix B in the NIH **Guidelines for Research Involving Recombinant DNA Molecules** at http://grants.nih.gov/grants/policy/recombinentdnaguidelines.htm.

SECTION 4 - FIRST AID MEASURES

Report to your Safety Office and Seek Medical Attention as Soon as Possible

SAFETY DATA SHEET

Ingestion: If person is unconscious seek emergency medical attention; never give anything by mouth to an unconscious person.
If the person is conscious wash mouth out with water and call a physician then administer three cups of water. Do not induce vomiting unless directed to do so by a physician.

Inhalation: If person is unconscious seek emergency medical attention, if person is conscious remove to fresh air and call a physician.

Dermal exposure: Immediately wash skin with water followed by washing with soap and water. Remove all contaminated clothing.

Eye exposures: Flush eyes with water for at least 15 minutes with eyelids separated and call a physician.

SECTION 5 - FIRE FIGHTING MEASURES

Flammability: Data not available

Suitable Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, Halon (where regulations permit), or appropriate foam.

Firefighting Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent inhalation, ingestion, skin and eye contact.

Specific Hazard(s): Responders should take into consideration the biohazard risk associated with responding to a fire in the area where the material may be stored or handled.

SECTION 6 - ACCIDENTAL RELEASE MEASURES

Procedure(s) of Personal Precaution(s): At a minimum use PPE listed in Section 8.
Wear laboratory coat, gloves and eye protection. Avoid all contact.
Methods for Cleaning Up

Patient/Victim: Wash with soap and water. Work clothes should be laundered separately. Launder contaminated clothing before re-use. Do not take clothing home.

Equipment/Environment: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the center; allow sufficient contact time before cleanup (30 min).

Note: The use of additional PPE may be necessary for cleaning solutions.

SECTION 7 - HANDLING AND STORAGE

Handle and store according to instructions on product information sheet and label.
Special Requirements:

SAFETY DATA SHEET

Follow established laboratory procedures when handling material.

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

Use Personal Protective Equipment: Including Eye Protection, Chemical Resistant Gloves, and appropriate clothing to prevent skin exposure. In addition, a Respiratory protection program that complies with OSHA 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Engineering Controls: The use and storage of this material requires user to maintain and make available appropriate eyewash and safety shower facilities. Use appropriate ventilation method to keep airborne concentrations a low as possible.

Exposure Limits: No exposure limits for this material have been established by ACGIH, NIOSH, or OSHA.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Data Not Available

SECTION 10 - STABILITY AND REACTIVITY

Hazardous polymerization will not occur.

SECTION 11 - TOXICOLOGICAL INFORMATION

Route of Exposure

Eye Contact: Data not available.
Skin Contact: Data not available.
Skin Absorption: Data not available.
Inhalation: Data not available.
Ingestion: Data not available.
Parenteral Exposure: Data not available.

Sensitization
Skin: Data not available
Respiratory: Data not available

Target Organ(s) or System(s): Data not available
Signs and Symptoms of Exposure
Skin and Mucous Membranes: Data not available
Respiratory: Data not available
Gastrointestinal: Data not available

Toxicity Data:	Data not available
Effects of Long Term or Repeated Exposure:	Data not available
Chronic Exposure–Teratogen:	Data not available
Chronic Exposure–Mutagen:	Data not available
Chronic Exposure–Reproductive Hazard:	Data not available

SECTION 12 - ECOLOGICAL INFORMATION

No ecological information available.

SECTION 13 - DISPOSAL CONSIDERATIONS

Decontaminate all wastes before disposal (steam sterilization, chemical disinfection, and/or incineration).

Dispose of in accordance with applicable regulations.

SECTION 14 - TRANSPORT INFORMATION

Contact ATCC for transport information.

SECTION 15 - REGULATORY INFORMATION

Contact ATCC for regulatory information.

SECTION 16 - OTHER INFORMATION

DATE REVISED: JUNE 1, 2016

THE INFORMATION PRESENTED IN THIS DOCUMENT IS BELIEVED TO BE CORRECT BASED UPON DATA AVAILABLE TO ATCC. USERS SHOULD MAKE AN INDEPENDENT DECISION REGARDING THE ACCURACY OF THIS INFORMATION BASED ON THEIR NEEDS AND DATA AVAILABLE TO THEM. ALL SUBSTANCES AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND ALL NECESSARY SAFETY PRECAUTIONS SHOULD BE TAKEN. ATCC ASSUMES NO LIABILITY RESULTING FROM USING OR COMING IN CONTACT WITH THIS SUBSTANCE.

American Type Culture Collection (ATCC®)
P.O. Box 1549
Manassas, VA 20110 USA
www.atcc.org

(800) 638-6597 or 703-365-2700
Fax: 703-365-2750
Doc ID: 8409
Effective Date: 08/13/2012
Revision: 4

SECTION 1: Identification of the substance/mixture and of the company/undertaking

1.1. Product identifier

Product form	: Substance
Substance name	: Ammonia, Anhydrous
CAS No.	: 7664-41-7
Product code	: AMM, AMMMET, AMMR
Formula	: NH_3
Synonyms	: Ammonia gas / Ammonia (anhydrous) / Free ammonia / Anhydrous, ammonia / Anhydrous ammonia / Ammonia anhydrous / Gaseous ammonia / AMMONIA

1.2. Relevant identified uses of the substance or mixture and uses advised against

Use of the substance/preparation	: Agricultural chemical Industrial use

1.3. Details of the supplier of the safety data sheet

PCS Sales (USA), Inc.
1101 Skokie Blvd.
Suite 400
Northbrook, IL 60062
T 800-241-6908 / 847-849-4200

Suite 500
122 1st Avenue South
Saskatoon, Saskatchewan Canada S7K7G3
T 800-667-0403 (Canada) / 800-667-3930 (USA)

SDS@PotashCorp.com - www.PotashCorp.com

1.4. Emergency telephone number

Emergency number	: 800-424-9300 CHEMTREC

SECTION 2: Hazards identification

2.1. Classification of the substance or mixture

GHS-US classification

Flammable Gas Category 2	H221
Gas Under Pressure: Compressed Gas	H280
Acute Toxicity Category 3 (Inhalation: gas)	H331
Skin Corrosive Category 1B	H314
Eye Damage Category 1	H318

2.2. Label elements

GHS-US labelling

Hazard pictograms (GHS-US) :

GHS05 GHS06 GHS07

Signal word (GHS-US) : Danger

Hazard statements (GHS-US) : H221 - Flammable gas
H280 - Contains gas under pressure; may explode if heated.
H314 - Causes severe skin burns and eye damage
H331 - Toxic if inhaled

Precautionary statements (GHS-US) : P210 - Keep away from heat, hot surfaces, open flames, sparks. - No smoking
P260 - Do not breathe gas.
P264 - Wash hands and forearms thoroughly after handling
P271 - Use only outdoors or in a well-ventilated area
P280 - Wear eye protection, face protection, protective gloves, protective clothing
P301+P330+P331 - IF SWALLOWED: Rinse mouth. Do NOT induce vomiting
P310 - Immediately call a POISON CENTER or doctor
P303+P361+P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water
P310 - Immediately call a POISON CENTER or doctor.
P363 - Wash contaminated clothing before reuse.
P304+P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing
P310 - Immediately call a POISON CENTER or doctor
P305+P351+P338 - If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P310 - Immediately call a POISON CENTER or doctor
P377 - Leaking gas fire: Do not extinguish, unless leak can be stopped safely
P381 - Eliminate all ignition sources if safe to do so
P403+P233 - Store in a well-ventilated place. Keep container tightly closed
P405 - Store locked up
P410 + P403 - Protect from sunlight. Store in a well-ventilated place.
P501 - Dispose of contents and container according to local, regional, national, and international regulations

2.3. Other hazards

Other hazards not contributing to the classification : Hazardous to the aquatic environment - Acute Hazard Category 1.
Very toxic to aquatic life.

SECTION 3: Composition/information on ingredients

3.1. Substances

Name : Ammonia, Anhydrous

CAS No.	:	7664-41-7
EC no	:	231-635-3
EC index no	:	007-001-00-5

Name	Product identifier	%	GHS-US classification
Ammonia	(CAS No.) 7664-41-7	99.5 - 100	Gas Under Pressure, H280 Flam. Gas 2, H221 Acute Tox. 3 (Inhalation: gas), H331 Skin Corr. 1B, H314 Eye Dam. 1 , H318

Full text of H-phrases: see section 16

3.2. Mixtures

Not applicable

SECTION 4: First aid measures

4.1. Description of first aid measures

First-aid measures after inhalation : Using proper respiratory protection, immediately move the exposed person to fresh air. Keep at rest and in a position comfortable for breathing. Give oxygen or artificial respiration if necessary. Seek immediate medical advice. Symptoms may be delayed.

First-aid measures after skin contact : Using proper respiratory protection, immediately move the exposed person to fresh air. Take off immediately all contaminated clothing. Rinse immediately with plenty of water (for at least 15 minutes). Seek medical attention immediately. Wash contaminated clothing before reuse.

First-aid measures after eye contact : Using proper respiratory protection, immediately move the exposed person to fresh air. Immediately rinse with water for a prolonged period (at least 20 minutes) while holding the eyelids wide open. Seek medical attention immediately.

First-aid measures after ingestion : Ingestion is an unlikely route of exposure for a gas. If swallowed, do not induce vomiting. Seek medical advice immediately.

4.2. Most important symptoms and effects, both acute and delayed

Symptoms/injuries after inhalation : Toxic if inhaled. Causes severe respiratory irritation if inhaled. Symptoms may include: Burning of nose and throat, constriction of airway, difficulty breathing, shortness of breath, bronchial spasms, chest pain, and pink frothy sputum. Contact may cause immediate severe irritation progressing quickly to chemical burns. May cause pulmonary edema. Symptoms may be delayed.

Symptoms/injuries after skin contact : Contact may cause immediate severe irritation progressing quickly to chemical burns.

Symptoms/injuries after eye contact : Contact may cause immediate severe irritation progressing quickly to chemical burns. Can cause severe eye damage or blindness.

Symptoms/injuries after ingestion	: Ingestion is an unlikely route of exposure for a gas. May cause burns or irritation of the linings of the mouth, throat, and gastrointestinal tract.
Chronic symptoms	: Repeated or prolonged inhalation may damage lungs. Prolonged and repeated contact will eventually cause permanent tissue damage.

4.3. Indication of any immediate medical attention and special treatment needed

Immediate medical attention is required for all routes of exposure.

SECTION 5: Firefighting measures

5.1. Extinguishing media

Suitable extinguishing media	: Water, foam, carbon dioxide, dry chemical.
Unsuitable extinguishing media	: None known.

5.2. Special hazards arising from the substance or mixture

Fire hazard	: Flammable gas. Heat may build pressure, rupturing closed containers, spreading fire and increasing risk of burns and injuries. Under conditions of fire this material may produce: Nitrogen oxides. Nitrogen. Withdraw immediately in case of rising sound from venting safety devices or discoloration of tank. Ammonia vapor concentrations between 16% and 25% can explode on contact with an ignition source.

5.3. Advice for firefighters

Firefighting instructions	: Keep upwind. In case of major fire and large quantities: Evacuate area. Fight fire remotely due to the risk of explosion. Remove containers from fire area if this can be done without risk. On heating, there is a risk of bursting due to internal pressure build-up. Cool down the containers exposed to heat with a water spray. Do not get water inside containers. Do not apply water stream directly at source of leak.
Protection during firefighting	: Firefighters must use full bunker gear including NIOSH-approved positive pressure self-contained breathing apparatus to protect against potential hazardous combustion or decomposition products and oxygen deficiencies. Evacuate area and fight the fire from a maximum distance or use unmanned hose holders or monitor nozzles. Cover pooling liquid with foam. Containers can build pressure if exposed to radiant heat; cool adjacent containers with flooding quantities of water until well after the fire is out. Withdraw immediately from the area if there is a rising sound from a venting safety device or discoloration of vessels, tanks, or pipelines. Be aware that burning liquid will float on water. Notify appropriate authorities if liquid enter sewers or waterways.
Other information	: Do not allow run-off from fire fighting to enter drains or water courses.

SECTION 6: Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

General measures	: Do not breathe gas. Stop leak if safe to do so. Eliminate ignition sources. Evacuate unnecessary personnel. Ventilate area. Keep upwind. Wear suitable protective clothing and equipment as described in Section 8 of this SDS.

6.2. Environmental precautions

If spill could potentially enter any waterway, including intermittent dry creeks, contact the U.S. COAST GUARD NATIONAL RESPONSE CENTER at 800-424-8802. In case of accident or road spill notify CHEMTREC at 800-424-9300. In other countries call CHEMTREC at (International code) +1-703-527-3887.

6.3. Methods and material for containment and cleaning up

For containment	: Provide adequate ventilation. Eliminate all ignition sources. Contain any spills with dikes or inert absorbents to prevent migration and entry into sewers or streams. Do not allow into drains or water courses or dispose of where ground or surface waters may be affected. Use cold water to absorb ammonia vapor from air.
Methods for cleaning up	: Eliminate all ignition sources. Ventilate area. Thoroughly wash down area with water. Dispose of materials in accordance with all local, regional, national, and international regulations. Practice good housekeeping – spillage can be slippery on smooth surface either wet or dry.

6.4. Reference to other sections

See Section 8 for Protective equipment and Section 13 for disposal.

SECTION 7: Handling and storage

7.1. Precautions for safe handling

Precautions for safe handling	: Ensure there is adequate ventilation. Keep away from open flames, hot surfaces and sources of ignition. Wear recommended personal protective equipment. Use only approved pressure vessels with appropriate safety devices. Never fill pressure storage tanks over 85% of vessel volume. Avoid copper or copper-containing alloys such as brass, for tanks, vessels, pipe, or valves. Use iron or steel tanks and piping, and valves especially designed for ammonia service. All equipment used to handle, store, transfer, or apply anhydrous ammonia must be properly engineered, constructed, and maintained in compliance with all applicable regulations, standards, and Recognized and Generally Accepted Good Engineering Practice [RAGAGEP]. Pressure vessels, piping, and appurtenances should be regularly inspected and tested using methods designed to reveal external and internal deterioration or defects that may impair the integrity of the equipment such that an unintended release of anhydrous ammonia may result. Consult with your State Department of Agriculture and other experts, as applicable, concerning the methods that would be most appropriate given the particular circumstances. Refer to 29 CFR 1910.111 Storage and Handling of Anhydrous Ammonia, 29 CFR 1910.119 Process Safety Management of Highly Hazardous Materials and the current ANSI standard K61.1, Safety Requirements for the Storage and Handling of Anhydrous Ammonia, for additional information.
Hygiene measures	: Handle in accordance with good industrial hygiene and safety procedures. Emergency eye wash fountains and safety showers should be available in the immediate vicinity of any potential exposure. Wash contaminated clothing before reuse.

7.2. Conditions for safe storage, including any incompatibilities

Storage conditions	: Detached outside storage is preferable.
Incompatible materials	: Avoid contact with: oxidizing gases, silver oxide, acids, copper, tin, and zinc. Hazardous reactions have been documented for contact of anhydrous ammonia with: acetaldehyde, acrolein, boron, boron trioxide, bromine, chlorine, chlorites, chromium trioxide, ethylene oxide, fluoride, gold, hypochlorous acid, iodine, mercury, nitric acid, nitrogen tetroxide, nitrogen trichloride, nitrogen trifluoride, phosphorus trioxide, picric acid, potassium chlorate, potassium ferricyanide, silver, and silver chloride. Liquefied gases in contact with water may explode violently.
Storage area	: Store in dry, cool area at ≤ 49 °C (120 °F). Store in a well-ventilated place. Keep away from combustible materials. Keep away from sources of ignition - No smoking. Protect from high temperatures.

