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BIONOMER PILOT PLANT

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Abstract
The purpose of this project is to develop a pilot-scale process for the bacterial production of methacrylic acid (MAA) and methyl ethyl ketone (MEK) from biomass feedstocks and the subsequent purification steps. The pilot plant will also be located on site at a sugar cane refinery in Brazil where the feedstock should be inexpensive and readily available. Although these sugar cane refineries only operate for 9 months each year, molasses can be stored so that the pilot plant runs year-round.

To obtain useful information about the feasibility and scalability of the process, 30 M kg/yr of each product will be produced. The products will be tested for purity and samples will be sent out to consumers to demonstrate the quality of the product. The MAA and MEK must be of the same purity generated by current commercial processes. The pilot plant will be designed in three major parts. The first part consists of the bacterial fermentors that are used to produce and scale up MAA and MEK production. Relatively little is currently known about the efficiency of production of MAA and MEK by *E. coli* and this part of the plant will provide critical data about conditions required for the bacteria as well as production rates. The second part of the plant consists of the MAA purification process. Many options will be considered for the purification steps, many of which will have to be modeled in ASPEN because MAA is usually not produced in the aqueous phase. The final section of the plant will be used for MEK purification. To reduce plant costs, the design will try to share equipment between the two purification processes.

The main goal of the plant is to obtain data and demonstrate feasibility, not to demonstrate sustainable profitability. Estimates for total capital investment and show that the plant will not be profitable for the first five years of operation, but the valuable data gained from the operation will be used to design the larger, more efficient, full-scale plant. The total capital investment required for the plant is approximately $ 6.33 million. Returns generated from sales are minimal compared to the capital investment and operating costs. A full scale plant is expected to be profitable over time because of economies of scale and the price of inputs and outputs of the process.

April 13, 2011

Dr. John Vohs
Professor Leonard K. Fabiano
University of Pennsylvania
School of Engineering and Applied Sciences
Department of Chemical Engineering

Dear Professor Fabiano and Dr. Vohs,

For our senior design project, we were tasked with developing a pilot plant capable of producing 30 M kg/year of methyl ethyl ketone and methacrylic acid as was proposed by Mr. Stephen Tieri of DuPont. These products are to be used in the synthesis of polymers and as solvents in many processes, so they must meet the rigorous standard of 99.5 wt% purity. The problem also says that these products will be made using novel microorganisms created Genomatica Inc. which in turn will be fed using cane sugar derivatives from a sugar mill in Brazil.

Based on the patents submitted by Genomatica, E.coli was chosen to produce the two products during two separate batch fermentation processes. We were also asked to examine the various technologies available to separate methyl ethyl ketone and methacrylic acid from the fermentation broth solution. Our report discusses the viability of all of the possibilities we considered and gives a detailed summary on the process we found most promising.

For MEK separation, we chose a pervaportion technique using a newly developed membrane by Pervatech, while MAA could be separated by combining liquid-liquid extraction and distillation.

The pilot plant is not expected to produce a profit, but it will be necessary to accurately estimate the cost, so that the company can know how much to invest in the plant. The total investment required for materials, utilities and personnel was estimated to be $5 million. Further discussion and analyses of these topics are contained in the following report.

Sifat Ahmad

Edward Eckels

Benjamin Galloway

Prosper Ndoro

BIOMONOMER PILOT PLANT

CONVERSION OF MOLASSES TO METHYL ETHYL KETONE AND METHACRYLIC ACID

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Industrial Consultant:
Mr. Steven M. Tieri

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April 5, 2011
Department of Chemical and Biomolecular Engineering
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ABSTRACT

The purpose of this project is to develop a pilot-scale process for the bacterial production of methacrylic acid (MAA) and methyl ethyl ketone (MEK) from biomass feedstocks and the subsequent purification steps. The pilot plant will also be located on site at a sugar cane refinery in Brazil where the feedstock should be inexpensive and readily available. Although these sugar cane refineries only operate for 9 months each year, molasses can be stored so that the pilot plant runs year-round.

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gained from the operation will be used to design the larger, more efficient, full-scale plant. The total capital investment required for the plant is approximately $ 6.33 million. Returns generated from sales are minimal compared to the capital investment and operating costs. A full scale plant is expected to be profitable over time because of economies of scale and the price of inputs and outputs of the process.

PART I: Introduction and Concept Stage for Overall Process

INTRODUCTION

BACKGROUND & MOTIVATION

As petroleum and natural gas prices rise around the world, there is a growing interest in using renewable resources to produce industrial chemicals. Recently, microorganisms have been engineered to produce chemicals like 1,4-butanediol, propanediol, and polyethylene. Two other chemicals that are produced in large quantities and from petroleum feedstocks are methacrylic acid (MAA) and methyl ethyl ketone (MEK).

MAA is currently used in a wide variety of applications and is usually converted to its methyl ester, methyl methacrylate (MMA). Poly(methacrylic acid) is used in some detergents and to produce soft polymers. MMA, on the other hand, can be used to produce hard but flexible polymers that are used in protective coatings and plastics. Because MMA is resistant to ultraviolet light, it can be used in both indoor and outdoor applications such as displays, building panels, and automotive compounds to plumbing fixtures, LCD screen coatings, and lighting fixtures. Demand for MMA has increased because of growing demand for LCD displays which use poly(methyl methacrylate). MMA is also used as an impact modifier used in polyvinyl chloride and pharmaceutical packaging (htt2).

There are a few pathways used today to make MMA. The earliest technologies used acetone, cyanohydrin, and sulphuric acid. The products of the process are MMA and ammonium bisulphate. Ammonium bisulphate has low value as a fertilizer and is very difficult to dispose of otherwise. Acetone is synthesized from petroleum or natural gas feedstocks, so it would be highly advantageous to move to a more price-stable feed. The

Alpha process for MMA production, developed by Lucite International, uses ethylene and methanol, which are also petrochemical derived feedstocks.

MEK is used as a low-boiling solvent for nitrocellulose, acrylic, and vinyl surface coatings. It is a very useful solvent because it can dissolve a large quantity of organic solids while maintaining low viscosity. This is ideal for paints and coatings, many of which are used for electronics, furniture, and automobiles. MEK is a popular solvent for printing inks because it evaporates readily, allowing the ink to dry quickly. It is also used in manufacturing rubber based cements and magnetic tape. MEK is a common solvent for polymerization processes for polystyrene, acrylonitrile-butadiene-styrene, and styrene-butadiene rubber. The current commercial synthesis of MEK uses the dehydration of secondary butanol. This reaction requires zinc or copper oxides if conducted in the gas phase, or the process can also be carried out in the liquid phase using Raney nickel or copper chromate at lower temperatures. Either way, the process employs a petroleum-derived feedstock and requires expensive metal catalysts that must be replaced or regenerated. Moving to a microorganism catalyzed process should greatly reduce the production cost of MEK.

As can be seen, all of the current commercial scale processes that make MAA and MEK come from some non-renewable petrochemical feedstock which introduces several disadvantages. First, petroleum prices have been on the rise over the past ten years for a variety of reasons including increased demand from automobile users and political instability in oil producing countries. Petroleum is the feedstock for many chemical processes or it generates intermediates that go into these processes. Growing demand for these intermediates also drives up the cost of petroleum. Finally, there is growing concern

that petroleum stocks will dry up in the near future. This is leading to growing efforts to find energy sources that sequester carbon from other "clean" sources, like corn or sugar cane.

Of the many pathways being researched, one (specifically the one examined in this paper) uses microorganisms to create MAA and MEK. The advantages of producing MEK and MAA from microorganisms allow manufacturers to side step some to the future problems that the petrochemical-derived products might face. Basic sugar feedstocks will provide the carbon needed to produce the chemicals of interest. Microorganisms are well known as highly efficient catalysts for some chemical production processes and can be adapted to produce a wide variety of chemicals. Yeast has been used to produce ethanol for thousands of years and *E. coli* has been manufactured to produce ethanol from corn and other sugar sources. Two different strains of bacteria have been engineered to produce MAA and MEK by modification of their enzymatic pathway. Patents are available for these organisms, and they are available for purchase from the manufacturer, Genomatica. The patents offer little to no data about the production efficiency of the bacteria, which will be one of the most important pieces of data obtained from the pilot plant designed in this report. The pilot plant will also be able to identify which metabolites and by-products of bacterial growth will interfere with the separation of the products. It is also unknown the exact concentration of MAA or MEK these bacteria can withstand, but the pilot facility would be the place to test this and other microorganisms that may have a higher tolerance for the products.

There are currently a few processes that harness microorganisms to make chemicals other than ethanol and methanol. Genomatica first developed a process for bacterial

production of 1,4-butanediol from sugar and water. Genomatica chose to expand to bacterial production of MEK because it could be made in ethanol manufacturing facilities that have been shut down since the demand for ethanol has dropped. This new technology was announced in 2008, but in mid-2010, Genomatica announced that they were temporarily halting any further development with their MEK production process. They were able to achieve proof of concept for the process, but the market demand was not sufficient to continue with the project (htt3). The design presented in this report has some advantages over the Genomatica approach because it incorporates two plants into one; MAA can be produced when MEK production is already meeting demand. As of January 2011, there exists no MAA or MMA process that uses biomass feedstocks. Many companies, including Evonik Industries and Arkema, are currently researching MMA from biomass processes. The consulting firm Nexant explains that many MMA plants produce 50 to 100 MM kg/yr MMA, which is in the range feasible for biomass-based production. Nexant also concluded that the biomass required per kg of MMA produced is higher than that of ethanol, but still not outrageous (htt4). If non-functioning bio-ethanol plants can be used to make MEK, it is possible that these plants could also be converted into MAA production plants. Incorporation of our design into existing bio-ethanol plants was out of scope for this project, but this report presents an original design for production and purification of MEK and MAA from biomass feedstocks by bioorganisms.

PROJECT CHARTER

Project Name	Renewable Bio-Monomer Pilot Plant
Project Champions	Sifat Ahmad, Edward Eckels, Benjamin Galloway, Prosper Ndoro
Project Leader	Stephen Tieri, Dr. John Vohs, and Professor Leonard Fabiano

Specific Goals

- Develop a functioning pilot-scale plant to produce methacrylic acid (MAA) and methyl ethyl ketone (MEK) from blackstrap molasses.
- Obtain data about the efficiency and kinetics of MAA/MEK production by the bacteria.
- Obtain information about the optimal operating parameters for all separation units included in the design.