7.3. Specific end use(s)

Agricultural chemical, Industrial use

SECTION 8: Exposure controls/personal protection

8.1. Control parameters

Ammonia (7664-41-7)		
USA ACGIH	ACGIH TWA	25 ppm
USA ACGIH	ACGIH STEL	35 ppm
USA NIOSH IDLH	NIOSH IDLH	300 ppm
USA NIOSH	NIOSH REL (TWA)	18 mg/m³; 25 ppm
USA NIOSH	NIOSH REL (STEL)	27 mg/m³; 35 ppm
USA OSHA	OSHA PEL (TWA)	35 mg/m³ ; 50 ppm
Alberta	TWA / STEL	25 ppm (TWA), 35 ppm (STEL)
British Columbia	TWA / STEL	25 ppm (TWA), 35 ppm (STEL)
Manitoba	TWA / STEL	25 ppm (TWA), 35 ppm (STEL)
New Brunswick	TWA / STEL	25 ppm (TWA), 35 ppm (STEL)
Newfoundland & Labrador	TWA / STEL	25 ppm (TWA), 35 ppm (STEL)
Northwest Territories	TWA / STEL	25 ppm (TWA), 35 ppm (STEL)
Nova Scotia	TWA / STEL	25 ppm (TWA), 35 ppm (STEL)
Nunavut	TWA / STEL	25 ppm (TWA), 35 ppm (STEL)
Ontario	TWA / STEL	25 ppm (TWA), 35 ppm (STEL)
Prince Edward Island	TWA / STEL	25 ppm (TWA), 35 ppm (STEL)
Quebec	TWAEV / STEV	25 ppm (TWAEV), 35 ppm (STEV)
Saskatchewan	TWA / STEL	25 ppm (TWA), 35 ppm (STEL)
Yukon	TWA / STEL	25 ppm (TWA), 40 ppm (STEL)

8.2. Exposure controls

Appropriate engineering controls : Provide sufficient ventilation to keep ammonia vapors below the permissible exposure limit. Ensure adequate ventilation, especially in confined areas.

Personal protective equipment :

Hand protection : Nitrile, Neoprene, Viton or Rubber gloves. Check glove manufacturer's permeation / degradation information.

Eye protection : Wear chemical goggles with a vapor-tight seal. Contact lenses should not be worn.

Skin and body protection : Wear impervious protective clothing and rubber gloves for small releases and normal loading and unloading operations.

Respiratory protection : For exposures at or below 300 ppm use a NIOSH-approved, full-face, negative-pressure respirator fitted with ammonia vapor cartridges. For exposure concentrations above 300 ppm, use a full-face, positive-pressure, self-contained breathing apparatus.

Environmental exposure controls	:	Emergency eye wash fountains and safety showers should be available in the immediate vicinity of any potential exposure.

SECTION 9: Physical and chemical properties

9.1. Information on basic physical and chemical properties

Physical state	:	Gas
Molecular mass	:	17.03 g/mol
Colour	:	Colorless
Odour	:	Ammonia. Pungent. Sharp.
Odour threshold	:	5 - 50 ppm
pH	:	11.6
pH solution	:	1 % aqueous solution
Relative evaporation rate (butylacetate=1)	:	No data available
Melting point	:	No data available
Freezing point	:	-77.7 °C (-108 °F)
Boiling point	:	-33.3 °C (-28 °F)
Flash point	:	No data available
Self ignition temperature	:	651 °C (1204 °F)
Decomposition temperature	:	No data available
Flammability (solid, gas)	:	Flammable gas.
Vapour pressure	:	7520 mm Hg at 25 °C (77 °F)
Relative vapour density at 20 °C	:	0.588
Relative density	:	0.682 at -33.35 °C (-28 °F)
Density	:	0.696 g/l at 20 °C (68 °F)
Solubility	:	510 – 530 g/L
Log Pow	:	-1.14 at 25 °C (77 °F)
Log Kow	:	No data available
Viscosity, kinematic	:	No data available
Viscosity, dynamic	:	0.00982 cP at 20 °C (vapour)
Explosive properties	:	Ammonia vapor concentrations between 16% and 25% can explode on contact with an ignition source
Oxidising properties	:	No data available
Explosive limits	:	16 - 25 vol %

9.2. Other information

% Volatiles: 100% at 20°C

SECTION 10: Stability and reactivity

10.1. Reactivity

May accelerate the burning of other combustible materials. Vapors dissolve easily in water. Large amounts of heat may be released as solution forms.

10.2. Chemical stability

Stable at standard temperature and pressure.

10.3. Possibility of hazardous reactions

Hazardous polymerization will not occur.

10.4. Conditions to avoid

Keep away from heat. Avoid ignition sources.

10.5. Incompatible materials

Ammonia vapor reacts with chlorine, bromine, mercury, silver, and hypoclorites to form explosive compounds. Avoid contact with: oxidizing gases, silver oxide, acids, copper, tin, and zinc. Hazardous reactions have been documented for contact of anhydrous ammonia with: acetaldehyde, acrolein, boron, boron trioxide, bromine, chlorine, chlorites, chromium trioxide, ethylene oxide, fluoride, gold, hypochlorous acid, iodine, mercury, nitric acid, nitrogen tetroxide, nitrogen trichloride, nitrogen trifluoride, phosphorus trioxide, picric acid, potassium chlorate, potassium ferricyanide, silver, and silver chloride.

10.6. Hazardous decomposition products

Under conditions of fire this material may produce: Nitrogen oxides. Nitrogen.

SECTION 11: Toxicological information

11.1. Information on toxicological effects

Acute toxicity :

Ammonia (7664-41-7)	
LD50 oral rat	350 mg/kg
LC50 inhalation rat (mg/l)	5.1 mg/l (Exposure time: 1 h)
LC50 inhalation rat (ppm)	2000 ppm/4h (Exposure time: 4 h)

Skin corrosion/irritation : Causes severe skin burns and eye damage.
pH: 11.6

Serious eye damage/irritation : Causes eye damage. Subacute and chronic exposure to 200 – 1000 ppm produced eye damage. 100 – 200 ppm produced moderate to severe eye irritation.
pH: 11.6

Respiratory or skin sensitisation : None of the components of this product are a sensitizer

Germ cell mutagenicity : None of the components of this product are a germ cell mutagen.

Carcinogenicity : None of the components of this product are listed as carcinogens by OSHA, IARC, or NTP.

Reproductive toxicity : Not classified

Specific target organ toxicity (single exposure) : Not classified

Specific target organ toxicity (repeated exposure)	: Not classified
Aspiration hazard	: Not an aspiration hazard

SECTION 12: Ecological information

12.1. Toxicity

Ecotoxicity	**Acute Toxicity to Fish:**	96-h: LC_{50}= 0.09 – 3.51 mg un-ionized NH_3/L
	Chronic Toxicity to Fish:	Various 12 d-5 yrs: NOEC=0.025-1.2 mg un-ionized NH_3/L.
	Acute Toxicity to Aquatic Invertebrates:	(*Daphnia magna*) 48 h LC_{50} = 2.94 mg un-ionized NH_3-N/L.
	Chronic Toxicity to Aquatic Invertebrates:	(*Daphnia magna* & others) 21 d-76 weeks: NOEC = 0.163-0.42 mg un- ionized NH_3/L.
	Acute Toxicity to Aquatic Plants:	(Benthic diatoms) Up to 25 days: LOEC = 0.5-1.0 mg N/L (*Chlorella vulgaris*) 21 days: LOEC = 500 mg N/L. Slightly toxic to aquatic organisms as defined by USEPA.
	Toxicity to Soil Dwelling Organisms:	No data available.
	Toxicity to Terrestrial Plants:	No data available.

12.2. Persistence and degradability

Environmental Fate:	**Stability in Water:**	Ke=25.6-47.3 cm/h at 15.2-15.0 ^{o}C. Removed from aquatic systems.
	Stability in Soil:	Mean sorption; sand: 19% loam: 28% clay, clay loam, and silt loam: 38%. Monitoring Data: levels of ammonia in urban areas are on average about 20 Φg/m^3. Non-urban sites have average levels of 4-5 Φg/m^3. Areas close to point sources (e.g., large animal feedlots or industrial sites) may have local atmospheric concentrations exceeding 200 Φg/m^3.
	Transport and Distribution:	**Transport:** the primary methods of transport in the atmosphere are via vertical and horizontal diffusion. **Distribution:** 99.98% to air, <0.1% each to water, soil, biota, and sediment
Toxicity:	No known toxicity.	
Degradation Products:	**Biodegradation:**	Inorganic. Undergoes photolytic degradation.
	Photodegradation:	Aerobic. BOD created within days. Rapidly biodegraded. Bioaccumulation: Rapidly assimilated by animals and plants.

12.3. Bioaccumulative potential

No additional information available

12.4. Mobility in soil

No additional information available

12.5. Other adverse effects

No additional information available

SECTION 13: Disposal considerations

13.1. Waste treatment methods

Sewage disposal recommendations	: This material is hazardous to the aquatic environment. Keep out of sewers and waterways.
Waste disposal recommendations	: Place in an approved container and dispose of contaminated materials at a licensed site.

Additional information	:	Dispose of waste material in accordance with all local, regional, national, and international regulations.

SECTION 14: Transport information

In accordance with DOT / TDG / ADR / RID / ADNR / IMDG / ICAO / IATA

14.1. UN number

UN-No.(DOT)	:	1005
DOT NA no.		UN1005

14.2. UN proper shipping name

US:

DOT Proper Shipping Name	:	Ammonia, anhydrous
Department of Transportation (DOT) Hazard Classes	:	2.2 - Class 2.2 - Non-flammable compressed gas 49 CFR 173.115
Hazard labels (DOT)	:	2.2 - Non-flammable compressed gas

Package Marking Requirements (49 CFR 173.330)	:	Ammonia, Anhydrous Inhalation Hazard
DOT Symbols	:	US - Proper shipping name for domestic use only
DOT Special Provisions (49 CFR 172.102)	:	13 - The words Inhalation Hazard shall be entered on each shipping paper in association with the shipping description, shall be marked on each non-bulk package in association with the proper shipping name and identification number, and shall be marked on two opposing sides of each bulk package. Size of marking on bulk package must conform to 172.302(b) of this subchapter. The requirements of 172.203(m) and 172.505 of this subchapter do not apply. T50 - When portable tank instruction T50 is referenced in Column (7) of the 172.101 Table, the applicable liquefied compressed gases are authorized to be transported in portable tanks in accordance with the requirements of 173.313 of this subchapter.
DOT Packaging Exceptions (49 CFR 173.xxx)	:	None
DOT Packaging Non Bulk (49 CFR 173.xxx)	:	304
DOT Packaging Bulk (49 CFR 173.xxx)	:	314;315

Canada:

DOT Proper Shipping Name	:	Ammonia, anhydrous
Transport Canada (TDG) Hazard Classes	:	2.3 - Class 2.3 – Poisonous Gas (Schedule 1) Subsidiary Hazard: 8 - Corrosive

Hazard labels (TDG)	:	2.3 - Poisonous gas 
Packaging Markings	:	 (Internationally Only)
Marine Pollutant Mark Requirements	:	49 CFR 172.322
Package Marking Requirements (49 CFR 173.330)	:	Ammonia, Anhydrous Inhalation Hazard
Shipping Papers Requirement (49 CFR 173.203 (I)	:	**Shipping Papers must have "Marine Pollutant" in near the basic shipping description**
DOT Special Provisions (49 CFR 172.102 – I)	:	**4 -** This material is poisonous by inhalation (see §171.8 of this subchapter) in Hazard Zone D (see §173.116(a) of this subchapter), and must be described as an inhalation hazard under the provisions of this subchapter; **N-87 -** The use of copper valves on UN pressure receptacles is prohibited; **T-50 -**When portable tank instruction T50 is indicated in Column (7) of the §172.101 Hazardous Materials Table, the applicable liquefied compressed gas and chemical under pressure descriptions are authorized to be transported in portable tanks in accordance with the requirements of §173.313 of this subchapter.
DOT Packaging Exceptions (49 CFR 173.xxx)	:	None
DOT Packaging Non Bulk (49 CFR 173.xxx)	:	304
DOT Packaging Bulk (49 CFR 173.xxx)	:	314;315
ERAP Required:	:	Shipments to, from or through Canada

14.3. Additional information

Emergency Response Guide (ERG) Number	:	125
Reportable Quantity	:	100 pounds

Other information : ***Shipping Papers for Transportation by highway***. 49 CFR §172.203 Following the basic description for a hazardous material in a Specification MC 330 or MC 331 cargo tank, there must be entered for—
(1) *Anhydrous ammonia*.
Applies to Product Code "AMM": (i) The words **"0.2 PERCENT WATER"** to indicate the suitability for shipping anhydrous ammonia in a cargo tank made of quenched and tempered steel as authorized by §173.315(a), Note 14 of this subchapter, or
Applies to Product Code "AMMR & AMMMET": (ii) The words **"NOT FOR Q and T TANKS"** when the anhydrous ammonia does not contain 0.2 percent or more water by weight.

Overland transport (International)

Class (ADR) : 2 - Gases

Hazard identification number (Kemler No.) : 268

Classification code (ADR) : 2TC

Danger labels (ADR) : 2.3 - Toxic gas
8 - Corrosive substances

Package Marking Requirements (49 CFR 173.330) : Ammonia, Anhydrous
Inhalation Hazard

Orange plates :

Tunnel restriction code : C/D

Excepted quantities (ADR) : E0

Transport by sea

DOT Vessel Stowage Location : D - The material must be stowed ''on deck only'' on a cargo vessel and on a passenger vessel carrying a number of passengers limited to not more than the larger of 25 passengers or one passenger per each 3 m of overall vessel length, but the material is prohibited on passenger vessels in which the limiting number of passengers is exceeded.

DOT Vessel Stowage Other : 40 - Stow ''clear of living quarters'',52 - Stow ''separated from'' acids,57 - Stow ''separated from'' chlorine

MFAG-No. : 125

Air transport

DOT Quantity Limitations Passenger aircraft/rail (49 CFR 173.27) : Forbidden

DOT Quantity Limitations Cargo aircraft only (49 CFR 175.75)	: Forbidden

SECTION 15: Regulatory information

15.1. US Federal regulations

All components listed on the United States TSCA (Toxic Substances Control Act) inventory

SARA Section 311/312 Hazard Classes: Classified as per OSHA HAZCOM 2012 GHS in Section 2 of this SDS.

Ammonia (7664-41-7)	
SARA Section 302 Threshold Planning Quantity (TPQ)	500 lb
SARA Section 304 and CERCLA (**(Comprehensive Environmental Response, Compensation, and Liability Act):** Designated as a hazardous substance. Reportable Quantity (RQ) is 100 lb (45.4 kg). Persons in charge of vessels or facilities are required to notify the National Response Center (NRC) immediately when there is a release in an amount equal to or greater than the RQ.	
SARA Section 313 - Emission Reporting	Ammonia, de minimis concentration by weight 1.0 % (includes anhydrous Ammonia and aqueous Ammonia from water dissociable Ammonium salts and other sources, 10% of total aqueous Ammonia is reportable under this listing)
CAA (Clean Air Act) :	Ammonia is listed as a regulated toxic substance under 112r for purposes of accidental release planning under the Risk Management Program. Threshold quantity is 10,000 lbs. for anhydrous ammonia and 20,000 lbs. for ammonia in solution (or aqua ammonia) at concentrations of 20% or greater.

15.2. US State regulations

The following states have an OSH program approved by OSHA. If you are located in any of these states you may be under state jurisdiction rather than federal jurisdiction and your state may have more stringent requirements than OSHA. You should consult your state regulations to ensure compliance.

Alaska	Indiana	Minnesota	North Carolina	Utah
Arizona	Iowa	Nevada	Oregon	Vermont
California	Kentucky	New Mexico	Puerto Rico	*Virgin Islands
*Connecticut	Maryland	*New Jersey	South Carolina	Virginia
Hawaii	Michigan	*New York	Tennessee	Washington
*Illinois				Wyoming

*The state plans in these states apply only to public sector employers. In these states private sector employers are subject to USOL – OSHA jurisdiction. All other state plans apply to both public and private sector employers.

Ammonia (7664-41-7)
U.S. - California - SCAQMD - Toxic Air Contaminants - Non-Cancer Acute
U.S. - California - SCAQMD - Toxic Air Contaminants - Non-Cancer Chronic
U.S. - California - Toxic Air Contaminant List (AB 1807, AB 2728)

U.S. - Connecticut - Hazardous Air Pollutants - HLVs (30 min)
U.S. - Connecticut - Hazardous Air Pollutants - HLVs (8 hr)
U.S. - Connecticut - Water Quality Standards - Acute Freshwater Aquatic Life Criteria
U.S. - Connecticut - Water Quality Standards - Acute Saltwater Aquatic Life Criteria
U.S. - Connecticut - Water Quality Standards - Chronic Freshwater Aquatic Life Criteria
U.S. - Connecticut - Water Quality Standards - Chronic Saltwater Aquatic Life Criteria
U.S. - Delaware - Accidental Release Prevention Regulations - Sufficient Quantities
U.S. - Delaware - Accidental Release Prevention Regulations - Threshold Quantities
U.S. - Delaware - Accidental Release Prevention Regulations - Toxic Endpoints
U.S. - Delaware - Pollutant Discharge Requirements - Reportable Quantities
U.S. - Florida - Essential Chemicals List
U.S. - Hawaii - Occupational Exposure Limits - STELs
U.S. - Hawaii - Occupational Exposure Limits - TWAs
U.S. - Idaho - Non-Carcinogenic Toxic Air Pollutants - Acceptable Ambient Concentrations
U.S. - Idaho - Non-Carcinogenic Toxic Air Pollutants - Emission Levels (ELs)
U.S. - Idaho - Occupational Exposure Limits - TWAs
U.S. - Louisiana - Reportable Quantity List for Pollutants
U.S. - Maine - Air Pollutants - Criteria Pollutants
U.S. - Massachusetts - Allowable Ambient Limits (AALs)
U.S. - Massachusetts - Allowable Threshold Concentrations (ATCs)
U.S. - Massachusetts - Oil & Hazardous Material List - Groundwater Reportable Conc. - Reporting Category 1
U.S. - Massachusetts - Oil & Hazardous Material List - Groundwater Reportable Conc. - Reporting Category 2
U.S. - Massachusetts - Oil & Hazardous Material List - Reportable Quantity
U.S. - Massachusetts - Oil & Hazardous Material List - Soil Reportable Concentration - Reporting Category 1
U.S. - Massachusetts - Oil & Hazardous Material List - Soil Reportable Concentration - Reporting Category 2
U.S. - Massachusetts - Right To Know List
U.S. - Massachusetts - Threshold Effects Exposure Limits (TELs)
U.S. - Massachusetts - Toxics Use Reduction Act
U.S. - Michigan - Occupational Exposure Limits - STELs
U.S. - Michigan - Polluting Materials List
U.S. - Michigan - Process Safety Management Highly Hazardous Chemicals
U.S. - Minnesota - Chemicals of High Concern
U.S. - Minnesota - Hazardous Substance List
U.S. - Minnesota - Permissible Exposure Limits - STELs
U.S. - New Hampshire - Regulated Toxic Air Pollutants - Ambient Air Levels (AALs) - 24-Hour
U.S. - New Hampshire - Regulated Toxic Air Pollutants - Ambient Air Levels (AALs) - Annual
U.S. - New Jersey - Discharge Prevention - List of Hazardous Substances
U.S. - New Jersey - Environmental Hazardous Substances List
U.S. - New Jersey - Right to Know Hazardous Substance List
U.S. - New Jersey - Special Health Hazards Substances List
U.S. - New Jersey - TCPA - Extraordinarily Hazardous Substances (EHS)
U.S. - New Jersey - Water Quality - Ground Water Quality Criteria
U.S. - New Jersey - Water Quality - Practical Quantitation Levels (PQLs)
U.S. - New Mexico - Precursor Chemicals
U.S. - New York - Occupational Exposure Limits - TWAs
U.S. - New York - Reporting of Releases Part 597 - List of Hazardous Substances
U.S. - North Carolina - Control of Toxic Air Pollutants
U.S. - North Dakota - Air Pollutants - Guideline Concentrations - 1-Hour

U.S. - North Dakota - Air Pollutants - Guideline Concentrations - 8-Hour
U.S. - Ohio - Accidental Release Prevention - Threshold Quantities
U.S. - Ohio - Extremely Hazardous Substances - Threshold Quantities
U.S. - Oregon - Permissible Exposure Limits - TWAs
U.S. - Oregon - Precursor Chemicals
U.S. - Pennsylvania - RTK (Right to Know) - Environmental Hazard List
U.S. - Pennsylvania - RTK (Right to Know) List
U.S. - Rhode Island - Air Toxics - Acceptable Ambient Levels - 1-Hour
U.S. - Rhode Island - Air Toxics - Acceptable Ambient Levels - 24-Hour
U.S. - Rhode Island - Air Toxics - Acceptable Ambient Levels - Annual
U.S. - Rhode Island - Water Quality Standards - Acute Freshwater Aquatic Life Criteria
U.S. - Rhode Island - Water Quality Standards - Acute Saltwater Aquatic Life Criteria
U.S. - Rhode Island - Water Quality Standards - Chronic Freshwater Aquatic Life Criteria
U.S. - Rhode Island - Water Quality Standards - Chronic Saltwater Aquatic Life Criteria
U.S. - Tennessee - Occupational Exposure Limits - STELs
U.S. - Texas - Effects Screening Levels - Long Term
U.S. - Texas - Effects Screening Levels - Short Term
U.S. - Vermont - Permissible Exposure Limits - STELs
U.S. - Virginia - Water Quality Standards - Acute Freshwater Aquatic Life
U.S. - Virginia - Water Quality Standards - Acute Saltwater Aquatic Life
U.S. - Virginia - Water Quality Standards - Chronic Freshwater Aquatic Life
U.S. - Virginia - Water Quality Standards - Chronic Saltwater Aquatic Life
U.S. - Virginia - Water Quality Standards - Public Water Supply Effluent Limits
U.S. - Virginia - Water Quality Standards - Surface Waters Not Used for the Public Water Supply Effluent Limits
U.S. - Washington - Permissible Exposure Limits - STELs
U.S. - Washington - Permissible Exposure Limits - TWAs
U.S. - Wisconsin - Hazardous Air Contaminants - All Sources - Emissions From Stack Height 25 Ft to Less Than 40 Ft
U.S. - Wisconsin - Hazardous Air Contaminants - All Sources - Emissions From Stack Height 40 Ft to Less Than 75 Ft
U.S. - Wisconsin - Hazardous Air Contaminants - All Sources - Emissions From Stack Heights 75 Feet or Greater
U.S. - Wisconsin - Hazardous Air Contaminants - All Sources - Emissions From Stack Heights Less Than 25 Feet
U.S. - Wyoming - Process Safety Management - Highly Hazardous Chemicals
U.S. - Alaska - Water Quality Standards - Acute Aquatic Life Criteria for Fresh Water
U.S. - Alaska - Water Quality Standards - Chronic Aquatic Life Criteria for Fresh Water
U.S. - Alaska - Water Quality Standards - Acute Aquatic Life Criteria for Marine Water
U.S. - Alaska - Water Quality Standards - Chronic Aquatic Life Criteria for Marine Water
U.S. - Alaska - Ambient Air Quality Standards

15.3. Canadian regulations

All components listed on the Canadian DSL (Domestic Substances List) inventory.

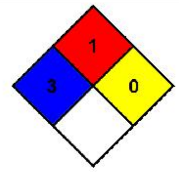
Ammonia (7664-41-7)
Listed on the Canadian Ingredient Disclosure List – Disclosure at 1 %

SECTION 16: Other information

NFPA health hazard : 3 - Short exposure could cause serious temporary or residual injury even though prompt medical attention was given.

NFPA fire hazard : 1 - Must be preheated before ignition can occur.

NFPA reactivity : 0 - Normally stable, even under fire exposure conditions, and are not reactive with water.



Full text of H-phrases:

Acute Tox. 3 (Inhalation: gas)	Acute toxicity (inhalation: gas) Category 3
Flam. Gas 2	Flammable gases Category 2
Skin Corr. 1B	Skin corrosive Category 1B
H221	Flammable gas
H280	Gases Under Pressure
H314	Causes severe skin burns and eye damage
H318	Causes serious eye damage
H331	Toxic if inhaled

Chemical Facility Antiterrorism Standards (6 CFR 27) : This product is listed as a Chemical of Interest in 6 CFR 27. Please determine if your use of this product meets the Screening Threshold Quantity as identified in Appendix A to this regulation. If so, you will be required to submit a Top Screen under DHS's Chemical Security Assessment Tool

REVISION DATE: 05/23/2017

REVISION SUMMARY: General review and format change. Changes to all sections

PREVIOUS REVISION DATE: 07/01/2015

Logo Change : No other information changes; kept same date

SDS US (GHS HazCom 2012)

ThermoFisher SCIENTIFIC

SAFETY DATA SHEET

Creation Date 07-Apr-2009 **Revision Date** 19-Jan-2018 **Revision Number** 4

1. Identification

Product Name	**Boric acid**
Cat No. :	**AC315180000, AC315180025; AC315181000, AC315185000;**
CAS-No	10043-35-3
Synonyms	Boracic acid; Orthoboric acid.; Hydrogen borate
Recommended Use	Laboratory chemicals.
Uses advised against	Food, drug, pesticide or biocidal product use.

Details of the supplier of the safety data sheet

Company
Fisher Scientific
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Acros Organics
One Reagent Lane
Fair Lawn, NJ 07410

Emergency Telephone Number
For information **US** call: 001-800-ACROS-01 / **Europe** call: +32 14 57 52 11
Emergency Number **US:**001-201-796-7100 / **Europe:** +32 14 57 52 99
CHEMTREC Tel. No.**US:**001-800-424-9300 / **Europe:**001-703-527-3887

2. Hazard(s) identification

Classification
This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Reproductive Toxicity	Category 1B

Label Elements

Signal Word
Danger

Hazard Statements
May damage fertility. May damage the unborn child

Precautionary Statements
Prevention
Obtain special instructions before use
Do not handle until all safety precautions have been read and understood
Use personal protective equipment as required
Do not breathe dust/fume/gas/mist/vapors/spray
Response
IF exposed or concerned: Get medical attention/advice
Storage
Store locked up
Disposal
Dispose of contents/container to an approved waste disposal plant
Hazards not otherwise classified (HNOC)
None identified

3. Composition/Information on Ingredients

Component	CAS-No	Weight %
Boric acid (H3BO3)	10043-35-3	<=100

4. First-aid measures

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention if symptoms occur.

Inhalation Remove to fresh air. Do not use mouth-to-mouth method if victim ingested or inhaled the substance; give artificial respiration with the aid of a pocket mask equipped with a one-way valve or other proper respiratory medical device. Get medical attention immediately if symptoms occur. If not breathing, give artificial respiration.

Ingestion Do NOT induce vomiting. Call a physician or poison control center immediately.

Most important symptoms and effects No information available.

Notes to Physician Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media Substance is nonflammable; use agent most appropriate to extinguish surrounding fire.

Unsuitable Extinguishing Media No information available

Flash Point No information available
Method - No information available

Autoignition Temperature No information available
Explosion Limits
Upper No data available
Lower No data available
Sensitivity to Mechanical Impact No information available
Sensitivity to Static Discharge No information available

Specific Hazards Arising from the Chemical
Non-combustible, substance itself does not burn but may decompose upon heating to produce corrosive and/or toxic fumes.

Hazardous Combustion Products
Oxides of boron.
Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

NFPA

Health	Flammability	Instability	Physical hazards
2	0	1	N/A

6. Accidental release measures

Personal Precautions Use personal protective equipment as required. Ensure adequate ventilation. Avoid dust formation. Do not get in eyes, on skin, or on clothing.
Environmental Precautions Should not be released into the environment. See Section 12 for additional Ecological Information.

Methods for Containment and Clean Up Sweep up and shovel into suitable containers for disposal. Avoid dust formation.

7. Handling and storage

Handling Wear personal protective equipment/face protection. Avoid dust formation. Do not get in eyes, on skin, or on clothing. Do not breathe dust. Do not ingest. If swallowed then seek immediate medical assistance.

Storage Keep containers tightly closed in a dry, cool and well-ventilated place.

8. Exposure controls / personal protection

Exposure Guidelines

Component	ACGIH TLV	OSHA PEL	NIOSH IDLH	Mexico OEL (TWA)
Boric acid (H3BO3)	TWA: 2 mg/m^3 STEL: 6 mg/m^3			TWA: 2 mg/m^3

Legend

ACGIH *- American Conference of Governmental Industrial Hygienists*

Engineering Measures Ensure that eyewash stations and safety showers are close to the workstation location.

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure.

Respiratory Protection Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Hygiene Measures Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

Physical State	Solid
Appearance	White
Odor	Odorless
Odor Threshold	No information available
pH	3.8-4.8 33 g/l aq.sol
Melting Point/Range	169 °C / 336.2 °F
Boiling Point/Range	No information available
Flash Point	No information available
Evaporation Rate	Not applicable
Flammability (solid,gas)	No information available
Flammability or explosive limits	
Upper	No data available
Lower	No data available
Vapor Pressure	2.7 mbar @ 20 °C
Vapor Density	Not applicable
Specific Gravity	No information available
Solubility	Soluble
Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	No information available
Decomposition Temperature	100 °C
Viscosity	Not applicable
Molecular Formula	H3 B O3
Molecular Weight	61.83

10. Stability and reactivity

Reactive Hazard None known, based on information available

Stability Moisture sensitive.

Conditions to Avoid Incompatible products. Excess heat. Avoid dust formation. Exposure to moisture.

Incompatible Materials Strong oxidizing agents, Strong bases

Hazardous Decomposition Products Oxides of boron

Hazardous Polymerization Hazardous polymerization does not occur.

Hazardous Reactions None under normal processing.

11. Toxicological information

Acute Toxicity

Product Information
Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Boric acid (H3BO3)	2660 mg/kg (Rat)	> 2000 mg/kg (Rabbit)	Not listed

Toxicologically Synergistic Products No information available

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Irritation Irritating to eyes and skin

Sensitization No information available

Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Component	CAS-No	IARC	NTP	ACGIH	OSHA	Mexico
Boric acid (H3BO3)	10043-35-3	Not listed	Not listed	Not listed	Not listed	Not listed

ACGIH: (American Conference of Governmental Industrial Hygienists)

A1 - Known Human Carcinogen
A2 - Suspected Human Carcinogen
A3 - Animal Carcinogen
ACGIH: (American Conference of Governmental Industrial Hygienists)

Mutagenic Effects No information available

Reproductive Effects Adverse reproductive effects have occurred in humans.

Developmental Effects May cause harm to the unborn child. Developmental effects have occurred in experimental animals.

Teratogenicity Teratogenic effects have occurred in experimental animals.

STOT - single exposure None known
STOT - repeated exposure None known

Aspiration hazard No information available

Symptoms / effects,both acute and delayed No information available

Endocrine Disruptor Information No information available

Other Adverse Effects The toxicological properties have not been fully investigated.

12. Ecological information

Ecotoxicity
Do not empty into drains. .

Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
Boric acid (H3BO3)	-	Gambusia affinis: LC50: 5600 mg/L/96h	-	EC50: 115 - 153 mg/L, 48h (Daphnia magna)

Persistence and Degradability Persistence is unlikely

Bioaccumulation/ Accumulation No information available.

Mobility . Will likely be mobile in the environment due to its water solubility.

Component	log Pow
Boric acid (H3BO3)	-0.757

13. Disposal considerations

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

14. Transport information

DOT Not regulated
TDG Not regulated
IATA Not regulated
IMDG/IMO Not regulated

15. Regulatory information

United States of America Inventory

Component	CAS-No	TSCA	TSCA Inventory notification - Active/Inactive	TSCA - EPA Regulatory Flags
Boric acid (H3BO3)	10043-35-3	X	ACTIVE	-

Legend:
TSCA - Toxic Substances Control Act, (40 CFR Part 710)
X - Listed
'-' - Not Listed

TSCA 12(b) - Notices of Export Not applicable

International Inventories
Canada (DSL/NDSL), Europe (EINECS/ELINCS/NLP), Philippines (PICCS), Japan (ENCS), Australia (AICS), China (IECSC), Korea (ECL).