Project Scope

<u>In-Scope:</u>

- Process which produces MEK and MAA at a comparable or higher rate and purity to petroleum based processes
  - 30 M kg/yr each of MEK and MAA
  - Identify most commercially viable separation technologies
  - Environmentally friendly, safe design which follows state and federal emissions legislation
  - Recycle materials to maximum extent
- Process which can be translated to a commercial scale

<u>Out-of-Scope:</u>

- Conversion of methacrylic acid to methyl methacrylate for industrial uses such as PVC
- Production of polymers, downstream intermediates, and polymeric derivatives
- "Showplace facility" which is used as a "sales device"
- Making the plant financially profitable
- Estimating Economic Feasibility of a Commercial Plant based on the chosen technology

Deliverables

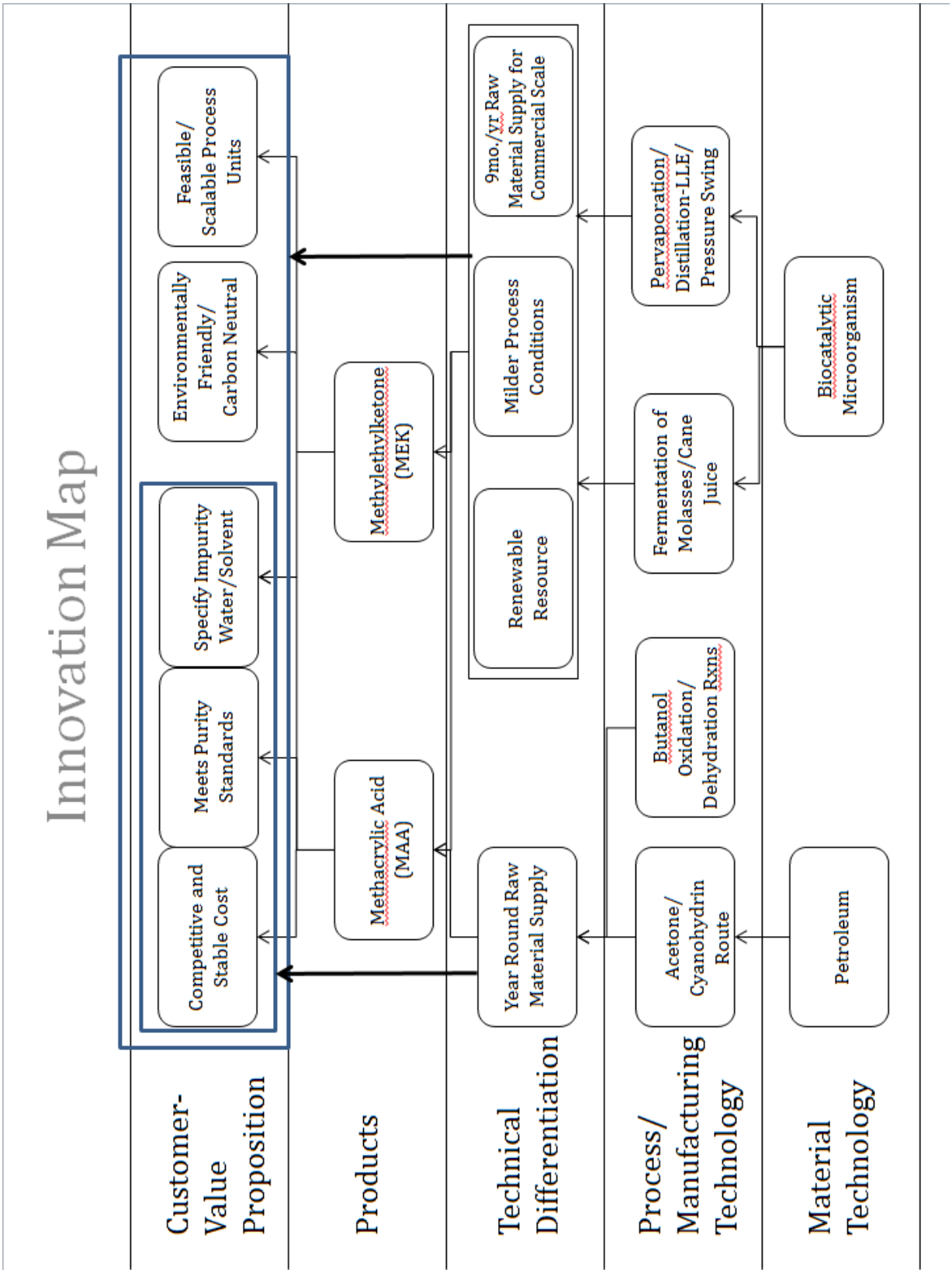
- Estimate of overall pilot program costs
- Manufacturing capability assessment

	• Data necessary to appropriately scale-up process to commercial scale in both the fermentation and separation process
Time Line	• 2 year startup, 5 year operation

INNOVATION MAP/TECHNOLOGY READINESS ASSESSMENT

This process is similar to many bioprocesses such as those used to make butanediol and ethanol. Therefore, many of the technologies required for this process already exist. Our process is simplified over those that use corn because molasses requires no further processing before introduction into the fermenters. The most unique aspect of this design is the microorganisms themselves. Their novelty shows great promise, but it is also one of the greatest challenges in designing a pilot plant. It is difficult to predict how quickly these bacteria will grow, how much product they will produce, and what types of by-products they will generate. All of these factors can greatly impact how the full-scale plant is designed and run. The purpose of the pilot plant will be to determine the conditions that optimize the growth and chemical production of these bacteria. The bacteria will use standard fermentation technology and the fermenters will have built in control apparatus to ensure that the bacteria will grow.

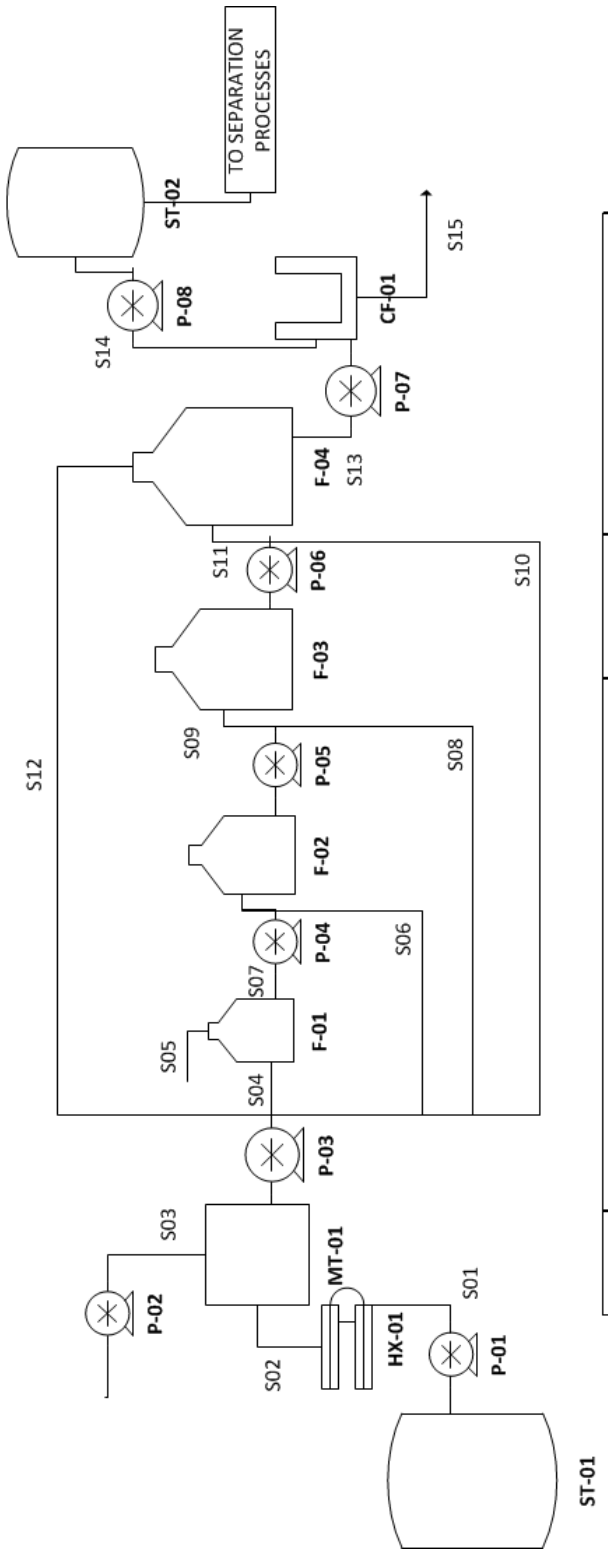
The first separation step is centrifugation. This technology is somewhat non-standard because it uses a continuous centrifuge, but these are readily available from a variety of manufacturers. A continuous centrifuge was chosen because of the large amounts of fluid that must be cleared of cellular debris. The other separation technologies are liquid-liquid extraction, distillation, and pervaporation. Of these technologies, only pervaporation is not readily modeled through correlations or simulations. Pervaporators use ceramic or polymer membranes that selectively allow one chemical to diffuse through while leaving the other chemical behind. There are many things to take into consideration in pervaporators including energy balances, mass fluxes, temperature gradients, and pressure gradients. These factors are all addressed in the individual unit operations sections later in the report. No new technologies had to be developed to achieve our goal. Existing technologies were combined to create a process that separates out mixtures not encountered in typical industrial processes.


Innovation Map
Customer-Value Proposition
Products
Technical Differentiation
Process/ Manufacturing Technology
Material Technology
Competitive and Stable Cost
Meets Purity Standards
Specify Impurity Water/Solvent
Environmentally Friendly/ Carbon Neutral
Feasible/ Scalable Process Units
Methacrylic Acid (MAA)
Methylethylketone (MEK)
Year Round Raw Material Supply
Renewable Resource
Milder Process Conditions
9mo./yr Raw Material Supply for Commercial Scale
Acetone/ Cyanohydrin Route
Butanol Oxidation/ Dehydration Rxns
Fermentation of Molasses/Cane Juice
Pervaporation/ Distillation-LLE/ Pressure Swing
Petroleum
Biocatalytic Microorganism

CONCEPT STAGE FOR OVERALL PROCESS

OVERALL FLOWSHEET

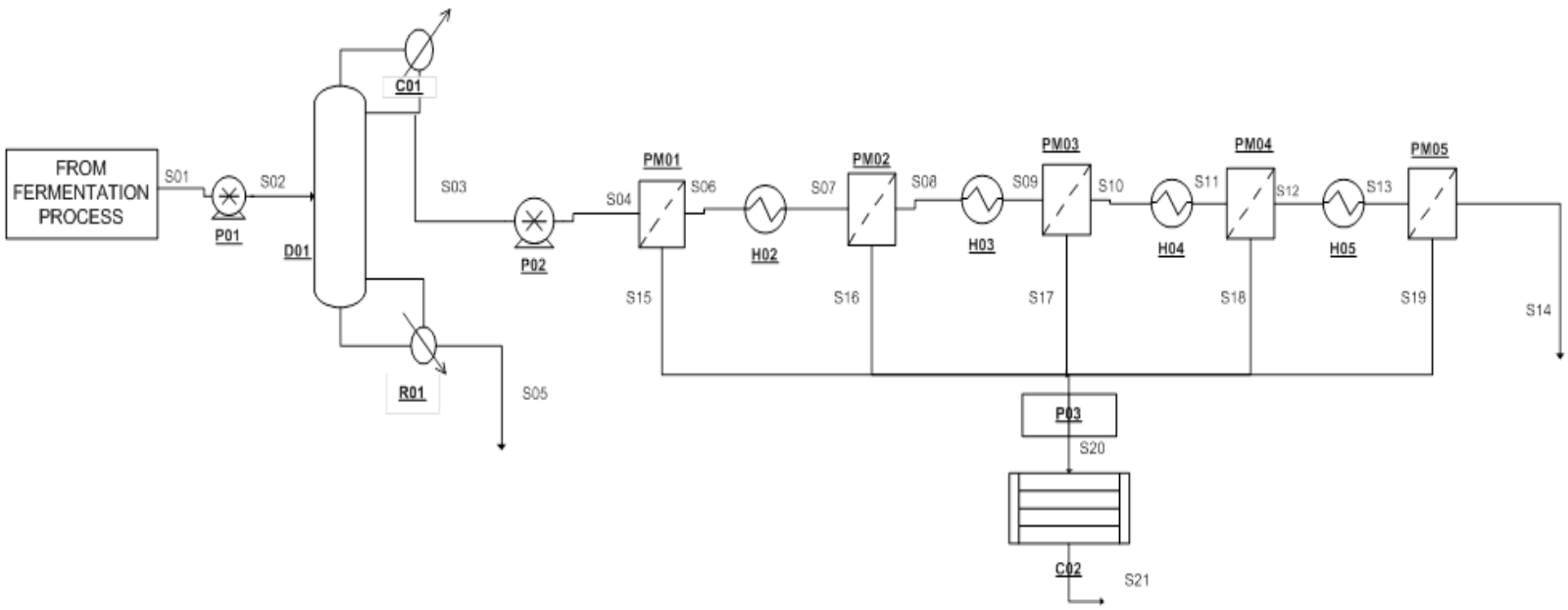
Fermentation Process Flowsheet



Stream No.	Stream Name
S01	Undiluted Molasses From Storage
S02	Undiluted Pasteurized Molasses
S03	Water/Micronutrient Feed
S04	Medium to Seed Fermentor (F-01)
S05	E. Coli Inoculum
S06	Medium to Small Fermentor (F-02)
S07	Fermentation Broth to Small Fermentor (F-02)
S08	Medium to Medium Fermentor (F-03)
S09	Fermentation Broth to Medium Fermentor (F-03)
S10	Medium to Chemical Production Fermentor (F-04)
S11	Fermentation Broth to Chemical Production Fermentor (F-04)
S12	Production Phase Medium Feed Stream
S13	Fermentation Product Stream to Microfilter (MF-01)
S14	Supernatant to Storage Tank (ST-01)
S15	Retentate Waste Stream