Component	CAS-No	DSL	NDSL	EINECS	PICCS	ENCS	AICS	IECSC	KECL
Boric acid (H3BO3)	10043-35-3	X	-	233-139-2	X	X	X	X	KE-03499

U.S. Federal Regulations

SARA 313 Not applicable

SARA 311/312 Hazard Categories See section 2 for more information

CWA (Clean Water Act) Not applicable

Clean Air Act Not applicable

OSHA - Occupational Safety and Health Administration Not applicable

CERCLA Not applicable

California Proposition 65 This product does not contain any Proposition 65 chemicals.

U.S. State Right-to-Know Regulations

Component	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Boric acid (H3BO3)	-	X	-	X	-

U.S. Department of Transportation
Reportable Quantity (RQ): N
DOT Marine Pollutant N
DOT Severe Marine Pollutant N

U.S. Department of Homeland Security This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade No information available

16. Other information

Prepared By Regulatory Affairs
Thermo Fisher Scientific

Email: EMSDS.RA@thermofisher.com

Creation Date	07-Apr-2009
Revision Date	19-Jan-2018
Print Date	19-Jan-2018
Revision Summary	This document has been updated to comply with the US OSHA HazCom 2012 Standard replacing the current legislation under 29 CFR 1910.1200 to align with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

End of SDS

ISO9001:2000 Certified

Material Safety Data Sheet
Dextrose

Section 1 - Chemical Product and Company Identification

MSDS Name:
Dextrose
Catalog Numbers:
LC13550
Synonyms:
D-glucose
Company Identification:
LabChem Inc
200 William Pitt Way
Pittsburgh, PA 15238
Company Phone Number:
(412) 826-5230
Emergency Phone Number:
(800) 424-9300
CHEMTREC Phone Number:
(800) 424-9300

Section 2 – Composition, Information on Ingredients

CAS#	Chemical Name:	Percent
50-99-7	Glucose	100

Section 3 - Hazards Identification

Emergency Overview

Appearance: *White solid*
Caution. May cause eye, skin, respiratory, and gastrointestinal tract irritation. This is expected to be a low hazard for usual industrial handling.
Target Organs: *None*

Potential Health Effects

Eye:
May cause eye irritation.
Skin:
May cause skin irritation. Low hazard for usual industrial handling.
Ingestion:
No hazard expected in normal industrial use. May cause irritation of the digestive tract.
Inhalation:
No hazard expected in normal industrial use. May cause respiratory tract irritation.
Chronic:
No information found.

Material Safety Data Sheet
Dextrose

Section 4 - First Aid Measures

Eyes:
Gently lift eyelids and flush continuously with water. If irritation develops, get medical aid.
Skin:
Get medical aid if irritation develops or persists. Wash clothing before reuse. Flush skin with plenty of soap and water.
Ingestion:
Get medical aid. Do NOT induce vomiting. If conscious and alert, rinse mouth and drink 2-4 cupfuls of milk or water. Get medical aid if irritation or symptoms occur.
Inhalation:
Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.
Notes to Physician:
Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information:
As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Dusts at sufficient concentrations can form explosive mixtures with air. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion.
Extinguishing Media:
Use agent most appropriate to extinguish fire. Use water spray, dry chemical, carbon dioxide, or appropriate foam.
Autoignition Temperature:
Not applicable.
Flash Point:
Not applicable.
NFPA Rating:
Not available.
Explosion Limits:
Lower: n/a Upper: n/a

Section 6 - Accidental Release Measures

General Information:
Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Vacuum or sweep up material and place into a suitable disposal container. Clean up spills immediately, observing precautions in the Protective Equipment section. Avoid generating dusty conditions. Provide ventilation.

Material Safety Data Sheet
Dextrose

Section 7 - Handling and Storage

Handling:
Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate ventilation to keep airborne concentrations low.
Storage:
Store in a cool, dry, well-ventilated area away from incompatible substances.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls:
Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate ventilation to keep airborne concentrations low.
Exposure Limits:

Chemical Name:	ACGIH	NIOSH	OSHA
Dextrose	none listed	none listed	none listed

OSHA Vacated PELs:
No OSHA Vacated PELs are listed for this chemical.

Personal Protective Equipment

Eyes:
Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin:
Wear appropriate gloves to prevent skin exposure.
Clothing:
Wear appropriate protective clothing to prevent skin exposure.
Respirators:
Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Section 9 - Physical and Chemical Properties

Physical State:	Solid
Color:	White
Odor:	Odorless
pH:	5.9 (0.5 M)
Vapor Pressure:	Negligible
Vapor Density:	Not applicable
Evaporation Rate:	Negligible
Viscosity:	Not applicable
Boiling Point:	Not available
Freezing/Melting Point:	295 F
Decomposition Temperature:	Not available
Solubility in water:	Soluble

Material Safety Data Sheet
Dextrose

Specific Gravity/Density: 1.54
Molecular Formula: $C6H12O6$
Molecular Weight: 180.08

Section 10 - Stability and Reactivity

Chemical Stability:
Stable under normal temperatures and pressures.
Conditions to Avoid:
Incompatible materials, dust generation, excess heat.
Incompatibilities with Other Materials:
Oxidizing agents.
Hazardous Decomposition Products:
Carbon monoxide, irritating and toxic fumes and gases, carbon dioxide.
Hazardous Polymerization:
Has not been reported.

Section 11 - Toxicological Information

RTECS:
CAS# 50-99-7: LZ6600000
LD50/LC50:
CAS# 50-99-7:
Oral, rat: LD50 = 25800 mg/kg
Carcinogenicity:
CAS# 50-99-7: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
Epidemiology:
No information found
Teratogenicity:
No information found
Reproductive:
No information found
Mutagenicity:
No information found
Neurotoxicity:
No information found

Section 12 - Ecological Information

No information available.

Section 13 - Disposal Considerations

Dispose of in accordance with Federal, State, and local regulations.

Material Safety Data Sheet
Dextrose

Section 14 - Transport Information

US DOT

Shipping Name: Not regulated
Hazard Class:
UN Number:
Packing Group:

Section 15 - Regulatory Information

US Federal

TSCA:
CAS# 50-99-7 is listed on the TSCA inventory.
SARA Reportable Quantities (RQ):
None of the chemicals in this material have an RQ.
CERCLA/SARA Section 313:
No chemicals are reportable under Section 313.
OSHA - Highly Hazardous:
None of the chemicals in this product are considered highly hazardous by OSHA.

US State

State Right to Know:
CAS# 50-99-7 is not present on state lists from CA, PA, MN, MA, FL, or NJ.
California Regulations:
None.

European/International Regulations

Canadian DSL/NDSL:
CAS# 50-99-7 is listed on Canada's DSL List.
Canada Ingredient Disclosure List:
CAS# 50-99-7 is not listed on the Canadian Ingredient Disclosure List.

Section 16 - Other Information

MSDS Creation Date: October 5, 2007
Revision Date: None

Magnesium Chloride, Hexahydrate

Safety Data Sheet

according to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations
Date of issue: 01/24/2014 Revision date: 11/08/2017 Supersedes: 11/08/2017 Version: 1.1

SECTION 1: Identification

1.1. Identification

Product form	:	Substance
Substance name	:	Magnesium Chloride, Hexahydrate
CAS-No.	:	7791-18-6
Product code	:	LC16380
Formula	:	MgCl2·6H2O

1.2. Recommended use and restrictions on use

Use of the substance/mixture	:	For laboratory and manufacturing use only.
Recommended use	:	Laboratory chemicals
Restrictions on use	:	Not for food, drug or household use

1.3. Supplier

LabChem Inc
Jackson's Pointe Commerce Park Building 1000, 1010 Jackson's Pointe Court
Zelienople, PA 16063 - USA
T 412-826-5230 - F 724-473-0647
info@labchem.com - www.labchem.com

1.4. Emergency telephone number

Emergency number	:	CHEMTREC: 1-800-424-9300 or 011-703-527-3887

SECTION 2: Hazard(s) identification

2.1. Classification of the substance or mixture

GHS-US classification

Not classified

2.2. GHS Label elements, including precautionary statements

Not classified as a hazardous chemical.

Other hazards not contributing to the classification	:	None under normal conditions.

2.4. Unknown acute toxicity (GHS US)

Not applicable

SECTION 3: Composition/Information on ingredients

3.1. Substances

Substance type	:	Mono-constituent

Name	Product identifier	%	GHS-US classification
Magnesium Chloride, Hexahydrate (Main constituent)	(CAS-No.) 7791-18-6	100	Not classified

Full text of hazard classes and H-statements : see section 16

3.2. Mixtures

Not applicable

SECTION 4: First-aid measures

4.1. Description of first aid measures

First-aid measures general	:	Never give anything by mouth to an unconscious person. If you feel unwell, seek medical advice (show the label where possible).
First-aid measures after inhalation	:	Allow victim to breathe fresh air. Allow the victim to rest.
First-aid measures after skin contact	:	Remove affected clothing and wash all exposed skin area with mild soap and water, followed by warm water rinse.
First-aid measures after eye contact	:	Rinse immediately with plenty of water. Obtain medical attention if pain, blinking or redness persists.
First-aid measures after ingestion	:	Rinse mouth. Do NOT induce vomiting. Obtain emergency medical attention.

4.2. Most important symptoms and effects (acute and delayed)

Symptoms/effects : Not expected to present a significant hazard under anticipated conditions of normal use.

4.3. Immediate medical attention and special treatment, if necessary

Obtain medical assistance.

SECTION 5: Fire-fighting measures

5.1. Suitable (and unsuitable) extinguishing media

Suitable extinguishing media : Foam. Dry powder. Carbon dioxide. Water spray. Sand.
Unsuitable extinguishing media : Do not use a heavy water stream.

5.2. Specific hazards arising from the chemical

No additional information available

5.3. Special protective equipment and precautions for fire-fighters

Firefighting instructions : Use water spray or fog for cooling exposed containers. Exercise caution when fighting any chemical fire. Prevent fire-fighting water from entering environment.
Protection during firefighting : Do not enter fire area without proper protective equipment, including respiratory protection.

SECTION 6: Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

6.1.1. For non-emergency personnel

Protective equipment : Safety glasses.
Emergency procedures : Evacuate unnecessary personnel.

6.1.2. For emergency responders

Protective equipment : Equip cleanup crew with proper protection.
Emergency procedures : Ventilate area.

6.2. Environmental precautions

Prevent entry to sewers and public waters. Notify authorities if liquid enters sewers or public waters.

6.3. Methods and material for containment and cleaning up

Methods for cleaning up : On land, sweep or shovel into suitable containers. Minimize generation of dust. Store away from other materials.

6.4. Reference to other sections

See Heading 8. Exposure controls and personal protection.

SECTION 7: Handling and storage

7.1. Precautions for safe handling

Precautions for safe handling : Wash hands and other exposed areas with mild soap and water before eating, drinking or smoking and when leaving work. Provide good ventilation in process area to prevent formation of vapor.

7.2. Conditions for safe storage, including any incompatibilities

Storage conditions : Keep container closed when not in use.
Incompatible products : silver nitrate.
Incompatible materials : Sources of ignition. Direct sunlight.

SECTION 8: Exposure controls/personal protection

8.1. Control parameters

No additional information available

8.2. Appropriate engineering controls

Appropriate engineering controls : Emergency eye wash fountains should be available in the immediate vicinity of any potential exposure.

8.3. Individual protection measures/Personal protective equipment

Personal protective equipment:

Safety glasses.

Hand protection:

Wear protective gloves

Eye protection:

Chemical goggles or safety glasses

Respiratory protection:

Respiratory protection not required in normal conditions

Other information:

Do not eat, drink or smoke during use.

SECTION 9: Physical and chemical properties

9.1. Information on basic physical and chemical properties

Physical state	: Solid
Color	: white
Odor	: None.
Odor threshold	: No data available
pH	: No data available
Melting point	: 116 - 118 °C
Freezing point	: No data available
Boiling point	: No data available
Flash point	: No data available
Relative evaporation rate (butyl acetate=1)	: No data available
Flammability (solid, gas)	: Non flammable.
Vapor pressure	: No data available
Relative vapor density at 20 °C	: No data available
Relative density	: No data available
Specific gravity / density	: 1.569 g/cm³
Solubility	: Soluble in water. Soluble in ethanol. Water: 167 g/100ml
Log Pow	: No data available
Auto-ignition temperature	: No data available
Decomposition temperature	: 116 - 118 °C
Viscosity, kinematic	: No data available
Viscosity, dynamic	: No data available
Explosion limits	: No data available
Explosive properties	: No data available
Oxidizing properties	: No data available

9.2. Other information

No additional information available

SECTION 10: Stability and reactivity

10.1. Reactivity

No additional information available

10.2. Chemical stability
Stable under normal conditions.

10.3. Possibility of hazardous reactions
Not established.

10.4. Conditions to avoid
Direct sunlight. Extremely high or low temperatures.

10.5. Incompatible materials
silver nitrate.

10.6. Hazardous decomposition products
Hydrogen chloride.

SECTION 11: Toxicological information

11.1. Information on toxicological effects

Likely routes of exposure : Skin and eye contact; Inhalation
Acute toxicity : Not classified

Skin corrosion/irritation : Not classified
Serious eye damage/irritation : Not classified
Respiratory or skin sensitization : Not classified
Germ cell mutagenicity : Not classified
Carcinogenicity : Not classified

Reproductive toxicity : Not classified
Specific target organ toxicity – single exposure : Not classified

Specific target organ toxicity – repeated exposure : Not classified

Aspiration hazard : Not classified

Potential Adverse human health effects and symptoms : Based on available data, the classification criteria are not met.

SECTION 12: Ecological information

12.1. Toxicity
No additional information available

12.2. Persistence and degradability

Magnesium Chloride, Hexahydrate (7791-18-6)	
Persistence and degradability	Not established.

12.3. Bioaccumulative potential

Magnesium Chloride, Hexahydrate (7791-18-6)	
Bioaccumulative potential	Not established.

12.4. Mobility in soil
No additional information available

12.5. Other adverse effects

Other information : Avoid release to the environment.

SECTION 13: Disposal considerations

13.1. Disposal methods
Waste disposal recommendations : Dispose in a safe manner in accordance with local/national regulations.
Ecology - waste materials : Avoid release to the environment.

SECTION 14: Transport information

Department of Transportation (DOT)

In accordance with DOT

Not regulated

SECTION 15: Regulatory information

15.1. US Federal regulations

Magnesium Chloride, Hexahydrate (7791-18-6)
Not listed on the United States TSCA (Toxic Substances Control Act) inventory

All components of this product are listed, or excluded from listing, on the United States Environmental Protection Agency Toxic Substances Control Act (TSCA) inventory except for:

Magnesium Chloride, Hexahydrate	CAS-No. 7791-18-6	100%

15.2. International regulations

CANADA

Magnesium Chloride, Hexahydrate (7791-18-6)
Not listed on the Canadian DSL (Domestic Substances List)

EU-Regulations

No additional information available

National regulations

Magnesium Chloride, Hexahydrate (7791-18-6)
Not listed on the Canadian IDL (Ingredient Disclosure List)

15.3. US State regulations

California Proposition 65 - This product does not contain any substances known to the state of California to cause cancer, developmental and/or reproductive harm

SECTION 16: Other information

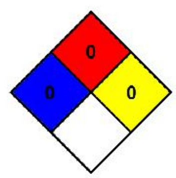
Revision date : 11/08/2017

Other information : None.

NFPA health hazard : 0 - Materials that, under emergency conditions, would offer no hazard beyond that of ordinary combustible materials.

NFPA fire hazard : 0 - Materials that will not burn under typical dire conditions, including intrinsically noncombustible materials such as concrete, stone, and sand.

NFPA reactivity : 0 - Material that in themselves are normally stable, even under fire conditions.



Hazard Rating

Health : 0 Minimal Hazard - No significant risk to health

Flammability : 0 Minimal Hazard - Materials that will not burn

Physical : 0 Minimal Hazard - Materials that are normally stable, even under fire conditions, and will NOT react with water, polymerize, decompose, condense, or self-react. Non-Explosives.

Personal protection : A

A - Safety glasses

SDS US LabChem

Material Safety Data Sheet
Potassium Hydroxide

ACC# 19431

Section 1 - Chemical Product and Company Identification

MSDS Name: Potassium Hydroxide
Catalog Numbers: P246-3, P250-1, P250-10, P250-3, P250-50, P250-500, P250-50LC, P250500LC, P251-3, P251-50, P251-500, P251-50KG, P258-12, P258-212, P258-50, P258-50LC, PFP25050LC
Synonyms: Caustic potash; Lye; Potassium hydrate
Company Identification:
Fisher Scientific
1 Reagent Lane
Fair Lawn, NJ 07410
For information, call: 201-796-7100
Emergency Number: 201-796-7100
For CHEMTREC assistance, call: 800-424-9300
For International CHEMTREC assistance, call: 703-527-3887

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
1310-58-3	Potassium hydroxide (KOH)	100.0	215-181-3

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: white or yellow solid.
Danger! Corrosive. Water-reactive. Causes severe eye and skin burns. Causes severe digestive and respiratory tract burns. Harmful if swallowed.
Target Organs: Respiratory system, eyes, skin.

Potential Health Effects
Eye: Causes severe eye burns. May cause irreversible eye injury. Contact may cause ulceration of the conjunctiva and cornea. Eye damage may be delayed.
Skin: Causes skin burns. May cause deep, penetrating ulcers of the skin.
Ingestion: Harmful if swallowed. May cause circulatory system failure. May cause perforation of the digestive tract. Causes severe digestive tract burns with abdominal pain, vomiting, and possible death.
Inhalation: Harmful if inhaled. Irritation may lead to chemical pneumonitis and pulmonary edema. Causes severe irritation of upper respiratory tract with coughing, burns, breathing difficulty, and possible coma.
Chronic: Prolonged or repeated skin contact may cause dermatitis. Prolonged or repeated eye contact may cause conjunctivitis.

Section 4 - First Aid Measures

Eyes: Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid imme diately.
Skin: Get medical aid immediately. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Discard contaminated clothing in a manner which limits further exposure.
Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water.

Never give anything by mouth to an unconscious person. Get medical aid immediately.
Inhalation: Get medical aid immediately. Remove from exposure and move to fresh air immediately. If breathing is difficult, give oxygen. If breathing has ceased apply artificial respiration using oxygen and a suitable mechanical device such as a bag and a mask.
Notes to Physician: Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. Use water with caution and in flooding amounts. Contact with moisture or water may generate sufficient heat to ignite nearby combustible materials.
Extinguishing Media: For small fires, use dry chemical, carbon dioxide, water spray or alcohol-resistant foam.
Flash Point: Not applicable.
Autoignition Temperature: Not applicable.
Explosion Limits, Lower: Not available.
Upper: Not available.
NFPA Rating: (estimated) Health: 3; Flammability: 0; Instability: 1

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Vacuum or sweep up material and place into a suitable disposal container. Avoid generating dusty conditions.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Use with adequate ventilation. Do not allow water to get into the container because of violent reaction. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale.
Storage: Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances. Keep away from strong acids. Keep away from water. Keep away from metals. Keep away from flammable liquids. Keep away from organic halogens.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Potassium hydroxide (KOH)	2 mg/m3 Ceiling	none listed	none listed

OSHA Vacated PELs: Potassium hydroxide (KOH): No OSHA Vacated PELs are listed for this chemical.
Personal Protective Equipment
Eyes: Wear safety glasses and chemical goggles or face shield if handling liquids.
Skin: Wear appropriate gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.
Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Section 9 - Physical and Chemical Properties

Physical State: Solid
Appearance: white or yellow
Odor: odorless
pH: 13.5 (0.1M solution)
Vapor Pressure: Not available.
Vapor Density: Not available.
Evaporation Rate: Not available.
Viscosity: Not available.
Boiling Point: 1320 deg C
Freezing/Melting Point:360 deg C
Decomposition Temperature: Not available.
Solubility: Soluble in water
Specific Gravity/Density:2.04
Molecular Formula:KOH
Molecular Weight:56.1

Section 10 - Stability and Reactivity

Chemical Stability: Stable. Readily absorbs carbon dioxide and moisture from the air and deliquesces (to absorb atmospheric water vapor and become liquid).
Conditions to Avoid: Moisture, contact with water.
Incompatibilities with Other Materials: Water, metals, acids.
Hazardous Decomposition Products: Oxides of potassium.
Hazardous Polymerization: Has not been reported.

Section 11 - Toxicological Information

RTECS#:
CAS# 1310-58-3: TT2100000
LD50/LC50:
CAS# 1310-58-3:
Draize test, rabbit, skin: 50 mg/24H Severe;
Oral, rat: LD50 = 273 mg/kg;
.

Carcinogenicity:
CAS# 1310-58-3: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: No data available.
Teratogenicity: No information reported.
Reproductive Effects: No data available.
Mutagenicity: No data available.
Neurotoxicity: No data available.
Other Studies:

Section 12 - Ecological Information

Ecotoxicity: Fish: Mosquito Fish: LC50 = 80.0 mg/L; 24 Hr.; Unspecified No data available.
Environmental: No information found.
Physical: No information found.
Other: No information available.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	POTASSIUM HYDROXIDE, SOLID	POTASSIUM HYDROXIDE
Hazard Class:	8	8
UN Number:	UN1813	UN1813
Packing Group:	II	II

Section 15 - Regulatory Information

US FEDERAL

TSCA
CAS# 1310-58-3 is listed on the TSCA inventory.
Health & Safety Reporting List
None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules
None of the chemicals in this product are under a Chemical Test Rule.
Section 12b
None of the chemicals are listed under TSCA Section 12b.
TSCA Significant New Use Rule
None of the chemicals in this material have a SNUR under TSCA.
CERCLA Hazardous Substances and corresponding RQs
CAS# 1310-58-3: 1000 lb final RQ; 454 kg final RQ
SARA Section 302 Extremely Hazardous Substances
None of the chemicals in this product have a TPQ.
SARA Codes
CAS # 1310-58-3: immediate, reactive.
Section 313 No chemicals are reportable under Section 313.
Clean Air Act:
This material does not contain any hazardous air pollutants.
This material does not contain any Class 1 Ozone depletors.
This material does not contain any Class 2 Ozone depletors.
Clean Water Act:
CAS# 1310-58-3 is listed as a Hazardous Substance under the CWA.
None of the chemicals in this product are listed as Priority Pollutants under the CWA.
None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
OSHA:
None of the chemicals in this product are considered highly hazardous by OSHA.
STATE
CAS# 1310-58-3 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Minnesota, Massachusetts.

California Prop 65

California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations
European Labeling in Accordance with EC Directives
Hazard Symbols:
C

Risk Phrases:
R 22 Harmful if swallowed.
R 35 Causes severe burns.

Safety Phrases:
S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S 36/37/39 Wear suitable protective clothing, gloves and eye/face pr otection.
S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

WGK (Water Danger/Protection)
CAS# 1310-58-3: 1
Canada - DSL/NDSL
CAS# 1310-58-3 is listed on Canada's DSL List.
Canada - WHMIS
This product has a WHMIS classification of D1B, E.
This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.
Canadian Ingredient Disclosure List
CAS# 1310-58-3 is listed on the Canadian Ingredient Disclosure List.

Section 16 - Additional Information

MSDS Creation Date: 6/21/1999
Revision #8 Date: 2/15/2008

SAFETY DATA SHEET

Revision Date 17-Jan-2018 **Revision Number** 3

1. Identification

Product Name **Sodium tripolyphosphate**

Cat No. : **S645-500**

Synonyms Sodium triphosphate; Sodium Tripolyphosphate.

Recommended Use Laboratory chemicals.
Uses advised against Not for food, drug, pesticide or biocidal product use

Details of the supplier of the safety data sheet

Company
Fisher Scientific
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number
CHEMTREC®, Inside the USA: 800-424-9300
CHEMTREC®, Outside the USA: 001-703-527-3887

2. Hazard(s) identification

Classification
This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Skin Corrosion/irritation	Category 2
Serious Eye Damage/Eye Irritation	Category 2
Specific target organ toxicity (single exposure)	Category 3
Target Organs - Respiratory system.	

Label Elements

Signal Word
Warning

Hazard Statements
Causes skin irritation
Causes serious eye irritation
May cause respiratory irritation

Precautionary Statements
Prevention
Wash face, hands and any exposed skin thoroughly after handling
Wear protective gloves/protective clothing/eye protection/face protection
Avoid breathing dust/fume/gas/mist/vapors/spray
Use only outdoors or in a well-ventilated area
Inhalation
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
Call a POISON CENTER or doctor/physician if you feel unwell
Skin
IF ON SKIN: Wash with plenty of soap and water
If skin irritation occurs: Get medical advice/attention
Take off contaminated clothing and wash before reuse
Eyes
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
If eye irritation persists: Get medical advice/attention
Storage
Store in a well-ventilated place. Keep container tightly closed
Store locked up
Disposal
Dispose of contents/container to an approved waste disposal plant
Hazards not otherwise classified (HNOC)
None identified

3. Composition/Information on Ingredients

Component	CAS-No	Weight %
Pentasodium triphosphate	7758-29-4	98 - 100

4. First-aid measures

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.

Inhalation Move to fresh air. If breathing is difficult, give oxygen. Get medical attention immediately if symptoms occur.

Ingestion Do not induce vomiting. Obtain medical attention.

Most important symptoms and effects No information available.

Notes to Physician Treat symptomatically

5. Fire-fighting measures

Unsuitable Extinguishing Media No information available

Flash Point No information available
Method - No information available

Autoignition Temperature No information available
Explosion Limits
Upper No data available
Lower No data available
Sensitivity to Mechanical Impact No information available

Sensitivity to Static Discharge No information available

Specific Hazards Arising from the Chemical
Keep product and empty container away from heat and sources of ignition.

Hazardous Combustion Products
None known
Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

NFPA

Health	Flammability	Instability	Physical hazards
2	0	1	N/A

6. Accidental release measures

Personal Precautions Use personal protective equipment. Ensure adequate ventilation. Avoid dust formation. Avoid contact with the skin and the eyes. Keep people away from and upwind of spill/leak.

Environmental Precautions See Section 12 for additional ecological information.

Methods for Containment and Clean Up Avoid dust formation. Sweep up and shovel into suitable containers for disposal. Keep container tightly closed in a dry and well-ventilated place.

7. Handling and storage

Handling Avoid contact with skin and eyes. Do not breathe dust. Ensure adequate ventilation. Wear personal protective equipment. Avoid dust formation.

Storage Keep containers tightly closed in a dry, cool and well-ventilated place.

8. Exposure controls / personal protection

Exposure Guidelines This product does not contain any hazardous materials with occupational exposure limitsestablished by the region specific regulatory bodies.

Engineering Measures Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location.

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure.

Respiratory Protection Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Hygiene Measures Handle in accordance with good industrial hygiene and safety practice.

9. Physical and chemical properties

Physical State	Powder Solid
Appearance	White
Odor	Odorless
Odor Threshold	No information available

pH	9.12 (1 %)
Melting Point/Range	622 °C
Boiling Point/Range	No information available
Flash Point	No information available
Evaporation Rate	No information available
Flammability (solid,gas)	No information available
Flammability or explosive limits	
Upper	No data available
Lower	No data available
Vapor Pressure	No information available
Vapor Density	No information available
Specific Gravity	> 1.5
Solubility	Partly soluble in water
Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	No information available
Decomposition Temperature	No information available
Viscosity	No information available
Molecular Formula	Na5P3O10
Molecular Weight	367.85

10. Stability and reactivity

Reactive Hazard None known, based on information available

Stability Hygroscopic. Stable under normal conditions.

Conditions to Avoid Avoid dust formation. Incompatible products.

Incompatible Materials Strong oxidizing agents

Hazardous Decomposition Products None under normal use conditions

Hazardous Polymerization Hazardous polymerization does not occur.

Hazardous Reactions None under normal processing.

11. Toxicological information

Acute Toxicity

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Pentasodium triphosphate	LD50 = 3120 mg/kg (Rat)	LD50 > 7940 mg/kg (Rabbit)	Not listed

Toxicologically Synergistic Products No information available

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Irritation Moderately irritating to eyes, skin and respiratory system

Sensitization No information available

Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Component	CAS-No	IARC	NTP	ACGIH	OSHA	Mexico
Pentasodium triphosphate	7758-29-4	Not listed	Not listed	Not listed	Not listed	Not listed

Mutagenic Effects No information available

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity No information available.

STOT - single exposure Respiratory system
STOT - repeated exposure None known

Aspiration hazard No information available

Symptoms / effects,both acute and delayed No information available

Endocrine Disruptor Information No information available

Other Adverse Effects The toxicological properties have not been fully investigated.

12. Ecological information

Ecotoxicity
Do not empty into drains.

Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
Pentasodium triphosphate	Not listed	LC50: = 1650 mg/L, 48h (Leuciscus idus)	Not listed	Not listed

Persistence and Degradability No information available

Bioaccumulation/ Accumulation No information available.

Mobility No information available.

13. Disposal considerations

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

14. Transport information

DOT Not regulated
TDG Not regulated
IATA Not regulated
IMDG/IMO Not regulated

15. Regulatory information

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	IECSC	KECL
Pentasodium triphosphate	X	X	-	231-838-7	-		X	X	X	X	X

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.

Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313 Not applicable

SARA 311/312 Hazard Categories See section 2 for more information

CWA (Clean Water Act) Not applicable

Clean Air Act Not applicable

OSHA Occupational Safety and Health Administration
Not applicable

CERCLA Not applicable

California Proposition 65 This product does not contain any Proposition 65 chemicals

U.S. State Right-to-Know Regulations

Component	Massachusetts	New Jersey	Pennsylvania	Illinois	Rhode Island
Pentasodium triphosphate	X	-	X	-	-

U.S. Department of Transportation

Reportable Quantity (RQ): N
DOT Marine Pollutant N
DOT Severe Marine Pollutant N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade No information available

16. Other information

Prepared By Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com

Revision Date 17-Jan-2018
Print Date 17-Jan-2018
Revision Summary This document has been updated to comply with the US OSHA HazCom 2012 Standard replacing the current legislation under 29 CFR 1910.1200 to align with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Disclaimer
The information provided in this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other materials or in any process, unless specified in the text

End of SDS

Material Safety Data Sheet
Sodium chloride

ACC# 21105

Section 1 - Chemical Product and Company Identification

MSDS Name: Sodium chloride
Catalog Numbers: AC194090000, AC194090010, AC194090050, AC1940900510, AC194090250, AC207790000, AC207790010, AC207790050, AC327300000, AC327300010, AC424290000, AC424290030, AC424290250, 42429-0010, 42429-5000, BP358-1, BP358-10, BP358-212, NC9269808, NC9380133, NC9460864, NC9634552, NC9780594, NC9821620, NC9826699, NC9919051, NC9974906, S271-1, S271-10, S271-10LC, S271-1LC, S271-3, S271-350LB, S271-50, S271-500, S271-50LC, S640-10, S640-10LC, S640-3, S640-350LB, S640-50, S640-500, S640SAM1, S640SAM2, S640SAM3, S641-212, S641-350LB, S641-500, S641P-350LB, S641P350LLC, S642-12, S642-212, S642-350LB, S642-500, S64212LC, S642350LBLC, S642SAM1, S642SAM2, S642SAM3, S671-10, S671-3, S671-500
Synonyms: Common salt; Halite; Rock salt; Saline; Salt; Sea salt; Table salt.
Company Identification:
Fisher Scientific
1 Reagent Lane
Fair Lawn, NJ 07410
For information, call: 201-796-7100
Emergency Number: 201-796-7100
For CHEMTREC assistance, call: 800-424-9300
For International CHEMTREC assistance, call: 703-527-3887

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
7647-14-5	Sodium chloride	99+	231-598-3

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: white solid.
Caution! May cause eye, skin, and respiratory tract irritation. Hygroscopic (absorbs moisture from the air).
Target Organs: None known.