Equipment No.	Equipment Name
CF-01	Centrifuge
F-01	Seed Fermentor
F-02	Small Fermentor
F-03	Medium Fermentor
F-04	Chemical Production Fermentor
HX-01	Pasteurizer
MT-01	Storage/Mixing Tank
P-01	Pump 1
P-02	Pump 2
P-03	Pump 3
P-04	Pump 4
P-05	Pump 5
P-06	Pump 6
P-07	Pump 7
P-08	Pump 8
ST-01	Pasteurized Molasses Storage Tank
ST-02	Fermentation Product Storage Tank

MEK Pervaporation Process Flowsheet



Stream Label	Stream Name	Stream Label	Stream Name	Equipmen Label	Equipment Name
S01	Distillation Column feed	S12	Perv. Feed 5	C01	Condenser 1
S02	Distillation Column feed	S13	Perv. Feed 5	C02	Condenser 2
S03	Distillate	S14	Retentate	D01	Distillation Column
S04	Bottoms	S15	Permeate 1	H01	Heater 1
S05	Perv. Feed 1	S16	Permeate 2	H03	Heater 2
S06	Perv Feed 2	S17	Permeate 3	MT01	Mixing Tank
S07	Perv Feed 2	S18	Permeate 4	P01	Pump 1
S08	Perv. Feed 3	S19	Permeate 5	P02	Pump 2
S09	Perv. Feed 3	S20	Vapor Permeate	P03	Vacuum Pump
S10	Perv. Feed 4	S21	Condensed Permeate (Pure MEK)	PM01-05	Pervaporation Membranes
S11	Perv. Feed 4				

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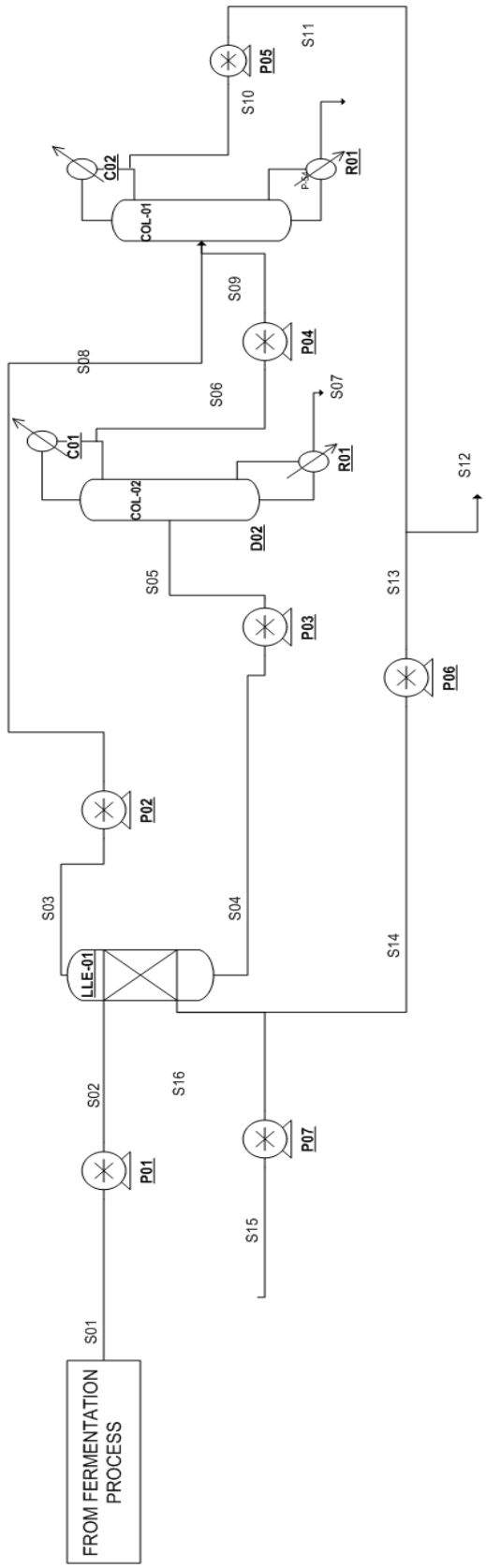
MAA Separation Process



MARKET AND COMPETITIVE ANALYSIS

MAA production estimates can be derived from MMA production because nearly all MAA produced is converted to MMA at the same plant. In 1992, worldwide production of MMA was estimated to be 490 MM kg/yr. MMA demand was expected to increase by 3-5% per year since 1992 and this trend has been confirmed by an increase in the market capacity. Between 2008 and 2011, new plants were slated to increase production of MMA by 605 MM kg/yr, so current production is probably around 1,000 MM kg/yr. The market is expected to continue to grow at 3-5% per year for the long term, which is supported by the fact that demand for MMA has been growing faster than the GDP around the world. This is a good market to break into, especially if you can use cheaper feedstocks, like molasses, to generate the monomer.

The current MEK production in the U.S. is around 180 MM kg/yr with another 45 MM kg imported each year. Worldwide MEK production capacity is approximately 1,360 MM kg/yr and the entire industry is valued at $ 2 billion per year (htt3). While applications of MEK are diverse, there is not much growth in the MEK markets in the United States. Most of the growth is in Asian markets and is fueled by a general increase in demand for consumer products. To break into the market, the new process will need to be able to produce MEK for much lower costs than the current methods. If the market is not ready for more MEK, the plant can be operated to produce only MAA.

A general estimate on the profitability of this process should be considered even before building the pilot plants to assess the long-term profitability of a full scale process. The cost of molasses in Brazil can be estimated by using the 2011 cost in North America, which is approximately $ 0.18 per kg molasses. The cost of molasses in Brazil will be much

cheaper because of the availability of sugar cane. The glucose content of molasses is approximately 60% sugar by mass, which is equal to $ 0.30 per kg of fermentable sugars. Assuming a conservative yield of two units of sugar to one unit of MAA or MEK, the effective cost becomes $ 0.60 per kg of product produced. With the selling price of MAA at $ 2.40 per kg and the price of MEK at $ 1.75 per kilogram, there is a large margin to cover operating costs. From this conclusion, it would seem that building a pilot plant is the next logical step in getting into this market.\

CUSTOMER REQUIREMENTS

Scope

This pilot plant is not intended to be a showplace facility to garner investor interest, nor is it intended to make secondary products like MMA or polymers.

Sterilization

It is important to maintain sterile conditions throughout the process because any contamination can result in fouled batches of bacteria, which can slow down the process, even to the point of stopping the process if adequate sterilization does not occur. There are many measures to consider in sterilization. All water injected into the system will be water for injection. No water is recycled to the process because it would require further storage tanks and sterilization that are more costly than using clean water. The other major source for contamination is the molasses feedstock itself. This feedstock will be autoclaved at 161 F for 15-20 seconds before injection into the system. This will be pumped into a storage unit and allowed to cool slightly before transfer into the fermetnors. The effluent exiting the fermentors will pass through a microfilter that removes all cellular debris. In the off

chance that some biomass passes through the filter, it will be denatured in the subsequent distillation steps because of the high temperatures in the columns. This biomass should contribute minimally to impurities in the final products because they will be flushed out with any water that is removed during the separation steps. The feed to the extractor in the MAA process contacts the product stream with n-butyl acetate, a hydrophobic solvent that disrupts cellular membranes and therefore kills off any remaining biological contaminants.

Molasses vs. Cane Juice

The problem statement for this problem mentioned that both molasses and sugar cane juice would be available as feedstocks for the fermentation process. However, it was determined that molasses would be more economically responsible. From the paper (insert citation here) a complete diagram of typical sugar mill can be seen. From the flowsheet of the sugar mill, the process makes two separate products, molasses and granulated sugar. The granulated sugar is a much more valuable product and is made from the intermediate cane juice. Any cane juice not consumed can be easily recycled. On the other hand molasses is a byproduct, that once formed cannot be recycled back into the system. So do to the value of the cane juice to the sugar mill, it was believed that molasses could be obtained at a lower cost than cane juice.

Being fully refined molasses carries several other advantages. Also cane juice, having not been fully refined, has a lower reducible sugar content than molasses, meaning that more would need to be purchased. From a processing standpoint, depending on the sugar concentration needed in the fermenters, it is easier to dilute than to concentrate. Also, in

order to run the separation process year round some of the sugar feedstock will need to be stored since the sugar mill is only operational 9 months/year. There were concerns about how long the cane juice would last before significant degradation occurred, while molasses will keep for up to 6 months if stored properly.

Environmental Concerns

There are a few sections of the plant that generate wastes that are potentially harmful to the environment. The fermentors generate CO_2 at relatively low rates, but this plant utilizes CO_2 scrubbers. At full scale, scrubbers will be necessary because the CO_2 production rates will be much higher. The CO_2 is not of a purity or quantity high enough to be sold as CO_2 or to soda companies. Other environmental concerns come from our waste streams. The water waste stream coming from the distillation column used to recover n-butyl acetate from the bottoms of the liquid-liquid extractor is 100 PPM in MAA, which meets EPA standards. This project assumes that U.S. regulations on the product are more stringent than those in Brazil and that this waste stream would be sufficiently clean to dump. There is also a purge stream coming off of the MAA purification column that contains n-butyl acetate with small amounts of water. This purge stream is only a small fraction of the n-butyl acetate fed into the process, so it is not cost effective to invest further resources into purification and recovery of this stream. The purge stream will be sent back to the on-site sugar refinery where it will be fed into a furnace to be combusted. In the pilot plant, the purge stream will be stored and shipped off to a waste handling company.

Data Production

Unlike its commercial counterpart which is designed to produce a profit, the pilot plant is meant to be a data collection exercise. The data produced is used to highlight any flaws in the theoretical design, whether they be errors in equipment size, fabrication, or any of the various operating parameters. The idea is that any problem in the process should be addressed and resolved before large amounts of money are invested in expensive separation equipment. Some of the various parameters that were estimated in the design included growth kinetics and viability of the patented cells to be used. ASPEN was also used to estimate activity coefficients for mixtures of MAA or MEK with solvents they might contact during separation; these coefficients were estimated within ASPEN via UNIFAC. After five years of data collection, any problems in the design should be resolved and solutions incorporated into the design of the full-scale plant.