Potential Health Effects
Eye: May cause eye irritation. Exposure to solid may cause pain and redness.
Skin: May cause skin irritation. May be harmful if absorbed through the skin.
Ingestion: May cause irritation of the digestive tract. May be harmful if swallowed. Ingestion of large amounts may cause nausea and vomiting, rigidity or convulsions. Continued exposure can produce coma, dehydration, and internal organ
Inhalation: May cause respiratory tract irritation. May be harmful if inhaled.
Chronic: No information found.

Section 4 - First Aid Measures

Eyes: Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. If irritation develo ps, get medical aid.
Skin: Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated

clothing and shoes. Get medical aid if irritation develops or persists.
Ingestion: Do not induce vomiting. Get medical aid if irritation or symptoms occur.
Inhalation: Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.
Notes to Physician: Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: Wear appropriate protective clothing to prevent contact with skin and eyes. Wear a self-contained breathing apparatus (SCBA) to prevent contact with thermal decomposition products. May be combustible at high temperatures.
Extinguishing Media: Use water spray, dry chemical, carbon dioxide, or chemical foam.
Flash Point: Not applicable.
Autoignition Temperature: Not available.
Explosion Limits, Lower: Not available.
Upper: Not available.
NFPA Rating: (estimated) Health: 1; Flammability: 0; Instability: 1

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Vacuum or sweep up material and place into a suitable disposal container. Avoid generating dusty conditions. Provide ventilation. Do not let this chemical enter the environment.

Section 7 - Handling and Storage

Handling: Use with adequate ventilation. Minimize dust generation and accumulation. Avoid contact with eyes, skin, and clothing. Avoid ingestion and inhalation.
Storage: Store in a cool, dry place. Store in a tightly closed container.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate ventilation to keep airborne concentrations low.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Sodium chloride	none listed	none listed	none listed

OSHA Vacated PELs: Sodium chloride: No OSHA Vacated PELs are listed for this chemical.
Personal Protective Equipment
Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.
Respirators: A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Section 9 - Physical and Chemical Properties

Physical State: Solid
Appearance: white

Odor: odorless
pH: 5.0 - 8.0 (5% aq.sol. 20°C)
Vapor Pressure: 1 mm Hg @ 865 deg C
Vapor Density: Not available.
Evaporation Rate: Not available.
Viscosity: Not available.
Boiling Point: 1461 deg C @ 760 mmHg
Freezing/Melting Point:801 deg C
Decomposition Temperature: Not available.
Solubility: 360 g/L (20°C)
Specific Gravity/Density:2.165
Molecular Formula: NaCl
Molecular Weight:58.44

Section 10 - Stability and Reactivity

Chemical Stability: Hygroscopic: absorbs moisture or water from the air.
Conditions to Avoid: High temperatures, incompatible materials, dust generation, exposure to moist air or water.
Incompatibilities with Other Materials: Metals, strong oxidizing agents, strong acids, bromine trifluoride, nitro compounds, dichloromaleic anhydride + urea.
Hazardous Decomposition Products: Hydrogen chloride, sodium oxide.
Hazardous Polymerization: Will not occur.

Section 11 - Toxicological Information

RTECS#:
CAS# 7647-14-5: VZ4725000
LD50/LC50:
CAS# 7647-14-5:
Draize test, rabbit, eye: 100 mg Mild;
Draize test, rabbit, eye: 100 mg/24H Moderate;
Draize test, rabbit, eye: 10 mg Moderate;
Draize test, rabbit, skin: 50 mg/24H Mild;
Draize test, rabbit, skin: 500 mg/24H Mild;
Inhalation, rat: LC50 = >42 gm/m3/1H;
Oral, mouse: LD50 = 4 gm/kg;
Oral, rat: LD50 = 3000 mg/kg;
Skin, rabbit: LD50 = >10 gm/kg;
.

Carcinogenicity:
CAS# 7647-14-5: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: The only adverse effect noted from occupational exposures have been mild nasal irritation with exposure to high dust levels and hypertension.
Teratogenicity: No information found
Reproductive Effects: No information found
Mutagenicity: No information found
Neurotoxicity: No information found
Other Studies:

Section 12 - Ecological Information

Ecotoxicity: No data available. No information available.
Environmental: No information available.

Physical: No information available.
Other: Do not empty into drains.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	Not regulated	Not Regulated
Hazard Class:		
UN Number:		
Packing Group:		

Section 15 - Regulatory Information

US FEDERAL

TSCA
CAS# 7647-14-5 is listed on the TSCA inventory.
Health & Safety Reporting List
None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules
None of the chemicals in this product are under a Chemical Test Rule.
Section 12b
None of the chemicals are listed under TSCA Section 12b.
TSCA Significant New Use Rule
None of the chemicals in this material have a SNUR under TSCA.
CERCLA Hazardous Substances and corresponding RQs
None of the chemicals in this material have an RQ.
SARA Section 302 Extremely Hazardous Substances
None of the chemicals in this product have a TPQ.
SARA Codes
CAS # 7647-14-5: immediate.
Section 313 No chemicals are reportable under Section 313.
Clean Air Act:
This material does not contain any hazardous air pollutants.
This material does not contain any Class 1 Ozone depletors.
This material does not contain any Class 2 Ozone depletors.
Clean Water Act:
None of the chemicals in this product are listed as Hazardous Substances under the CWA.
None of the chemicals in this product are listed as Priority Pollutants under the CWA.
None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
OSHA:
None of the chemicals in this product are considered highly hazardous by OSHA.
STATE
CAS# 7647-14-5 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

California Prop 65

California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives

Hazard Symbols:

Not available.

Risk Phrases:

Safety Phrases:

S 24/25 Avoid contact with skin and eyes.

WGK (Water Danger/Protection)

CAS# 7647-14-5: 0

Canada - DSL/NDSL

CAS# 7647-14-5 is listed on Canada's DSL List.

Canada - WHMIS

This product has a WHMIS classification of Not controlled..

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.

Canadian Ingredient Disclosure List

Section 16 - Additional Information

MSDS Creation Date: 7/12/1999
Revision #13 Date: 2/15/2008

Safety Data Sheet

Infosafe No™	1CH71	Issue Date : August 2014	RE-ISSUED by CHEMSUPP

Product Name : **SULFUR**

Classified as hazardous

1. Identification

GHS Product Identifier	SULFUR
Company Name	CHEM-SUPPLY PTY LTD (ABN 19 008 264 211)
Address	38 - 50 Bedford Street GILLMAN SA 5013 Australia
Telephone/Fax Number	Tel: (08) 8440-2000 Fax: (08) 8440-2001
Recommended use of the chemical and restrictions on use	Sulfuric acid manufacture, paper and pulp manufacture, carbon disulfide, rubber vulcanization, detergents, petroleum refining, dyes and chemicals, drugs and pharmaceuticals, explosives, insecticides, rodent repellents, soil conditioner, fungicide, coating for controlled-release fertilisers, nucleating agent for photographic film, cement sealant, binder and asphalt extender in road paving, base material for low-temperature mortars, and laboratory reagent.

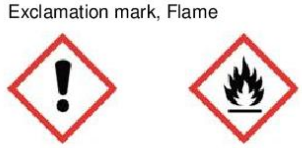
Other Names

Name	Product Code
SULFUR Small Pastilles	ST262
SULFUR Roll	ST053
Brimstone	
Flowers of sulfur	
SULFUR LR	SL006
SULFUR TG	ST006

Additional Information	Sulfur is not subject to the provisions of the Australian Dangerous Goods Code entry Sulfur UN 1350 when it is transported in quantities of less than 400 kg per package, or when it has been formed to a specific shape (e.g. prills, granules, pellets, pastilles or flakes). Sulfur is not subject to the provisions of the International Maritime Dangerous Goods Code entry Sulfur UN 1350 when it has been formed to a specific shape (e.g. prills, granules, pellets, pastilles or flakes).
Other Information	EMERGENCY CONTACT NUMBER: +61 08 8440 2000 Business hours: 8:30am to 5:00pm, Monday to Friday. Chem-Supply Pty Ltd does not warrant that this product is suitable for any use or purpose. The user must ascertain the suitability of the product before use or application intended purpose. Preliminary testing of the product before use or application is recommended. Any reliance or purported reliance upon Chem-Supply Pty Ltd with respect to any skill or judgement or advice in relation to the suitability of this product of any purpose is disclaimed. Except to the extent prohibited at law, any condition implied by any statute as to the merchantable quality of this product or fitness for any purpose is hereby excluded. This product is not sold by description. Where the provisions of Part V, Division 2 of the Trade Practices Act apply, the liability of Chem-Supply Pty Ltd is limited to the replacement of supply of equivalent goods or payment of the cost of replacing the goods or acquiring equivalent goods.

2. Hazard Identification

GHS classification of the substance/mixture	Flammable Solids: Category 2 Skin Corrosion/Irritation: Category 2
Signal Word (s)	WARNING
Hazard Statement (s)	H228 Flammable solid. H315 Causes skin irritation.
Pictogram (s)	Exclamation mark, Flame



Precautionary statement – Prevention	P210 Keep away from heat/sparks/open flames/hot surfaces. – No smoking. P264 Wash thoroughly after handling. P280 Wear protective gloves/protective clothing/eye protection/face protection.
Precautionary statement – Response	P302+P352 IF ON SKIN: Wash with plenty of soap and water. P332+P313 If skin irritation occurs: Get medical advice/attention. P362 Take off contaminated clothing and wash before reuse. P370+P378 In case of fire: Use dry chemical, CO2, water spray or foam.

3. Composition/information on ingredients

Chemical Characterization	Solid				
Ingredients	**Name**	**CAS**	**Proportion**	**Hazard Symbol**	**Risk Phrase**
	Sulfur	7704-34-9	100 %	Xi, F	R36/38, R11

4. First-aid measures

Inhalation If inhaled, remove from contaminated area to fresh air immediately. Apply artificial respiration if not breathing. If breathing is difficult, give oxygen. Get medical aid if cough or other symptoms appear.

Ingestion Rinse mouth thoroughly with water immediately. Give plenty of water to drink. Do not induce vomiting. Seek medical attention.

Skin Wash with plenty of soap and water. Remove contaminated clothing and wash before re-use. Seek immediate medical advice.

Eye contact Immediately irrigate with copious quantity of water for at least 15 minutes. Eyelids to be held open. Seek medical attention.

First Aid Facilities Maintain eyewash fountain and safety shower in work area.

Advice to Doctor Treat symptomatically or consult a Poisons Information Centre.

Other Information For advice, contact the National Poisons Information Centre (Phone Australia 13 11 26; New Zealand 0800 764 766) or a doctor.

5. Fire-fighting measures

Hazards from Combustion Products Librates toxic fumes in fire (sulfur oxides, hydrogen sulfide gas).

Specific Methods Small fire: Use dry chemical, CO2, water spray or foam.
Large fire: Use water spray, fog or foam.
If safe to do so, move undamaged containers from fire area. Cool containers with flooding quantities of water until well after fire is out.

Specific hazards arising from the chemical May be ignited by friction, heat, sparks or flame. Vapours, dust, borings or turnings may form combustible mixtures with air. May burn fiercely. May re-ignite after fire is extinguished. Fire may produce irritating, poisonous and/or corrosive gases. Containers may explode when heated. Runoff may pollute waterways. May be transported in a molten form. Solids may melt and flow when heated or involved in a fire.

Hazchem Code 1Z

Precautions in connection with Fire Wear SCBA and chemical splash suit. Structural firefighter's uniform may provide limited protection.

6. Accidental release measures

Spills & Disposal Eliminate all ignition sources (no smoking, flares, sparks or flames) within at least 15m. Do not touch or walk through spilled material. Prevent entry into waterways, drains or confined areas. Obtain expert advice on use of water as spilled material may be water-reactive. Prevent dust cloud. Use clean non-sparking tools to collect absorbed material and place it into loosely-covered metal or plastic containers for later disposal. SEEK EXPERT ADVICE ON HANDLING AND DISPOSAL.

Personal Precautions Avoid substance contact. Avoid generation of dusts: do not inhale dusts. Ensure supply of fresh air in enclosed rooms.

Personal Protection Wear protective clothing specified for normal operations (see Section 8)

Clean-up Methods - Small Spillages Sweep up (avoid generating dust) and remove to a suitable, clearly labelled container for disposal in accordance with local regulations.

Clean-up Methods - Large Spillages Seek expert advice on handling and disposal.

7. Handling and storage

Precautions for Safe Handling Avoid generation or accumulation of dusts. Avoid prolonged or repeated contact with skin, eyes and clothing . Take precautionary measures against static discharges. Use in well ventilated areas away from all ignition sources. In case of insufficient ventilation, wear suitable respiratory equipment. Contaminated clothing should be removed and washed before reuse.

Conditions for safe storage, including any incompatabilities	Store away from sources of heat or ignition. Store away from combustible materials. Store in well ventilated area. Store in a cool dry place out of direct sunlight. Avoid contact with incompatible materials that support combustion such as strong oxidising agents. Keep containers securely sealed and protected against physical damage.
Other Information	A bulk cargo of sulfur has a liability to dust discharge during cleaning. Explosion may be avoided by preventing the atmosphere becoming dust-laden by adequate ventilation or by hosing-down instead of sweeping.

8. Exposure controls/personal protection

Other Exposure Information	A time weighted average (TWA) concentration for an 8 hour day, and 5 day week has not been established by Safe Work Australia for this product. There is a blanket limit of 10 mg/m³ for dusts or mists when limits have not otherwise been established.
Appropriate engineering controls	In industrial situations maintain the concentrations values below the TWA. This may be achieved by process modification, use of local exhaust ventilation, capturing substances at the source, or other methods.
Respiratory Protection	Where ventilation is not adequate, respiratory protection may be required. Avoid breathing dust, vapours or mists. Respiratory protection should comply with AS 1716 - Respiratory Protective Devices and be selected in accordance with AS 1715 - Selection, Use and Maintenance of Respiratory Protective Devices. Filter capacity and respirator type depends on exposure levels. In event of emergency or planned entry into unknown concentrations a positive pressure, full-facepiece SCBA should be used. If respiratory protection is required, institute a complete respiratory protection program including selection, fit testing, training, maintenance and inspection.
Eye Protection	The use of a face shield, chemical goggles or safety glasses with side shield protection as appropriate. Must comply with Australian Standards AS 1337 and be selected and used in accordance with AS 1336.
Hand Protection	Hand protection should comply with AS 2161, Occupational protective gloves - Selection, use and maintenance. Recommendation: Rubber or plastic gloves.
Personal Protective Equipment	Final choice of personal protective equipment will depend on individual circumstances and/or according to risk assessments undertaken.
Footwear	Safety boots in industrial situations is advisory, foot protection should comply with AS 2210, Occupational protective footwear - Guide to selection, care and use.
Body Protection	Clean clothing or protective clothing should be worn, preferably with an apron. Clothing for protection against chemicals should comply with AS 3765 Clothing for Protection Against Hazardous Chemicals.
Hygiene Measures	Always wash hands before smoking, eating or using the toilet. Wash contaminated clothing and other protective equipment before storing or re-using.