Another important point for data collection is the fermentation process. As discussed earlier, many assumptions had to be made concerning the growth of the cells. Exact growth rates are not known for *E. coli* using molasses as a feedstock. Molasses itself contains many sugars, each of which will be processed at different rates by the bacteria. Each sugar may be processed along a different biosynthetic pathway, some of which may not end with MAA and MEK. It is important to understand that in addition to producing MEK and MAA, these microorganisms must sustain themselves, producing all of the proteins, lipids, and other molecules needed for growth and reproduction. This system is far too complicated to model on paper, so many assumptions were made. Exact parameters will be obtained by the pilot plant.

Scalability

In line with the data production part of the pilot plant, the operations and equipment used must be scalable. Columns must be sized and design appropriately or the produced data will not translate to full size column. An example is a standard distillation tower. Even with moderate insulation, heat loss through the sides of the tower is still a significant concern for most processes. The relative rate of heat loss through the sides becomes more pronounced and more alarming as column diameter decreases and the ratio of column surface area to column cross-sectional area becomes smaller. As this ratio becomes smaller, more and more fluid is contacting the side of the column allowing for more condensation to occur. The excess of condensation and liquid falling down through the column is a standard operating problem called weeping that can halt production, requiring shut down and start-up of the column, and limiting total production. This problem is generally considered minimal for columns with diameters greater than 8 inches, which was a specific design goal for the distillation columns of this process.

Another scalability issue arises with the pervaporation equipment used in the MEK separation process. Membrane separation is generally only used as last resort for most separation processes. Membranes are more costly and have greater maintenance concerns than normal distillation columns. Also most membranes that can be applied to the compounds of interest have only been recently developed making their reliability and performance to this application an unknown that should only be adapted if other technologies prove unsuccessful in achieving the desired product. The pilot plant will be essential in assessing the performance, maintenance costs, and lifetimes of the pervaporation membranes.

The final scalability concern facing this pilot plant was the choice of packing versus trays for distillation and LL extraction towers. Standard commercial towers use trays for mixing rising vapor and falling vapor. However, the distance between stages (HETP) is larger than is generally desired for pilot scale towers. To rectify this, random or structured packing is used in pilot scale separation columns. Packing allows for a lower HETP and a smaller column which will be easier to maintain and construct. This advantage is generally greater than the added cost of the packing which will cost more than traditional trays. These design concerns are discussed in depth in the subsequent sections, but deserved mention here because they are very important for someone unfamiliar with designing pilot plants.

Return/Economic Viability

A commercial plant is design to be able to handle quantities that offset the cost of equipment and operations and eventually turn a profit. A pilot plant operates under very different rules. Any product produced is not likely to be sold to defray the cost of construction and operation. The limited amount produced is not enough to warrant any sales contract that a full scale plant might have. The basic costs associated with sending of funding a sales agent to sell the insignificant amount of product and the transportations cost from sugar refineries in Brazil, more than negate any income the product might yield. Instead, any product made will be given away as free sample to companies that further refine the bio-monomers to their desired polymer-consumer form or for purity testing. The idea is that if the product is adequate for a company's specific refinement process, the company will place a large order and purchase the product from the commercial plant. Since the product is not being sold, there is no real return on the pilot plant that produces

on this scale. Instead all economic analysis will be done to simply examine the overall cost of operating the plant for the expected lifetime of 5-years.

Discrete vs. Continuous Operation

Another consideration that must be examined for a pilot plant is whether to run the separation process continually or in batches. The fermentation process will be capable of running continuously as it will be completely automated via various control mechanisms. The separation, however, will need workers present to monitor the process and collect the necessary data. However, due to the size of the process and the desired production goal that is expected of this plant, it will not be necessary to run the plant 24 hours a day. Instead, it is likely that the plant will only be operational for the first shift of the day (from 9 am to 5 pm) or, as recommended to by Mr. Tieri, another common shift structure is 12 hour shifts 5 days a week. This discrete operation will significantly reduce labor cost, but would require an apparent increase in storage capacity because greater volumes are produced per hour. Fortunately, flow rate in each process are small enough that a day's worth of product can be stored in one or two 55-gallon storage drums.

Running for only a few hours each day does have its disadvantages. The process will now have significant startup and shut-down times each day. This start-up is the time it takes the process to reach steady state output. Since there is no way of knowing or predicting this time, it will be assumed to be one hour for both the MEK and MAA separations, which will further shorten the production time from 12 to 11 hours a day. During the start-up, period the normal outlet streams will be recycled back to the beginning of the process, essentially making the process a closed loop system until steady-state is reached. With the discrete flow, the system will likely need to be flushed and sent

back to the storage tanks when the process is shut down. This is done because the high purity MAA remaining in the second distillation column might react overnight, damaging equipment and leading to costly repairs. After the MAA is stored, the entire system will be flushed with a cleaning solvent to remove any trace amounts of product. This entire shut-down time is assumed to take one hour, making the total operation time 50 hours per week.

On-Site of Sugar Refinery

Most pilot plants are built on-site, or as a sister process near a larger pre-existing plant. The proximity carries with it several advantages. First, it allows for easy access to the pilot plant's feedstock, which in this case will be the blackstrap molasses used to feed the fermentation process. Blackstrap molasses is a low value by-product of the sugar refinery, which can be easily bought from the refinery as feed for the bacteria.

Secondly, the sugar refinery has a built in furnace to incinerate bagasse, a solid organic by-product of the sugar refinery. Since the sugar mill is nearby, it is probable that the pilot plant and sugar mill will enter into a contract whereby the sugar mill will handle certain waste products of the pilot plant. The process will produce on average ~70kg/day of 90% n-butyl acetate in water as a waste by-product. N-butyl acetate, being highly combustible, can be transported to the sugar mill incinerators. This arrangement will likely be cost neutral. It is advisable for a commercial plant to contain its own furnace to deal with waste and help defray heating cost or have an additional column to re-purify the n-butyl acetate and recycle it. However, for the pilot plant scale the cost of adding and running another column would outweigh the decrease in fresh n-butyl acetate that would be required.

PART II: Batch Processes

Cultivation, Cell Separations, and Storage

CONCEPT STAGE

The design of a fermentation process must be considered at great length, as growth and product formation are sensitive to numerous parameters, including reactor design, composition of growth medium, cell density, pH, and temperature. Selection of these parameters is specific to a particular strain; thus conceptual development for the fermentation process begins with selection of a host microorganism. The following sections describe subsequent preliminary design decisions, including selection of reactor design and separation method, and end with the synthesis of a preliminary process design.

SELECTION OF HOST ORGANISM

As MEK and MAA are simple industrial chemicals whose production does not require complex host machinery, it is economically inefficient to consider a host more complex than a microorganism. In selecting a microorganism for an industrial process, it is important to consider the organism's growth kinetics, minimal growth requirements, and ease of transforming the host cells. These considerations are pertinent to optimizing product yield while minimizing costs.

Growth Kinetics

The host microorganism should grow rapidly and grow to high cell densities. Rapid microorganism growth reduces the process time that must be developed to cell growth rather than product formation. In batch processes, longer growth times also increase the turnover time between batches, a particularly undesirable quality in a pilot facility which should be able to rapidly test multiple operating conditions. Slow growth time also diverts feedstock to cell formation rather than product formation. High cell density is also

desirable because it increases volumetric productivity and thus reduces the amount of space the bioreactor must occupy for a particular production rate.

Minimal Growth Requirements

The proposed facility's primary feedstock is to be molasses, so it is important to identify a host organism that requires few additional nutrients to the sugars and ions present in molasses. Supplementation of the medium can become costly and complicates the separations process by introducing additional components. The most optimal microorganism has minimal nutrient requirements aside from glucose and

Ease of Transformation

In conventional processes to produce chemicals from an organism, such as the production of ethanol from yeast, the product naturally occurs in the organism. The production of MEK and MAA differs in that though both products can be derived from glucose, a source of energy for all organisms, this conversion requires a complement of enzymes which does not naturally occur in full in microorganisms. In order to be capable of converting glucose to MEK or MAA, the selected host microorganism must be provided with DNA sequences for each missing enzyme in a process known as transformation.[1] These sequences are described in full by the Genomatica patents which are the basis for this project.[2]

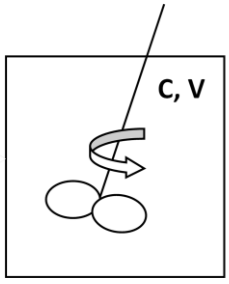
The nature of MEK and MAA production thus requires a microorganism that is amenable to transformation. *Saccharomyces cerevisiae* (Baker's yeast) and *E. coli* are examples of host cells which are easy to transform, due the large body of literature on transformation protocols involving each species and the wide availability of strains optimized for transformation and plasmid stability.[3]

SELECTION OF BIOREACTOR DESIGN

The design of the bioreactor or fermentor is a critical factor to consider in developing a fermentation process, as it affects, among other factors, the flexibility of the process, product yield, and ease of modeling and operating the fermentation. The principle bioreactor designs utilized in fermentation processes are batch, continuous, and fed-batch. Each design comes with a unique set of advantages and disadvantages.

Batch

In batch fermentation, there is no mass transfer in or out of the fermentor once the process has begun other than gas exchange and addition of pH control solutions.[4] As a result, it is not possible for the system to reach a steady state. However, in a well-mixed batch fermentor, conditions are uniform throughout the fermentor at a particular time. Aside from the inability to reach steady-state, batch fermentors are also disadvantageous for the inevitable down time between batches in order to charge, discharge, sterilize, or start the process. [5] In fermentation processes where there is substrate inhibition, growth and production will also be lower than that for other reactor designs. Despite these drawbacks, most fermentation processes are initially developed in batch reactors because they are "versatile, well characterized, and easy to operate."[6]

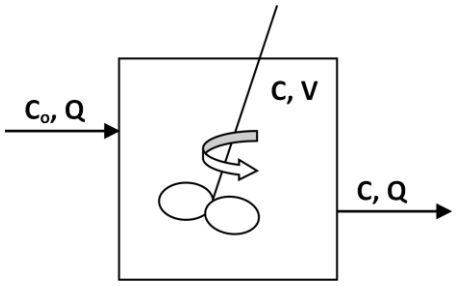


Pros: Versatile, well characterized, easy to operate
Cons: Long down times, lower yield of cells and product

Figure 1. Features of Batch Bioreactors.

Continuous

Unlike batch fermentation, continuous bioreactors, also known as chemostats, are designed for long-term operation. Long-term operation is possible in continuous cultures because there is a constant influx of fresh nutrients and cells. There is also a continuous effluent stream that removes cells, unused nutrients, and waste products which may otherwise inhibit cell growth and product formation. Due to the constant inlet and outlet streams, chemostats can reach steady state. Chemostats can maintain a culture in the exponential phase of growth because of constant nutrient replenishment and waste removal, unlike a batch culture wherein nutrients are limited. Chemostats are able to produce microbial products more efficiently because of this feature. Operating conditions of chemostats, such as the growth rate, can be more finely controlled. However, chemostats are rare in commercial processes because maintaining sterility throughout the process is difficult and foaming can cause overflow in full-scale chemostats.[7] [8] In chemostats, the feed rate can also be controlled to promote production of growth-associated products over secondary metabolites or non-growth associated products, and vice versa.



Pros: Long-term operation possible, avoids substrate and product inhibition, reaches steady state, high productivity, fine control possible
Cons: Difficult to maintain sterility over long periods of time, foaming

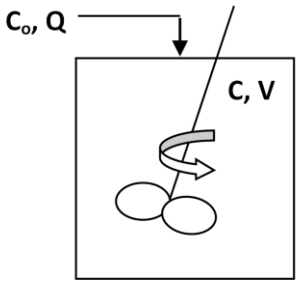
Figure 2. Features of Continuous Bioreactors.

Fed-Batch

Fed-batch fermentation offers a middle ground between continuous and batch operation. In a fed-batch reactor, a batch phase is followed by a feed phase wherein mass transfer is allowed into the system but not out of the system. This type of fermentation is

advantageous because the possibility of mass transfer into the system allows for higher productivity than is possible in a purely batch fermentor, particularly in systems where there is substrate inhibition. Substrate inhibition occurs when a feed, such as glucose, inhibits growth of the cells once it is present above a certain concentration. In a batch reactor, substrate inhibition can dramatically lower productivity because it imposes a maximum limit on the amount of substrate that can be introduced to the fermentor throughout the entire process.

Fed-batch reactors avoid this problem by allowing a feed stream to replenish the consumed substrate, which extends the maximum growth or production time. The feed stream also allows for replenishment of antibiotics, which are commonly used in fermentation medium but degrade over time. Typically, the flow rate of the feed stream is controlled to supply nutrients at a rate equal to their consumption so that a constant substrate concentration can be maintained in the reactor without exceeding the concentration at which inhibition occurs. Production of byproducts associated with high substrate concentration is also avoided by maintaining a constant concentration at a sub-inhibitory level. Continuous fermentations can also avoid problems of substrate inhibition, but fed-batch fermentors are not as susceptible to contamination.



Pros: Versatile, higher productivity than batch, avoids substrate and product inhibition
Cons: Down times, lower yields than CSTRs

Figure 3. Features of Fed-Batch Bioreactors.

There are two approaches to fed-batch fermentation: constant volume or variable volume. The distinction between the two approaches lies in the dilution of the inlet stream during the feed phase. In constant volume fed-batch fermentation, the limiting substrate is

introduced at a high concentration so as to maintain a relatively constant volume within the fermentor. In variable volume fed-batch fermentation, the limiting substrate is introduced at a concentration that results in non-negligible volume changes with fermentation time.[9]

CELL SEPARATION METHODS

The primary methods employed on a large scale for separation of cells from fermentation broths are microfiltration and centrifugation. In some processes, cells are also lysed or homogenized prior to separations of cellular material.

Homogenization

Homogenization is generally unnecessary in cases where the product is extruded by the cell. In these cases, it is generally only employed when the additional yield and selling cost of the product (such as expensive recombinant protein drugs) is high enough to justify the additional separations processes for separating cellular components from the product. As MEK and MAA are small organic and soluble molecules, it can be assumed that the majority will pass through cell membranes into the fermentation broth. Homogenization would thus only complicate separations without contributing significantly to product yield.

Centrifugation

Centrifugation, is the use of centrifugal force to separate materials of different densities. It is the simpler of the cell separations methods discussed herein, as it only provides one level of separation.[10] Aside from its simplicity, centrifugation is advantageous because centrifuges can easily be cleaned with steam and do not incur consumable costs for membranes or filter aids. Centrifuges also require little floor space and accomplish

separations faster and with greater consistency than filters. However, centrifugation is expensive, both in terms of initial capital costs, operation costs due to high electricity consumption, and maintenance costs.[11] Centrifuges also produce bioaerosols, or airborne particles containing living organisms or materials released from organisms, which puts plant workers at risk of exposure.[12]

Microfiltration

Filtration removes solids from fluid by passing the fluid through a porous membrane. In microfiltration, pore size ranges from 0.1 to 10μm. Finer filtration methods than this are unnecessary for removal of microorganisms. Filtration is favored for some processes because of the high level of purity it can offer, the lower energy requirements, and quiet operation. The finer separation provided by microfiltration also simplifies downstream processing. Filtration is safer for handling microorganisms as it is a contained system which does not produce bioaerosols.[13] Disadvantages of filtration include membrane resistance which increases as solids accumulate on the filter. Increasing membrane resistance decreases flow through the filter which has the undesirable effect of increasing process time. [14] Filtration is also expensive because the membranes must be regularly replaced as they develop leaks and leach chemicals.

PROPOSED ORGANISM, REACTOR TYPE AND SEPARATION METHOD

Proposed Organism: Escherichia Coli (E. coli)

Though a number of microorganism species fit our criteria to some extent, our investigations were limited primarily to yeast (specifically *Saccharomyces cerivisiae* and *Pichia pastoris*) and *E. coli,* the two most commonly used microorganisms for industrial

processes. Our inability to collect experimental data imposed this limitation. Without the ability to collect experimental data on important characteristics of potential microorganisms, such as growth kinetics, it was necessary to select a microorganism about which a sufficient amount of information was readily available.

For the purposes of this process design, *E. coli* and yeast were competitive choices. Both have minimal growth requirements and favorable growth kinetics, particularly the ability to rapidly grow to high cell densities. The genetics of both are well characterized and both species are easily transformable. However, *E. coli* is cheaper and faster growing than yeast; the doubling time for *E. coli* is as fast as 20 minutes, whereas it is 90 minutes for yeast. *E. coli* can also grow to a higher cell density than yeast yet has a slightly lower maximum glucose uptake rate, meaning that it requires less glucose to grow and thus to make product.[15] [16]

Additionally, while yeast medium is relatively inexpensive, wild type *E. coli* can subsist exclusively on molasses supplemented with micronutrients.[17] Yeast, on the other hand, requires the presence of various growth factors in addition to micronutrients. *S. cerivisiae* requires biotin, pantothenic acid, inositol, and thiamine, while *P.pastoris* has variable growth factor requirements.[18] The presence of additional components in the fermentation process, as required by yeast, would increase costs and could complicate downstream separations.

The increased metabolic requirements for yeast are generally justified in the use of recombinant technology only when a eukaryotic protein product is being produced and intron splicing is necessary. *E. coli,* as a prokaryote, is incapable of intron splicing and is unable to make these products. In this case, the necessity of the yeast's transcription

machinery justifies the additional costs of using yeast. As the production of MEK and MAA which does not require a eukaryotic host, *E. coli*'s low cost, fast growth, tolerance for high cell density, and minimal growth requirements make it a superior microorganism to yeast for the production of MAA and MEK. This process specifically makes use of the BL21 strain of *E. coli* which was selected for its minimal acetate production relative to the commonly used K-12 strain. The BL21 strain has also been optimized for transformation and can grow at a satisfactory growth rate on minimal medium supplemented with ferrous sulfate.[19]

Proposed Bioreactor Design: Fed-Batch

One of the major concerns that informed selection of a bioreactor design was product and substrate inhibition of *E. coli*. Though glucose is the primary carbon source for *E. coli,* high glucose concentrations will inhibit rather than promote growth and product formation. This inhibition results from acetate excretion by certain strains of *E. coli*. *E. coli* growth is also inhibited by the formation of the product MEK, as shown in Figure 4. Given other research which indicates that high concentrations of chemical in the culture medium will inhibit microorganism growth, such as is the case with *E. coli* and 2-butanol or yeast and ethanol, it was also assumed that MAA would inhibit growth at similar concentrations.

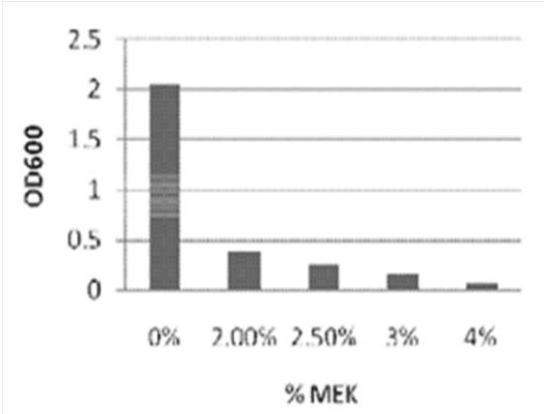


Figure 4. Product inhibition of *E. coli* growth in LB medium containing various concentrations of MEK. Obtained from Figure 6 of Genomatica patent "Microorganisms for Production of Methylethylketone."

Additionally, as the project's purpose is to design a pilot scale facility which must study and optimize the process technologies, it was important to identify a bioreactor design that

allows for testing multiple operating conditions within a short span of time while still meeting the production quotas for market demonstration and customer qualification testing.

Given these objectives, fed-batch bioreactors were identified as the reactor design for the fermentation process. Fed-batch reactors combine many of the best features of continuous and batch bioreactors: they are versatile like a batch bioreactor while allowing for a higher level of production like a continuous bioreactor. However, unlike a batch bioreactor, substrate and product inhibition can be avoided. Fed-batch reactors are also much less susceptible to contamination than a continuous bioreactor. A variable volume fed-batch reactor design was selected, as the ability to dilute the fermentation broth once the production phase begins will reduce product concentration within the culture and thus toxicity to the cells.

Proposed Separation Method: Centrifugation

A centrifuge was chosen to separate out the cells from the aqueous solution of MAA or MEK. Vendors were not forthcoming with quotations for microfilters, which would have been ideal for the pilot plant, so other options were explored. The final fermentor contains up to 7600 L of solution. It would be impossible to centrifuge all of this liquid in one batch, especially on the pilot plant scale. To maintain scalability, a continuous centrifuge was chosen. This centrifuge is capable of centrifuging 14.26 gallons per hour, which is sufficient for purifying the supernatant from one batch of the fermentation process for both MAA and MEK production. The centrifuge is expensive but it provides a convenient solution because it requires no loading or unloading like a conventional “batch” centrifuge. The fluid fed into

the centrifuge will experience 17,000 rpm of acceleration which is sufficient for removing cellular debris and other particles on the micron size or larger.

Storage Considerations

While the molasses feedstock will be supplied by the partner sugar and ethanol facility for only 9 months a year, the fermentation and distillation processes will operate year round in order to reduce vessel sizes and initial capital investment. Year round operation requires two storage tanks: one to provide a continuous molasses feed to the fermentation section and another to continuously supply clarified fermentation broth to the distillation section. Both storage tanks must be designed so that an excess supply is always available in case of contaminated batches or other issues requiring disposal of a batch.

Bioreactor Operating Conditions

As previously mentioned, optimal cultivation conditions are specific to particular strains of organisms. For most strains of *E. coli,* including the selected BL21 strain, the optimal culture temperature is 37°C and the optimal pH is a neutral 7.0. The cultures will be grown anaerobically. Typical cell densities for *E. coli* fermentation range from 10 to 100 $\frac{g}{L}$ of cell dry weight, which is roughly equivalent to $3x10^{13}$ to $3x10^{14}$ cells/L.[20] In the interest of exercising caution, a target

Component	Concentration (g/L H_2O)
Glucose	44.92
Fructose	51.33
Sucrose	114.08
SiO_2	2.50
K_2O	12.45
CaO	6.77
MgO	0.36
P_2O_5	0.71
Fe_2O_3	1.43
Sulfates as SO_3	6.42
Cl	1.43
Organic Non-Sugars	49.91
Na_2HPO_4	33.90
KH_2PO_4	15.00
NaCl	2.50
NH_4Cl	5.00
$MgSO_4$	5.00
$FeSO_4*7H_2O$	$3.0x10^{-5}$
Kanamycin	$2.5\ x10^{-5}$

Table 1. Composition of Growth Medium.

cell density of $5x10^{13}$ cells/L was selected from the lower end of this range. Finally, the growth medium will consist of molasses diluted 1:4 in water and supplemented with ferrous sulfate and minimal salts according to the BD Science's M9 formulation.[21] Ferrous sulfate is added because researchers have shown that BL21 *E. coli* grows best on minimal medium when ferrous sulfate is present. [22] Finally, kanamycin is added to the medium in order to kill undesirable microbes which may enter the fermentors or *E. coli* which have lost the transformations which enable MAA or MEK production. Table 1 shows the precise composition of the growth medium, including the various components present in molasses.