9. Physical and chemical properties

Form	Solid
Appearance	Yellow powder, granules, flakes, discs, pastilles or roll.
Odour	Pure sulfur is odorless, but traces of hydrocarbon impurity may impart an oily and/or rotten egg odor.
Melting Point	113-119 °C
Boiling Point	444 - 445 °C
Solubility in Water	Insoluble.
Solubility in Organic Solvents	Soluble in toluene, carbon disulfide, carbon tetrachloride and benzene. Slightly soluble in acetone, ether, alcohol.
Specific Gravity	1.96 - 2.07
Vapour Pressure	< 0.01 hPa (20 °C)
Vapour Density (Air=1)	8.9
Flash Point	160°C closed cup.
Flammability	Flammable solid category 2.
Auto-Ignition Temperature	235 °C
Explosion Limit - Upper	40 % vol
Explosion Limit - Lower	1 % vol

Explosion Properties	Sulfur is a poor conductor or electricity causing charges of static electricity to build up during transport or processing. Static discharge may lead to ignition of sulfur dust. Sulfur may cause an explosion upon contact with ammonia, ammonia nitrate, ammonium perchlorate, tetraphenyllead, stannic iodide with sodium, sodium, phosphorus, iodine pentaoxide, potassium perchlorate. Combination of finely divided sulfur and finely divided bromates (also chlorates or iodates) of barium, calcium, magnesium, potassium, sodium or zinc will explode with heat, percussion and sometimes, light friction.
Molecular Weight	32.06
Other Information	Refractive index: 2.038

10. Stability and reactivity

Reactivity	Risk of dust explosion.
Chemical Stability	Stable under normal use conditons.
Conditions to Avoid	Exposure to moisture. Heat, flames, ignition sources and incompatibles.
Incompatible Materials	Alkali metals, alkaline earth metals, metals, metallic oxides, non metals, nonmetallic oxides, fluorine, halogen-halogen compounds, oxidizing agents, peroxi compounds, nitrites, hydrides, nitrides, carbides, sulfides, lithium silicide, silicon compounds, carbon disulfide, ethers, acetylidene, organic nitro compounds; with mineral acids and oxidizing agent (formed could be: sulfuric acid); violent reactions possible with: chlorates, nitrates, perchlorates and permanganates.
Hazardous Decomposition Products	Sulfur oxides.
Possibility of hazardous reactions	Can react violently with halogens, carbides, halogenates, halogenites, zinc, uranium, tin, sodium, lithium, nickel, palladium, gadolinium, phosphorus, potassium, indium, calcium, boron, aluminium, ammonia, ammonium nitrate, ammonium perchlorate, chlorine dioxide, potassium permanganate, silver nitrate, silver oxide and sodium hydride. Forms explosive and sensitive mixtures with most oxidising substances such as chlorates, nitrates, perchlorates or permanganates.
Hazardous Polymerization	Will not occur.
Other Information	Transitions temperature, between alpha and beta crystalline forms, is ~ 95 °C. The conversion is slow.

11. Toxicological Information

Acute Toxicity - Oral	LD50 (rat): > 5000 mg/kg
Acute Toxicity - Dermal	LD50 (rabbit): > 2000 mg/kg
Acute Toxicity - Inhalation	LC50 (rat): > 9.23 mg/l/4 h.
Ingestion	May be harmful if ingestion. May cause gastrointestinal tract irriation with symptoms including nausea, vomiting and diarrhea. Poorly absorbed. Ingestion of large amounts may cause sore throat, headache, nausea and possible unconsciousness in severe cases. May be converted to toxic hydrogen sulfide in the intestines. Excessive amounts that are ingested may affect the central nervous system, behaviour and kidneys.
Inhalation	May be harmful if inhaled. Inhalation of dusts causes irriation to the mucous membranes and upper respiratory tract. Inhalation of sulfur causes irritation to the mucous membranes of the respiratory tract (nose, throat and lungs), causing coughing, sneezing, wheezing and laboured breathing. Inflammation of the respiratory tract may result in bronchitis, pulmonary edema, pneumonia, asthma. However, this reaction is potentially reversible and leaves no scar tissue.
Skin	May cause irritation, rash and dermatitis.
Eye	Contact causes irritation to the eyes. Symptoms include of tearing, redness, pain, burning, scratchy discomfort and blurred vision. Prolonged or repeated exposure may lead to possible eye damage.
Carcinogenicity	No evidence of carcinogenic properties.
Chronic Effects	Chronic exposure may lead to irritation of mucous membranes, chronic bronchitis, emphysema and bronchial asthma. May cause possible skin sensitization and permanent eye damage (clouding of lens and chronic irritation).
Serious eye damage/irritation	Eye irritation (human): 8 ppm.

Infosafe No™	1CH71	Issue Date : August 2014	RE-ISSUED by CHEMSUPP

Product Name : **SULFUR**

Classified as hazardous

Mutagenicity	No evidence of mutagenic properties.

12. Ecological information

Acute Toxicity - Fish	LC50 (Br. rerio): 866 mg/l/96 h.
Acute Toxicity - Daphnia	EC50 (Daphnia magna): > 10000 mg/l/24 h.
Acute Toxicity - Bacteria	EC50 (activated sludge): 1900 mg/l/3 h.
Acute Toxicity - Other Organisms	EC50 (Protozoa, Tetrahymen pyriformis): 0.16 mg/l/24 h.

13. Disposal considerations

Disposal Considerations	Whatever cannot be saved for recovery or recycling should be disposed of according to relevant local, state and federal government regulations.

14. Transport information

Transport Information	Dangerous Goods of Class 4.1 Flammable Solids, are incompatible in a placard load with any of the following: - Class 1, Class 2.1, Class 4.2, Class 5 and Class 7
U.N. Number	1350
UN proper shipping name	SULFUR
Transport hazard class(es)	4.1
Hazchem Code	1Z
Packaging Method	3.8.4.1
Packing Group	III
EPG Number	4A1
IERG Number	20

15. Regulatory information

Regulatory Information	Listed in the Australian Inventory of Chemical Substances (AICS).
Poisons Schedule	Not Scheduled

16. Other Information

Date of preparation or last revision of SDS	September 2009.
Literature References	'Standard for the Uniform Scheduling of Medicines and Poisons No. 4', Commonwealth of Australia, June 2013. Lewis, Richard J. Sr. 'Hawley's Condensed Chemical Dictionary 13th. Ed.', Rev., John Wiley and Sons, Inc., NY, 1997. National Road Transport Commission, 'Australian Code for the Transport of Dangerous Goods by Road and Rail 7th. Ed.', 2007. 'Labelling of Hazardous Workplace Chemicals, Code of Proctice' Safe Work Australia. Standards Australia, 'SAA/SNZ HB 76:2010 Dangerous Goods - Initial Emergency Response Guide', Standards Australia/Standards New Zealand, 2010. Safe Work Australia, 'Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(2004)]'. Safe Work Australia, 'Hazardous Substances Information System, 2005'. Safe Work Australia, 'National Code of Practice for the Labelling of Safe Work Hazardous Substances (2011)'. Safe Work Australia, 'National Exposure Standards for Atmospheric Contaminants in the Occupational Environment [NOHSC:1003(1995)]'.
Contact Person/Point	Paul McCarthy Ph. (08) 8440 2000 DISCLAIMER STATEMENT: All information provided in this data sheet or by our technical representatives is compiled from the best knowledge available to us. However, since data, safety standards and government regulations are subject to change and the conditions of handling and use, or misuse, are beyond our control, we make no warranty either expressed or implied, with respect to the completeness or accuracy to the information

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Empirical Formula & Structural Formula S

...End Of MSDS...

Appendix D: Patents

United States Patent [19]

Neubeck

[11] **4,233,405**

[45] **Nov. 11, 1980**

[54] **PROCESS FOR SPRAY DRYING ENZYMES**

[75] Inventor: **Clifford E. Neubeck**, Hatboro, Pa.

[73] Assignee: **Rohm and Haas Company**, Philadelphia, Pa.

[21] Appl. No.: **83,449**

[22] Filed: **Oct. 10, 1979**

[51] **Int. Cl.**[3] **C12N 9/98**

[52] **U.S. Cl.** **435/187**; 435/203; 435/222

[58] **Field of Search** 435/187, 203, 222

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,180,917 1/1980 Neubeck 34/5

FOREIGN PATENT DOCUMENTS

40-17169 2/1979 Japan .

OTHER PUBLICATIONS

Chemical Abstracts 76, 32816f (1972).
Chemical Abstracts 81, 62097t (1974).

Primary Examiner—Lionel M. Shapiro

[57] **ABSTRACT**

This invention involves a process for the preparation of spray dried enzymes.

10 Claims, No Drawings

PROCESS FOR SPRAY DRYING ENZYMES

BACKGROUND OF THE INVENTION

The concept of using spray drying for preparing dry enzyme compositions is known in the art. A protease unhairing enzyme in the form of a whole crude culture of bacteria (*Pseudomonas effusa*) has been successfully spray dried. A crude culture of *Bacillus subtilis* to which wood flour was added has also been spray dried. The stability of the activity was poor in the latter case and together with the lack of any cost advantage made spray drying over tray drying impractical. A protease (Bacillus spp.) was spray dried using ultrafilter concentrates but odor, high bacteria count, and hazards related to inhalation of alkaline protease dust made this method of manufacture impractical.

Subsequently attempts were made to develop a process for spray drying enzyme concentrates (primarily from surface culture) obtained by ultrafilter concentration of several culture filtrates. These trials indicated that spray drying of enzymes was possible but activity yields and solids recovery were frequently low, a large amount of product collected on the chamber walls, moisture in the final product was very high, caking and darkening of the dried concentrate was frequently observed and activity retention was poor during storage at room temperature.

Freeze drying has also been utilized to obtain a dry enzyme composition. Initially, freeze drying gave better yields than spray drying. Several of the concentrates still gave problems with freeze drying until it was noted that supplementation of the enzyme concentrates with selected combinations of inorganic salts and insoluble ingredients gave improved results. These findings became the basis of U.S. application Ser. No. 939,745 filed Sept. 9, 1978 by Clifford E. Neubeck assigned to a common assignee U.S. Pat. No. 4,180,917, issued Jan. 1, 1980.

In initial studies, several different formulations of enzyme concentrates were tested in an attempt to select the type and composition of additives to be used in spray drying. These experiments, involved in vacuo drying of bacterial alpha-amylase concentrate to which various salts and insoluble ingredients were added, demonstrated the critical nature of the additives. The variation of the additives had a significant effect on the physical nature, activity recovery, moisture level, lumping, color, etc., of the product. As a result of the above experimentation, initial spray drying experiments were conducted.

SUMMARY OF THE INVENTION

This invention relates to a process for spray drying enzymes which comprises concentrating a liquid enzyme solution by ultrafiltration, adding to said concentrate water-insoluble salts, optionally in the presence of water-insoluble suspenders and thickeners, intimately mixing the concentrate composition, and spray-drying said concentrate composition at air inlet temperatures of from about 120° C. to about 180° C., and air outlet temperatures from about 68° C. to about 85° C.

In accordance with the process of this invention any conventional ultrafiltration equipment can be utilized as long as it fills the requirements of being able to not only concentrate the liquid enzyme solution but also remove low molecular weight sugars, amino acids, and peptides which are the major cause of discoloration, hygroscopicity, odor, gumming, caking and other similar factors which lead to deterioration of the product and in some cases to unmarketable products.

Water insoluble salts that can be utilized in the process of this invention include tribasic calcium phosphate, tribasic magnesium phosphate, calcium carbonate, calcium hypophosphate, calcium magnesium silicate, calcium silicate, calcium sulfite, calcium tartarate, magnesium oxide and magnesium silicate and the like. Any water soluble salts which when combined form water insoluble salts can also be utilized. By water soluble salt it is meant any salt which is at least slightly soluble in the media in which it is to be dissolved.

Water insoluble suspenders and thickeners which may optionally be employed in the process of this invention include cornstarch, potatoe starch, tapioca, cellulose and the like.

In accordance with the process of this invention the air inlet temperatures which can be utilized for the addition of the liquid enzymes concentrate composition are from about 120° C. to about 180° C. However, a preferred temperature range for the air inlet temperature is from about 150° C. to about 177° C. more preferably from about 155° C. to about 165° C. The air outlet temperatures which can be utilized in the process of this invention for the spray dried enzyme compositions obtained by this process are from about 65° C. to about 90° C. However, a preferred temperature range for the air outlet temperature is from about 70° C. to about 85° C. more preferably from about 75° C. to about 83° C.

A preferred process of this invention relates to the preparation of a spray dried enzyme composition which comprises concentrating a liquid enzyme solution obtained from a submerged culture of an enzyme selected from Bacillus sps protease, Aspergillus sps protease or Bacillus sps amylase, by ultrafiltration, forming water insoluble salts in situ by the reaction of a water soluble phosphate salt and a water soluble calcium salt, intimately mixing the concentrate composition and spray drying the concentrate composition at air inlet temperatures from about 155° C. to about 175° C. and air outlet temperatures from about 75° C. to about 83° C.

DETAILED DESCRIPTION OF THE INVENTION

Table I gives a summary of eleven test runs made in small scale using an *Aspergillus oryzae* protease without any additives to illustrate the type of result obtained. Similar data were also obtained on other *Aspergillus oryzae* proteases.

TABLE 1

Summary of Preliminary Spray Drying Study using *Aspergillus oryzae* protease Concentrates Without Additives[1]

Test Number	1	2	3	4	5	6	7	8	9	10	11
Starting Material											
Activity(HU)[2]	17100	17100	16400	16400	17100	17100	17100	36000	36000	47000	47000
% Solids	12.5	12.5	12.5	12.5	12.0	12.0	12.0	16.0	16.0	17.2	17.2
Drying Conditions											

TABLE 1-continued

	Summary of Preliminary Spray Drying Study using *Aspergillus oryzae* protease Concentrates Without Additives[1]										
Test Number	1	2	3	4	5	6	7	8	9	10	11
Air T in °C.	160	160	130	120	149	149	121	120	130	120	130
Air T out °C.	75	75	70	70	71	82	68	70	70	70	70
Product activity HU[2]	73000	113000	122000	125000	117000	108000	137000	184000	193100	216000	206000
% Moisture	13.5	14.1	10.1	11.3	11.3	11.5	12.3	9.4	9.4	9.4	10.1
% Weight Recovered[3]	16.4	29.8	49.2	62.8	41.6	51.0	57.2	77.1	78.2	70.7	66.1
% Activity Recovered	8.8	27.3	45.5	60.7	38.5	40.0	57.6	65.0	72.5	54.9	51.0

[1]Most of these spray dried products were gummy, dark in color and hygroscopic.

[2]An enzyme has an activity of 1000 HU per g. if 11.18 mg. of it produces an increase in soluble nitrogen of 5.0 mg. from 0.417 g. hemoglobin in 5 hours at 40° C. and pH 4.7.

[3]% Weight recovery of solids $= \frac{\text{weight out} \times \text{\% solids}}{\text{weight in} \times \text{\% solids}}$ 100. Solids recovery includes solids in product collected plus product in chamber plus chamber dustings.

Spray Drying Studies With Additives

The relatively poor experience with spray drying as summarized in Table I (for *Aspergillus oryzae* protease) led to the testing of a number of additives as supplements to the enzyme concentrate utilizing in vacuo drying in order to determine the effect on the physical properties of the final product. This experimentation was done on Bacterial alpha-amylase Concentrate since it was the material considered for initial testing with various additives.

Table II summarizes these results. Surprisingly the best mixture of additives from the standpoint of physical appearance (#17) did not give an exceptional yield of activity. The formation of calcium phosphate in the concentrate by mixing ammonium phosphate and lime gave distinctly better properties than adding calcium phosphate alone (#18) with starch (#14 and #190) or with ammonium phosphate, lime, and starch (#15). Starch alone was not effective. The results suggested that the manner of adding substances and the composition had a profound effect on the physical nature of the product.

Spray drying tests were made on Bacterial alpha-amylase concentrate using the concentrate alone, concentrate plus cornstarch (5%) and tricalcium phosphate (7%) added sequentially and finally a third mixture containing concentrate plus cornstarch (5%) and a dry mixture of ammonium phosphate (7%) and lime (3.6%). The dry mixture of ammonium phosphate and lime was added to the concentrate after the starch has been thoroughly suspended.

In Table II, aliquots of the enzyme Concentrate at 16% solids and 41000 FM Activity were mixed with various additives and then dried in vacuo at 45° C. The dried material was examined for texture, color, and activity in cases where the physical nature of the dried material was good. An enzyme with 1,000 starch liquifying units (FM) per g. reduced the viscosity of 300 times its weight of potato starch by 90% in 10 minutes at 70° C. and pH 6.7.

TABLE II

Laboratory Drying Experiments on Bacterial alpha-amylase Concentrate									
	Materials Added to Concentrate				Appearance of Dried Product				
Test Number	% $NH_4H_2PO_4$	% CaO	% $Ca_3(PO_4)_2$	% Starch	Color	Stickiness	Grinding	Activity	Activity Recovery
12	—	—	—	—	Dark	Gummy	Very Difficult		
13	—	—	—	10	Dark	Gummy	Very Difficult		
14	—	—	14	10	Light	None	Hard	102000	91.3
15	5.2	3.6	7.0	10	Dark	Slightly Gummy	Difficult		
16	2.6	1.8	10.8	10	Light	None	Hard	69600	108
17	10.4	7.2	—	10	Very Light	None	Soft	37500	65
18	—	—	14.0	—	Light	Sl. Gummy	Hard		
19	—	—	10.0	14	Dark	None	Hard		

Table III summarizes the results obtained when the various mixtures disclosed therein were spray dried.