MODELING THE GROWTH PHASE

The fermentation process was modeled as two distinct phases: a growth phase and a subsequent production phase. Design of the fermentation process thus began with modeling the growth of *E. coli*. As shown in **Figure 5,**[23] bacterial growth in culture occurs as a series of four phases: lag, log, stationary, and death. In the first growth phase, the lag phase, bacteria adapt to the new culture conditions and are unable to divide. In the log phase, the cells begin growing exponentially and there is a linear relationship between the log of cell concentration and time. The slope of this line is known as μ, or the growth rate. During the stationary phase, growth rate slows as nutrients are exhausted and toxic products accumulate. In the death phase, cells die due to a lack of nutrients.

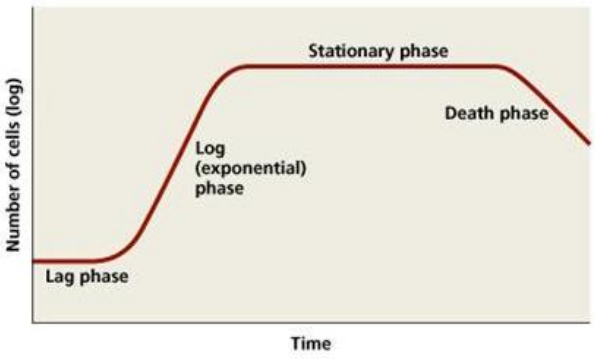


FIGURE 5. Growth phases of bacteria in culture.

Within each bioreactor, a lag phase of 1 hour was assumed. This assumption is consistent with research on the kinetics of BL21 *E. coli* on minimal media.[24] Growth of the bacteria during the log phase was then modeled as:

$$X = X_0 e^{-\mu(t-t_L)} \quad \text{Eq. 1}$$

where X is the cell concentration, X_0 is the initial cell concentration, μ is the growth rate (hr^{-1}), t is the time since inoculation of the culture, and t_L is the duration of the lag phase (hr). The growth rate was equal to $0.7 hr^{-1}$.[25] As estimated by the patent, the glucose uptake rate of *E. coli* was 10mmol/g cell dry weight-hr, or $3x10^{-15}$ mol/cell-hr given a dry cell weight of $3x10^{-13}$ g/cell.[26] The amount of glucose consumed in a particular time interval can thus be calculated as follows:

$$\Delta S = X * v * V * \Delta t \quad \text{Eq. 2}$$

where ΔS is the change in glucose substrate (mol), X is the cell concentration (cells/L), v is the glucose uptake rate (mol/cell-hr), V is the volume of the bioreactor (L), and Δt is the length of the time interval. As the cell concentration within the bioreactor is constantly changing, the amount of glucose consumed during each fermentation was calculated by summing the amount of glucose consumed in 0.1hr increments.

A number of simplifying assumptions were made in applying these equations. Growth inhibition by acetate formation was neglected due to BL21's limited acetate formation, as discussed in the sections on selection of reactor design and microorganism. Fructose and sucrose were also assumed to have no affect on acetate formation, as research has shown that acetate formation can be reduced by replacing glucose with fructose, indicating that glucose triggers acetate formation more so than other sugars.[27]

In order to account for plasmid instability, i.e. the loss of a transformation by a recombinant cell, it was also assumed that a 90% yield of cells would be obtained from each bioreactor. This yield is may be optimistic, however; engineering a cell to express a foreign product places a significant metabolic burden upon the host by increasing demand for resources, and resulting plasmid instability can cause a great proportion of cells to be lost. A yield of 90% generally occurs only when a transformation is relatively stable. [28]

Other assumptions were that the growth rate would be unaffected up to a glucose concentration of 45g/L and that fructose and sucrose concentrations would have no effect on growth. Finally, though *E.coli* are known to also metabolize fructose and sucrose, limited information on the uptake and metabolism of sucrose and fructose by BL21 *E.coli* is available. Consequently, it was assumed that all of the sucrose and fructose in the molasses is taken up at the same rate as glucose and converts completely to glucose. Calculations of sugar consumption were thus in terms of "glucose equivalents", wherein 1 mole of sucrose was equal to 2 moles of glucose equivalent, and 1 mole of fructose was equal to 1 mole of glucose equivalent. A lower bound of 5g/L glucose equivalent was also assumed; glucose equivalent within the fermentors was not allowed to drop below this level so that cell death due to nutrient depletion could be avoided. A summary of these assumptions is presented in Figure 6**.**

FIGURE 6. Assumptions for Modeling Fermentation Growth Phase

- Lag phase = 1hr
- Growth rate is unaffected by glucose concentrations < 45g/L
- Acetate formation is negligible and does not impact growth/production
- Sucrose and fructose are taken up by *E.coli* at an equal rate to glucose
- Sucrose and fructose convert completely to glucose
- 1 mol sucrose = 2 mol glucose equivalent, 1 mol fructose = 1 mol glucose equivalent
- Plasmid instability will result in a 90% yield of cells from each bioreactor

Sample calculations for cell concentration and glucose consumption in the fermentors

designed for the process are presented in Appendix B.

MODELING THE PRODUCTION PHASE

MEK and MAA were treated as secondary metabolites, compounds produced by an organism that are not required for primary metabolic processes, in the fermentation model. The foremost reason for this assumption was that MEK and MAA are foreign products in *E.coli,* and thus are unlikely to be a component of its primary metabolic pathways. Treating MEK and MAA as secondary metabolites also simplified calculations. MEK and MAA producing microorganisms are novel, and thus there is no information available on how much of the glucose taken up by the cells goes to MEK or MAA production versus growth. Without this information, it was impossible to determine how much MEK and MAA are produced while the cells are still growing. As a result, it was assumed that production of MEK and MAA would begin only once cells reached an optimal cell density of $5x10^{13}$ cells/L. As the fermentation broth is to be immediately transferred out of a fermentor once reaching this density until it reaches the final fermentor in the series, there will be no MEK and MAA production until the fermentation broth reaches the final fermentor.

Calculations of MEK and MAA production were based on the maximum theoretical yields supplied in the Genomatica patents: 1 mol of MEK per mol of glucose and 1.33 mol of MAA per mol of glucose. However, due to the improbability of obtaining the theoretical yields, it was assumed that only 50% of glucose equivalent would actually be converted to MEK or MAA. The other 50% of glucose equivalent was assumed to be lost to thermodynamic inefficiencies, metabolic demands of the cells, metabolic byproduct

formation, and potential growth of the cells. This correction also allowed for the possibility that sucrose and fructose give lower yields of MEK and MAA. While this assumption may be an overly cautious estimate of MEK and MAA production, it is better to underestimate rather than overestimate given that certain production quotas for product quality testing must be met.

It was also necessary to make assumptions to account for product inhibition, or inhibition of cell productivity and survival by MEK and MAA, during the production phase. The Genomatica patent on the MEK producing microorganisms included preliminary data demonstrating that *E.coli* growth is significantly reduced by 48 hours at high MEK concentrations. The patent for MAA producing microorganisms did not show similar data on toxicity of MAA to cells, but it was also assumed to be potentially toxic to *E.coli* at similar concentrations. The charts provided in the patent did not specify whether the percentage of MEK that resulted in a decrease in cell concentration was by volume or by mass, but so as to account for the likelihood of product inhibition, the production phase was designed such that an MEK or MAA mass percent greater than 5% is never reached in the largest and final fermentor. Without dynamic data on how the time varying MEK and MAA concentrations affect growth and product formation throughout the culture time, an accurate account of the effects of product inhibition was impossible.

In order to obtain a high yield of MEK and MAA without increasing the MEK and MAA mass percents to a toxic level (>5%), it was necessary to use a dilute rather than a concentrated feed stream during the fed-batch production phase. If a concentrated feed stream was provided, the high amount of glucose would allow for a large amount of MEK and MAA to be produced in a limited volume. As a result, MEK and MAA concentrations

would skyrocket past the toxic limit after production of only a small amount of MEK and MAA. A dilute feed stream also allows for more flexibility in the process, a quality which is desirable for a pilot facility. For instance, if the assumption that only 50% of glucose equivalent converts to MEK and MAA is an underestimate and MEK and MAA yields end up being significantly higher, the dilution of the feed streams can be adjusted by a controller or operator in order to maintain subtoxic concentrations of MEK and MAA. A summary of these assumptions is presented in Figure 7.

FIGURE 7. Assumptions for Modeling Fermentation Production Phase

- MEK and MAA are secondary metabolites which are produced only after the *E. coli* cells reach an optimal density of $5x10^{13}$
- Only 50% of the theoretical maximum MAA and MEK yields will be obtained
- Product inhibition will be negligible when MEK and MAA are present at 5% mass or less within the system

Sample calculations for MEK and MAA production, flow rates, and batch times for the final process design are presented in Appendix B.

CLEANING: CLEAN-IN-PLACE (CIP) AND STERILIZATION-IN-PLACE (SIP) PROCESSES

In high-quality fermentation processes, a cleaning system is essential for preventing microbial contamination and eliminating residues. If fermentors are left unclean, organic residues from the fermentation broth will supply nutrients for growth of unwanted organisms which can then contaminate the product. In large-scale industrial processes, it is also essential for cleaning systems to be automated. An automated CIP/SIP process requires minimal operator involvement, which reduces operator exposure to potentially harmful cleaning materials and operating costs.

The most commonly used cleaning systems are clean-in-place (CIP) and sterilization-in-

place (SIP), both of which allow for cleaning without dismantling the fermentor or ceasing plant operations. CIP involves introduction of caustic solutions like sodium hypochlorite, and SIP involves introduction of steam into the fermentors. A typical SIP/CIP system includes a solution reservoir, supply pump, a spray device, and a return circuit. CIP liquids can be recycled if microfiltered. The amount of time required for CIP is generally longer than that for SIP; this general rule was used to estimate CIP/SIP times based on fermentor size. CIP/SIP times were kept to the minimum; unlike a process for production of pharmaceuticals, cleaning standards are not high for an industrial process for production of chemicals. CIP and SIP times are not considered for the seed fermentor and the small fermentor, as they are too small to justify the expense of these systems.

PRELIMINARY PROCESS SYNTHESIS

Once models for the growth and production phases were established, the fermentors were sized in accordance with those models to meet the production quota of 30,000kg/yr each of MAA and MEK. Simulations of the process were conducted in SuperPro. A number of reactor configurations were possible, but in the interests of reducing costs, the first design aimed to meet the production quota with a series of three bioreactors with the following culture volumes: 5L, 50L, and 500L. A scale up factor of ten was used between fermentors in accordance with convention. This initial design required a feed lasting at least 80 hours in the largest fermentor, however; which would result in a final volume of 8090L of fermentation broth in the largest reactor. One of the assumptions of the production phase model assumed that there would be no growth of cells during the production phase, but diluting a 500L culture to a final volume of 8090L would almost

certainly spur cell growth as the *E. coli cells* attempt to regain optimal cell density. Additionally, the exorbitantly long batch time would require the purchase of multiple fermentors given that there are only 330 operating days in a year. In the interest of minimizing costs, this design was thus rejected. Given that the final volume of the third fermentor in the initial design was calculated to be 8090L, the need for an additional scale up fermentor in the process became obvious. The next design attempted to meet the production quota with a series of four bioreactors with the following initial culture volumes: 5L, 50L, 500L, and 2500L. In order to minimize the size of the largest fermentor and decrease turnover time, a small fed-batch time of 3 hours was selected. As expected, this reduced the final culture volume to 3905L, a big improvement over the largest fermentor size in the prior design. Assuming the fed-batch phase begins one hour before remaining glucose from culture is consumed, scheduling considerations only allow for 364 batches during an operating year of 330 days. However, a total of at least 386 batches is

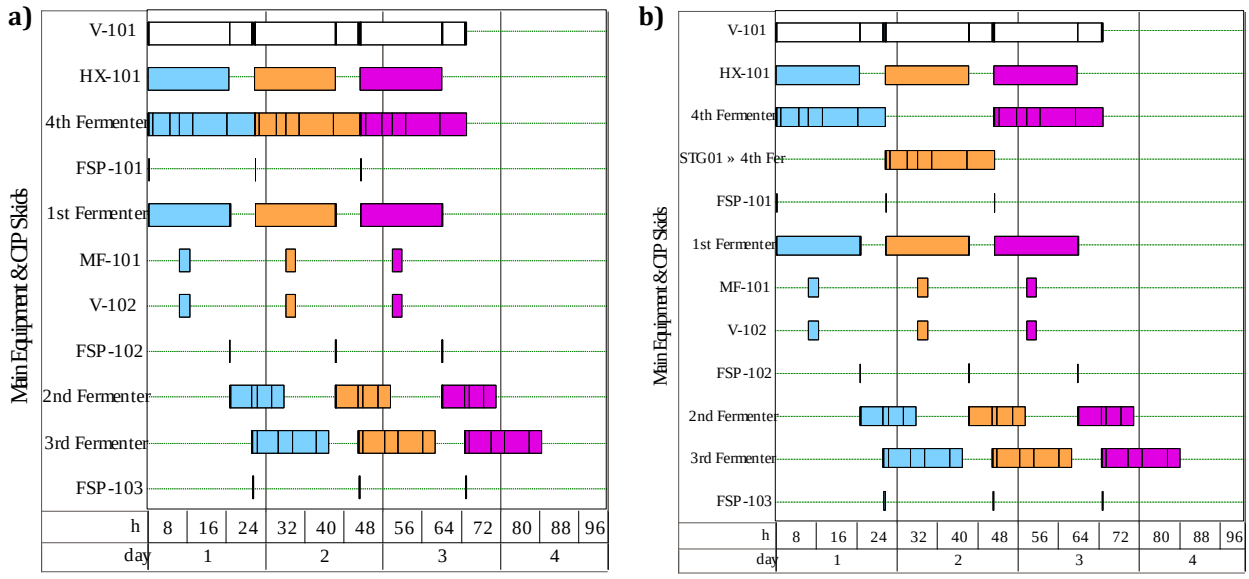


FIGURE 8. Equipment occupancy charts displaying three consecutive batches for fermentation process when duration of the fed-batch phase is 3hrs. a) Only one fermentor of the largest size is available. Process is bottlenecked. b) Two fermentors of the largest size are available. Process is no longer bottlenecked.

necessary in order to obtain the desired yield. As shown by the equipment occupancy chart, or Gantt chart, for this process in Figure 10.a, there is a bottleneck in the fourth fermentor. In order to increase the number of batches possible in a year, a duplicate 4th fermentor was added to the process design so that production could be staggered between the two largest fermentors. However, as Figure 8.b shows, there was hardly any overlap between the staggered units' cycles, indicating that the addition of a second large fermentor had little effect on the number of batches available. The scheduling summary provided by SuperPro confirmed this, as the additional fermentor only increased the number of available batches by two to 366. This design was thus unsuitable for meeting the production quotas.

These observations suggested that a small fed-batch phase did not take full advantage of the process time devoted to expanding the cells, as it reduced the production time of each batch of cells. Subsequent designs increased the duration of the fed-batch phase in order to increase the product yield per batch. Increasing the fed-batch phase to about 6-7

hours enabled the process to meet production quotas; however, this design required two fermentors of the largest size. Further adjustments to the fed-batch phase were made in order to obtain a design where only one fermentor of the largest size was necessary in the hopes that this might reduce initial capital costs and reduce the amount of resources wasted in the growth phase rather than the production phase.

Figure 9.a shows the Gantt chart for the resulting process, wherein the fed-batch phase lasts 11hrs and the entire production phase lasts 16.67hrs. The final culture volume in the largest fermentor is 7650L for MAA and 5934L for MEK. As the fermentors are to be shared between the MAA and MEK processes, the largest fermentor must be sized to the larger volume. The culture volume is much higher for MAA because the fed-batch stream for its

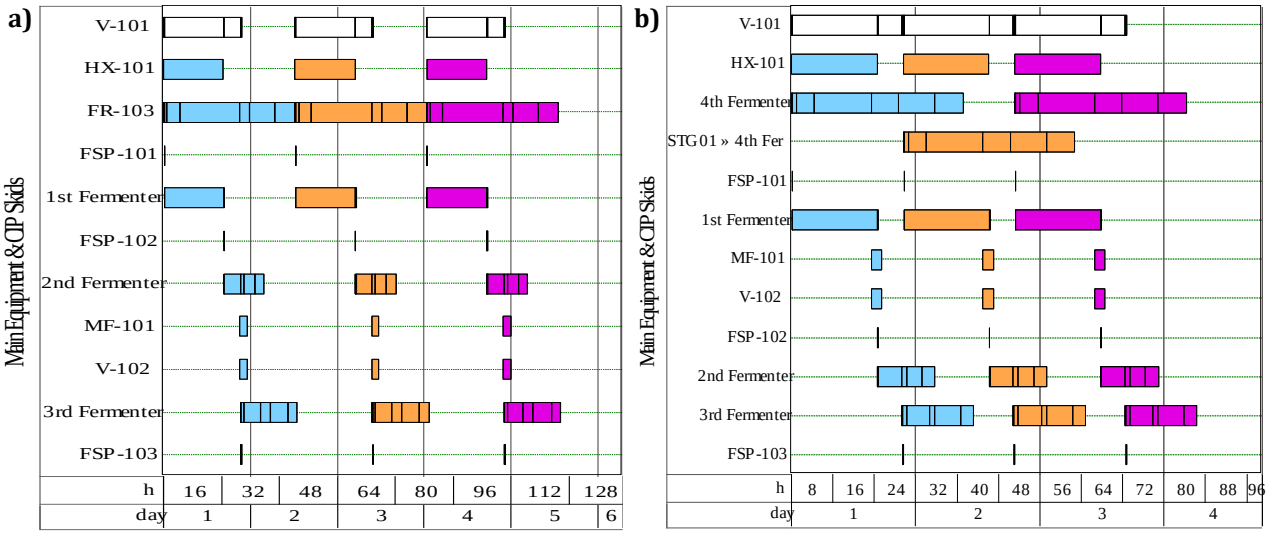


FIGURE 9. Equipment occupancy charts displaying three consecutive batches for fermentation process when duration of the fed-batch phase is 11hrs. a) Only one fermentor of the largest size is available. Process is bottlenecked. Available batches = 217. Cycle time = 36.37hrs. b) Two fermentors of the largest size are available. Process is no longer bottlenecked. Available batches = 366. Cycle time = 21.58hrs.

culture is diluted 1:6 instead of 1:4 as is the case for MEK. This additional dilution is necessary because yield of MAA per mol of glucose is higher than that for MEK from glucose, so MAA concentration increases more rapidly. Cultures containing MAA-producing microorganisms thus need to be diluted more rapidly in order to avoid product inhibition.

However, aside from the dilution of the fed-batch inlet stream, the fermentation process for MAA and MEK producing organisms is virtually the same.

While the process shown in Figure 9.a has a bottleneck, the number of batches available in this design is sufficient for meeting production quotas given the increased per batch yield. As shown in Figure 9.b, adding a second fermentor would add capacity for almost 1.5x as many batches. However, in a pilot plant design, the objective is not simply to maximize product formation to the fullest extent. In fact, in this pilot plant, it is unlikely that any of the product will actually be sold due to the requirements of quality testing and the absence of a sales staff. A second fermentor with a working volume of 7650L is thus an unnecessary expense as there are enough available batches for data collection without its addition.

Working volume in a fermentor should not exceed 90% of the fermentor's capacity. The fermentors were thus sized by dividing the culture volume by 0.9. As fermentors come in standard sizes, however, market availability dictated the following sizes: 7L, 100L, 1000L, and

BENCH-SCALE LABORATORY WORK

As previously mentioned in consideration of microorganisms, fermentation design is sensitive to a number of parameters such as cell density, pH, and media composition. However, due to our inability to collect experimental data, we utilized growth parameters that were not necessarily tailored for this process. When obtaining growth parameters and extrapolating observations from published research, there is the risk that unknown caveats of the research make it inappropriate for application to a particular process. Though *E. coli*

is a relatively simple microorganism, there is still a great deal of variation among strains and the presence of recombinant DNA introduces yet more variability. For instance, we assumed growth rates roughly equivalent to the background strain, BL21, but the introduction of recombinant DNA imposes a metabolic burden on the cell which very likely slows growth and product formation.

However, without the ability to conduct laboratory work to measure an accurate growth rate, we were unable to account for these deviations. Furthermore, many of our simplifying assumptions, such as treating both sucrose and fructose as "glucose equivalents," could be corrected with bench-scale work to measure the actual rates of fructose and sucrose utilization in the recombinant *E. coli.* Bench-scale lab work could also reveal to what extent plasmid instability affects the recombinant microorganisms and how much product inhibition takes place at various culture times and MEK or MAA concentrations.

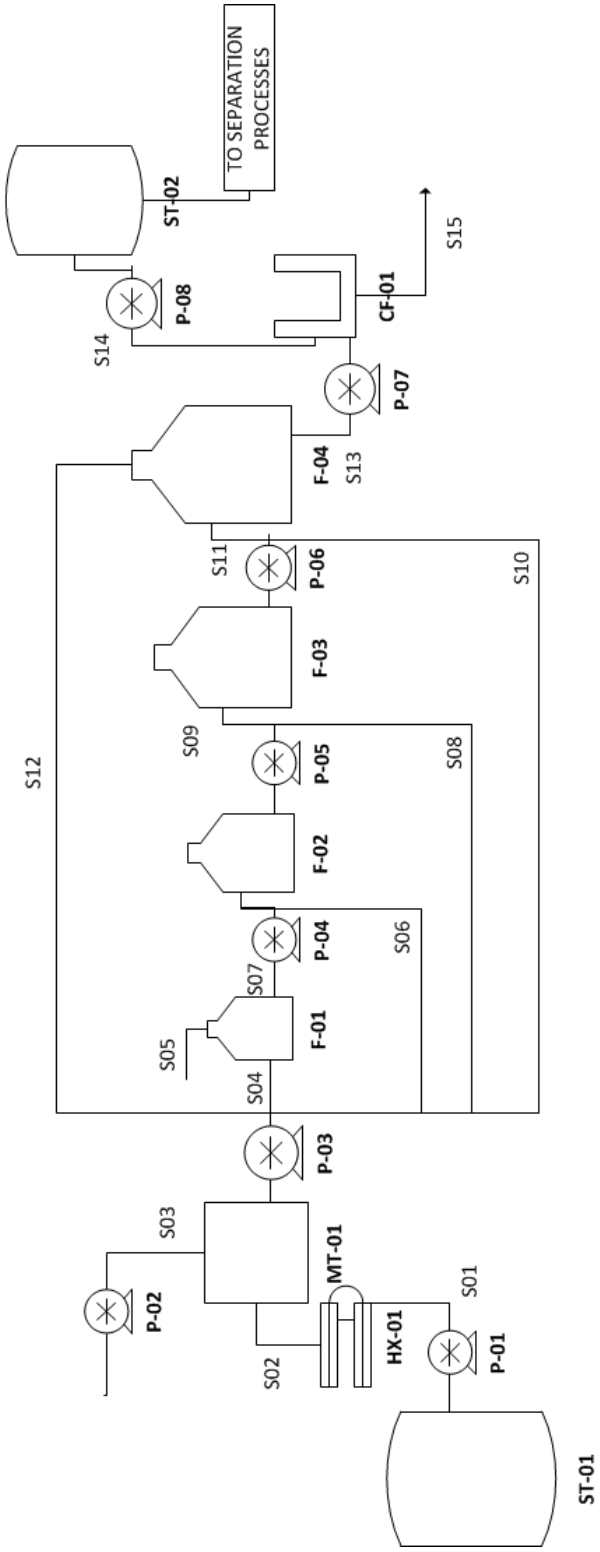
In further development of this process design, it is absolutely essential that a bench-scale laboratory program confirm the validity of the operating parameters (including, but not limited to, pH, medium composition, growth rate, efficiency of glucose utilization) presented in this report, as a slight deviation could dramatically affect the project design and result in a failure to meet production quotas. This process design employed a cautious approach to estimating most parameters in order to avoid this scenario, so bench-scale work could alternately reveal inefficiencies in our assumptions, such as that cell density only reaches the lower end of the normal range and that a 10% loss in yield of cells occurs at the end of each fermentation due to plasmid instability. The fermentations could possibly reach higher cell densities, in which case the current batch times are

overestimates and the process design is inefficient.