Bacterial alpha-amylase Ultrafilter Concentrate at 25050 FM (diastase activity) and 8.0% soluble solids was divided into 3 parts:

Mix 1. No additives

Mix 2. Plus 5% cornstarch and 7% tricalcium phosphate

Mix 3. Plus 5% cornstarch, 7% $NH_4H_2PO_4$ and 3.6% CaO. (Ammonium phosphate and lime mixed dry before addition to the concentrate containing starch)

Each of the compositions was then spray dried at 16,600–18,700 RPM wheel speed for atomization and air temperatures of 310° F. (154° C.) inlet and 175° F. (80° C.) outlet. Mix 1 was also dried at other combinations of wheel speed and temperature.

As noted in Table III, spray dried product prepared from Mix 1 (i.e. no additives) gave poor solids recovery and caking of the product was observed after storage for several days. Change in atomizer speed or drying temperatures did not alter the results significantly for Mix 1. Best recovery of activity and best appearance was obtained with Mix 3 but Mix 2 was more readily handled in the pumping system to the atomizer. Mix 3 gave a lower specific activity.

TABLE III

Spray Dry Test Runs With Bacterial alpha-amylase Concentrate

Composition Dried	Wheel Speed RPM	Air Temp. °F. In	Air Temp. °F. Out	Dry Product Quality	Dry Product Activity (FM)[1)]	Recovery Solids	Recovery Activity
Mix 1	12,000	310	173	Poor Drying		Poor recovery	
Mix 1	13,300	310	175	Still Wet	287,000	42.3	40.8
Mix 1	16,600	310	175	Some Caking[2)]	303,500	62	60.3
Mix 1	16,600	330	177	Some Caking[2)]	292,500	62	58.1
Mix 1	16,600	340	179	Some Caking [2)]	294,000	62	58.4
Mix 1	16,600	350	180	Some Caking[2)]	291,000	62	57.8
Mix 2	18,700	310	175	Smooth flowing Light Color	130,000	87	80
Mix 3[3)]	18,700	310	180	Very good Light color	102,000	100	100

1)An enzyme with 1,000 starch liquifying units per g. reduces the viscosity of 300 times its weight of potato starch by 90% in 10 minutes at 70° C. and pH 6.7.
2)Samples prepared from Mix 1 caked into a lump of material with dark color after storage.
3)Mix 3 had a tendency to clog the nozzles of wheel atomizer.

Large scale runs were made with Bacterial alpha-amylase Concentrate in two different size driers. Basically the additive composition of Mix 2 (Table III) was used but slightly different ratios of starch to calcium phosphate were employed and the ultrafiltration concentration was slightly more efficient in the larger run so that a larger amount of low molecular weight soluble solids were removed. Example 1 gives the details of the smaller run while Example 2 gives the details of the larger run.

EXAMPLE 1

Bacterial alpha-amylase Concentrate Spray Dried (Dryer has a water evaporation rate of 343 lbs./hour operating at 420° F./200° F. inlet/outlet temperature

A strain of *Bacillus subtilis* selected to produce alpha amylase was grown on 24,000 pounds of medium containing cornstarch, corn steep water and inorganic salts using deep tank conditions to produce a culture with 21% dry solids and having 19,500 starch liquefying units (FM) per gram[1]. After filtration and washing on a rotary vacuum filter, 30,800 pounds of filtrate were obtained having 12,800 FM units per g and a solids content of 13.1%. Activity recovery was 85% with a reduction in solids from 5,000 lbs. to 4,035 lbs. The culture filtrate was concentrated by ultrafiltration to an activity of 45,900 FM/g in a weight of 7,055 pounds at a solids content of 19.0%. Solids (primarily in the form of low molecular weight solubles) were reduced from 4,035 lbs. to 1,340 lbs.—about 27% of the solids in the original crude culture or 33% of that in the filtrate before concentration. Only 18% enzyme activity was lost.

[1] An enzyme with 1,000 starch liquefying units per g reduces the vicosity of 300× its weight of potato starch by 90% in 10 minutes at 70° C. and pH 6.7.

Insoluble solids in the form of 400 pounds of cornstarch (5%) and 525 pounds of tricalcium phosphate (7%) were added to the concentrate to give a total weight of 8080 pounds at an activity of 39,900 FM/g. The concentrate plus additives was spray dried using a 160 mm wheel for atomization rotating at 18,700 RPM. Inlet temperature was set at 310° F. and the outlet temperature was set at 170° F. Product in the amount of 1889 pounds at an activity of 146,000 FM/g was collected plus 40 pounds of drying chamber dustings at 146,000 FM/g. Solids recovery was 94.6% in the product plus 2.0% in the dustings. Activity recovery was 85.3% in the product plus 1.8% in the dustings. The moisture content in the light colored free-flowing product was 5.26%. The dry concentrate has retained full activity and good flow character after storage for several months at room temperature.

EXAMPLE 2

Bacterial alpha-amylase concentrate Spray Dried (Dryer has a water evaporation rate of 1,400 lbs. per hour operating at 420° F./200° F. inlet/outlet temperatures)

A strain of *Bacillus subtilis* selected to produce alpha amylase was grown under deep tank conditions on 31,400 pounds of medium containing cornstarch, corn steep water and inorganic salts to produce a culture containing 21% total solids and 16,500 FM per g. After filtration and washing, 40,900 pounds of filtrate at an activity of 10,360 FM/g. and a solids content of 13% were obtained. Activity recovery was 82% while solids were reduced from 6,594 pounds to 5,317 pounds. The culture filtrate was concentrated by ultrafiltration at 10° C. to a final weight of 8,600 pounds having a solids content of 15.4% and 43,300 FM. Solids, primarily in the form of highly colored, gummy, low molecular weight material were reduced from 5,317 pounds to 1,315 pounds. The discarded soluble solids of 4,002 pounds reduced the soluble solids in the concentrate by 75% with a loss in activity of only 12%.

Insoluble solids in the form of 450 pounds of cornstarch (5.6%) and 300 pounds of tricalcium phosphate (4.0%) were added to the concentrate to give 9,350 pounds of concentrate plus additives at 38,000 FM/g. The 9,350 pounds of mixture were spray dried using a 260 mm radial wheel rotating at 11,000 RPM for atomization. Inlet temperature was set at 315° F. and outlet temperature was 155° F. Product in the amount of 1,975 pounds at an activity of 170,000 FM/g was recovered. The activity recovery was 94.5% and the solids recovery was 99.6%. The light colored free flowing powder contained 4.0% moisture and remained free flowing when stored at room temperature for several months.

EXAMPLE 3

Neutral Fungal Protease Concentrate

This enzyme is prepared by growing a selected strain of *Aspergillus flavus*-oryzae on a medium containing a mixture of liver and soybean meals and ammonium phosphate under deep tank conditions to produce a fungal protease which can be used for protein modification, e.g. meat tenderization. Fungal mycelium and medium debris are removed from the crude culture by filtration on a rotary vacuum filter using diatomaceous earth as a filter aid. Proteolytic activity is measured as casein solubilization units (EE)[1]. The filtered cell free solution containing enzyme is concentrated by ultrafiltration and spray dried to give a dry stable free flowing solid of high activity as described below.

[1] Casein solubilization units (EE) are defined as follows: An enzyme with an activity of 1000 EE solubilizes nine times its weight of casein in 1 hour at 40° C. and pH 8.0.

The crude enzyme concentrate, 15,000 lbs., containing a total dry solids of 12.8% and 1,590 EE was filtered and washed on a rotary vacuum filter to give 18,480 lbs. of filtrate plus wash at 1,300 EE. Filtrate plus wash in the amount of 3,450 lbs. (equivalent to 2,800 lbs. of culture and 358 lbs. solids) was then concentrated to 440 lbs. at 9,250 EE and containing 12.3% solids in an ultrafiltration unit operating at 10° C.±1° C. The total solids were reduced to 54 lbs. or 15% of the starting culture solids. The concentrate at 9,250 EE which contained about 90% of the activity present in 2,800 lbs. of culture was divided into two parts of 120 lbs. and 320 lbs. The second part was supplemented with 21 pounds of cornstarch and 24 pounds of calcium acetate added in sequence. Good mixing was employed to permit formation of finely divided water insoluble calcium phosphate intimately mixed with the starch. A total of 365 lbs. of supplemented concentrate was obtained. Activity recovery in the supplemented concentrate was 87.5% of that expected. The unsupplemented part 1 (120 lbs.) and supplemented part 2 (365 lbs. after addition) were then spray dried in the same drier separately using the same drying conditions: viz. 310° F./175° F. inlet and outlet air temperatures and atomized at 18,700 RPM with 160 mm wheel. Table IV gives the results obtained in this experiment.

TABLE IV

Spray Dried Neutral Fungal Protease

	Concentrate without Supplementation	Concentrate with Supplementation
Dry Solids	12.3%	23.0%
Activity before spray drying	9,250 EE	7,100 EE
Pounds Concentrate dried	119	365
Pounds of spray dry material produced		
Collected from		
Cyclone	9.5	79
Chamber sweepings	2.5	4
Activity	72,500 EE	28,500 EE
Recovery of solids	82%	99%
Recovery of Activity	79%	91%
Moisture in Product	6.54%	6.79%
Product Physical Appearance	Dark Color Forms lumps on storage	Light in Color Free flowing character retained on storage

EXAMPLE 4

Fungal Acid Protease Concentrate

This enzyme is produced by growth of a selected strain of *Aspergillus oryzae* under deep tank conditions on a medium containing a mixture of liver, soybean, and blood meals. The fungus produces a protease active on hemoglobin at pH 4.7 and other proteins which may be measured by its action on hemoglobin at pH 4.7 in terms of HU activity[1]. The protease is well suited for the modification of gluten in bakery operations.

For example, 106,000 lbs. of crude culture at 5850 HU and 12.6% total solids were filtered and water washed on a rotary vacuum filter to remove fungal mycelium and culture debris. Diatomaceous earth was used as a filter aid. The clear filtrate plus wash at 129,850 lbs. had an activity of 4,774 HU.

[1] An enzyme has an activity of 1,000 HU per g if 11.18 mg of it produces an increase in soluble nitrogen of 5.0 mg from 0.417 g hemoglobin in 5 hours at 40° C. and pH 4.7.

The culture filtrate plus wash was concentrated to 9,765 lbs. with an activity of 67,000 HU and a solids content of 18.1%. Activity recovery in the concentrate was about 90% of that in the crude culture but the total solids in the concentrate were reduced to 13.2% of that originally present in the culture.

The 9,756 lbs. of concentrate was supplemented with 60 lbs. of cornstarch and 60 lbs. of tricalcium phosphate added sequentially to give 9,885 lbs. of supplemented concentrate at 68290 HU and 20.94% solids. The mixture was spray dried at 330° F./175° F. inlet/outlet temperature with a radial atomizer of 260 mm rotating at 11,000 RPM to give 1,765 lbs. of free flowing light tan powder at 314,600 HU. The moisture level in the final product was 3.67%. Activity and solids recovery were 82% and 85% respectively.

Another lot of this enzyme Concentrate prepared from a different batch but processed in the same way was spray dried under the same conditions without any supplemental additives. The spray dried product was darker in color and exhibited very poor flow properties as compared to the above example with additives. Recovery of activity and solids were very satisfactory however, e.g. activity and solids recovery were 98% and 99% respectively.

The spray dried product produced from concentrate without supplements required the addition of flow aids to prepare a useable product. Preparation of a useable product after storage of the spray dried material prepared from unsupplemented concentrate required both grinding and addition of flow aids. Loss in activity and solids accompanied these operations. Caking and lumping of the spray dried material prepared without additives occurred during storage for several months.

EXAMPLE 5

Neutral Bacterial Protease Concentrate

This enzyme is prepared by growing a selected strain of *Bacillus subtilis* on a medium containing hominy, cornstarch and soybean meal under deep tank conditions to produce neutral bacterial protease. Activity is measured in terms of casein solubilization units (EE). The crude culture is filtered and washed using a rotary vacuum filter. The filtrate is concentrated by ultrafiltration and the concentrate is spray dried as described below.

The crude culture, 113,000 pounds, containing neutral bacterial protease with a solids content of 12.8%

and an activity of 1,330 EE was filtered and washed on a rotary vacuum filter to give 137,000 pounds of filtrate plus wash at 950 EE. Filtrate in the amount of 5,800 pounds (eqivalent to the use of 4,785 pounds of culture and 612 pounds of dry solids) was concentrated at 10° C.±1° C. in an ultrafilter unit to 867 pounds of concentrate containing 11.2% solids and an activity of 6030 EE. The dry solids in the ultrafilter concentrate (867 lbs.) amounted to 97 pounds equivalent to about 16% of the solids present in the starting culture medium.

One part of the concentrate (450 lbs.) was spray dried without any additives while the second part (417 lbs.) was treated with a mixture of 13.9 pounds of cornstarch and 10.2 pounds of calcium phosphate added sequentially. The two parts of concentrate were spray dried separately in the same drier under the same conditions namely, 310° F./170° F. inlet/outlet air temperatures, atomized by centrifugal wheel of 160 mm turning at 18700 RPM. Table V below gives the results obtained from this experiment.

TABLE V

	Concentrate without Supplementation	Concentrate with Supplementation
Dry solids	11.2%	16.0%
Activity	6030 EE	5620 EE
Pounds liquid concentrate dried	450	441
Pounds Spray dry Product	40.0	54
Chamber Sweepings	2.5	10
Activity Product	49600 EE	Product 28700 EE
Sweepings	49600 EE	Sweepings 30100 EE
Recovery solids Product + Sweepings	84%	90.7%
Activity recovery Product + Sweepings	77.7%	74.7%
Physical Appearance of Spray Dried Product	Light Color Good flow Became lumpy on storage in sealed container Moisture = 5.07	Light Color Good flow Retains good flow property on storage = 4.78

As noted this culture concentrate could be spray dried either with or without supplementation but the product caked up under storage unless the supplements were present at the time of spray drying. Addition of supplements after drying can be accomplished but in this case an added step of mixing is required and this must be accomplished before the spray dried material has caked. If caking occurs a grinding step is also necessary.

I claim:

1. A process for the preparation of a spray-dried enzyme composition which comprises: concentrating a liquid enzyme solution by ultrafiltration; adding to said concentrate water-insoluble salts, optionally in presence of water, insoluble suspenders and thickeners, intimately mixing the concentrate composition, and spray-drying the concentrate composition at air inlet temperatures from about 150° C. to about 180° C. and air outlet temperatures from about 65° C. to about 90° C.

2. A process according to claim **1** wherein the water insoluble salts are selected from the group consisting of tribasic calcium phosphate, tribasic magnesium phosphate, calcium carbonate, calcium hypophosphate, calcium magnesium silicate, calcium silicate, calcium sulfite, calcium tartarate, magnesium oxide and magnesium silicate.

3. A process according to claim **2** wherein the water insoluble salt is tribasic calcium phosphate.

4. A process according to claim **3** wherein the water insoluble salt is formed in situ by the reaction of a water soluble phosphate salt and a water soluble or insoluble calcium salt.

5. A process according to claim **4** wherein the liquid enzyme solution is obtained from a submerged culture.

6. A process according to claim **5** wherein the water soluble phosphate salt is selected from the group consisting of sodium potassium and ammonium phosphates or mixtures thereof and the calcium salt is selected from the group consisting of acetate, carbonate, chloride, hydroxide and sulfate or mixtures thereof.

7. A process according to claim **4** wherein suspenders and thickeners are added which are selected from the group consisting of cornstarch, tapioca, potatoe starch, and cellulose.

8. A process according to claim **6** wherein the enzyme is a protease derived from a Bacillus sps.

9. A process according to claim **6** wherein the enzyme is a protease derived from an Aspergillus sps.

10. A process according to claim **6** wherein the enzyme is an amylase derived from a Bacillus sps.

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