Aside from the necessity of confirming our operating consumptions, a bench-scale laboratory program would be beneficial for optimization of the microorganism itself. The *E. coli* strains, for instance, could be engineered to withstand high concentrations of MEK and MAA, as has previously been accomplished with yeast and ethanol.[29] *E. coli* with higher MEK and MAA tolerance would increase the volumetric productivity of the fermentation section and require less introduction of water into the system during the production phase for the purpose of avoiding product inhibition. Engineering the microorganism could thus save utilities costs while simultaneously improving batch production.

FEASIBILITY AND DEVELOPMENT STAGES

PROCESS FLOW DIAGRAM AND MATERIAL BALANCES



Stream No.	Stream Name
S01	Undiluted Molasses From Storage
S02	Undiluted Pasteurized Molasses
S03	Water/Micronutrient Feed
S04	Medium to Seed Fermentor (F-01)
S05	E. Coli Inoculum
S06	Medium to Small Fermentor (F-02)
S07	Fermentation Broth to Small Fermentor (F-02)
S08	Medium to Medium Fermentor (F-03)
S09	Fermentation Broth to Medium Fermentor (F-03)
S10	Medium to Chemical Production Fermentor (F-04)
S11	Fermentation Broth to Chemical Production Fermentor (F-04)
S12	Production Phase Medium Feed Stream
S13	Fermentation Product Stream to Microfilter (MF-01)
S14	Supernatant to Storage Tank (ST-01)
S15	Retentate Waste Stream

Equipment No.	Equipment Name
CF-01	Centrifuge
F-01	Seed Fermentor
F-02	Small Fermentor
F-03	Medium Fermentor
F-04	Chemical Production Fermentor
HX-01	Pasteurizer
MT-01	Storage/Mixing Tank
P-01	Pump 1
P-02	Pump 2
P-03	Pump 3
P-04	Pump 4
P-05	Pump 5
P-06	Pump 6
P-07	Pump 7
P-08	Pump 8
ST-01	Pasteurized Molasses Storage Tank
ST-02	Fermentation Product Storage Tank

Figure 10: Process Flow Diagram for Fermentation Steps

Detailed material balances on each unit operation are included in the specification sheets.

PROCESS DESCRIPTION

The fermentation process begins in the molasses storage tank (**ST-01**), in which a two month supply of molasses is held at any one time. From this storage tank, raw molasses is pumped by **P-01** as stream **S01** to the pasteurizer (**HX-01**). In the pasteurizer, the molasses is raised to a temperature of 71.7°C for about 20 seconds in order to kill any microbial contamination within the molasses. The pasteurized molasses exists in stream **S02** and enters the water/molasses mixing tank (**MT-01**). Micronutrients, which are necessary for optimal growth of the *E.coli,* and water for injection, a type of water suitable for cell cultures, is pumped into **MT-01** by **P-02** via stream **S03**. An agitator within the mixing tank ensures that the molasses, micronutrients, and water are uniformly mixed.

A particular batch culture begins when 5L of the molasses-water mixture, henceforth known as medium, is pumped by **P-03** into the seed fermentor (**F-01**) through stream **S04**. An inoculum of *E.coli* cells is injected into the seed fermentor via stream **S05** to provide an initial concentration of 1.00×10^9 cells/L. The *E.coli* population is expanded within the seed fermentor for 16.5 hrs until the final cell concentration is 5×10^{13} cells/L. Once this cell concentration is reached, the fermentation broth is transferred to the small fermentor (**F-02**) by pump **P-05** through stream **S07**. Pump **P-03** transfers fresh medium from the water/molasses mixing tank (MT-01) through stream **S06** into **F-02**. After the cells grow to a final concentration of 5×10^{13} cells/L in 4.5 hrs, the fermentation broth is transferred to yet another fermentor for further expansion of the cell population. The fermentation broth is transferred to the medium fermentor (**F-03**) through stream **S09**.

Fresh medium enters from **MT-01** via stream **S08**. The cells are expanded in 4.5 hrs again, and the fermentation broth is then transferred by pump **P-06** into the chemical production fermentor **F-04** via stream **S11**. Stream **S10** brings in fresh medium from **MT-01**. The cells grow for 3.45 hours. This final expansion completes the growth phase of the batch, in which enough cells are produced to ensure sufficient production of MEK or MAA per unit time in order to achieve production quotas.

Once the cell expansion ends, the production phase begins. The glucose remaining within the medium after the growth pahse is converted to MAA or MEK. One hour before the glucose is completely consumed, a feed stream of medium enters from **MT-01** via stream **S12**. This stream is fed into **F-04** for a period of 11 hours. In order to maintain a constant glucose concentration, the flow rate of the stream is controlled so that glucose is supplied at a rate equivalent to that at which it is consumed by the cells. The feed stream extends the amount of product that can be formed in the largest fermentor. If the glucose present within the feed stream was present from the beginning of the batch time, the glucose concentration would be too high and cell growth and production would be inhibited.

After a production phase lasting 16.67 hours, the resulting fermentation broth is sent through stream **S13** to a continuous centrifuge (**CF-01**) in order to separate the cells from the product stream. The cells exit the process in waste stream **S15**. The supernatant is pumped into a storage tank (**ST-02**) through stream **S14**. This storage tank can hold supernatant from three batches at a time; this buffer of supernatant is maintained so that the downstream separation processes may be run continuously even in the case of a contaminated batch.

ENVIRONMENTAL AND SAFETY CONCERNS

The fermentation process poses some risks to the environment and to the workers at the pilot plant. The main environmental concern comes from the carbon dioxide generated by the cultivation of *E.coli*. The emissions generated at the pilot scale are negligible, but may become a greater issue after scale-up. At present Brazil lacks an overt carbon emissions tax, but some counties in the U.S. levy a tax of up to $5 per ton of carbon emissions.[30] This pilot plant facility produces approximately 150 tons of carbon dioxide per year, which would warrant an emissions tax of $750. This amount is negligible, especially in comparison to the utilities costs alone for the batch processes. Even after scale up, the plant may not need CO_2 scrubbers. Consider a full scale process that is 1000 times larger than the pilot plant. Even then, the emissions tax would only be $750,000 which would likely be less than the utilities required to run the plant.

The workers at the pilot plant must take necessary precautions when working with recombinant strains of E. coli. These bacteria may have mutations that make them especially virulent or contagious, and thus they should be handled with extreme care. Appropriate clothing, masks, eyewear, and gloves should be worn when starting up the seed fermentor and during any transfer steps that require handling by a technician. The lab workers should wash their hands after working with the bacteria. The waste stream of bacteria produced by the centrifuge will be disposed of by incineration in order to ensure that none of the recombinant bacteria escape the plant.

ENERGY BALANCE AND UTILITY REQUIREMENTS

Low Pressure Steam (50 psig, $3/1000 lb)				
Equipment	Unit	Flowrate (lb/hr)	Annual Consumption (lb/yr)	Annual Cost
Seed Fermentor (MAA/MEK)	F-01	1.78	6376	$19.13
Small Fermentor (MAA/MEK)	F-02	126.99	122352	$367.00
Medium Fermentor (MAA/MEK)	F-03	568.79	548013	$1,644.04
Chemical Production Fermentor (MAA)	F-04	1716.77	497521	$1,492.56
Chemical Production Fermentor (MEK)	F-04	1716.77	787742	$2,363.23
Pasteurizer	HX-01	5.01	28920	$86.76
			TOTAL	$5,972.72
Electricity ($0.06/kWh)				
Equipment	Unit	Power (kW)	Annual Consumption (kWh)	Annual Cost
Agitator	AG-01	2.24	24.61	$1.23
Centrifuge	CF-01	6.60	52272.00	$3,136.32
Pump 1	P-01	0.02	123.04	$7.38
Pump 2	P-02	0.45	97.09	$5.83
Pump 3	P-03	1.0320	358.35	$21.50
Pump 4	P-04	0.0008	0.22	$0.01
Pump 5	P-05	0.0050	3.24	$0.19
Pump 6	P-06	0.0155	33.71	$2.02
Pump 7 (MAA)	P-07	1.1185	258.39	$15.50
Pump 7 (MEK)	P-07	0.7457	272.74	$16.36
Pump 8 (MAA)	P-08	0.9694	244.29	$14.66
Pump 8 (MEK)	P-08	0.5966	238.03	$14.28
Water/Molasses Mixing Tank	MT-01	9.6900	76,744.80	$4,604.69
			TOTAL	$7,839.98

Figure 11. Utilities and Material Cost Estimates for the fermentation Process

Utility requirements for the batch processes are summarized in Figure 11. Total utilities costs for the batch processes are $13812.70. For the fermentation process, the required utilities are electricity and low pressure steam.

The predominant consumers of low pressure steam are the medium and chemical production fermentors (F-03 and F-04), which must remain at a temperature of 37°C to support optimal *E.coli* growth. Due to convection between the fermentors and the surrounding air, heat is constantly being lost from the fermentors. In the large fermentors, a large supply of steam is necessary to overcome this heat loss and maintain constant temperature. High utilities costs due to temperature control are a problem intrinsic to large fermentors and are thus difficult to avoid. If this pilot plant was implemented in full scale, insulation of the fermentors may be worth consideration.

The major electric costs of the batch processes are agitation of the water/molasses mixing tank and operation of the centrifuge. The requirement of mixing the molasses and water was responsible for 60% of the electricity costs. This high utility requirement suggests that for a full scale implementation of this pilot plant, it will be important to find a cheaper method of ensuring uniformity of the molasses/water medium. High utility expenses due to centrifugation, while manageable at the pilot scale, may mandate a microfiltration system in a full scale process.

Table 2. Detailed Overview of Process Units in Fermentation Process

Fermentation Process Equipment Summary								
Unit Name	**Unit No.**	**Function**	**Size/ Capacity**	**Construction Material**	**Estimated Cost**	**Source of Cost Estimate**	**Operating Temp.**	**Operating Pressure**
Centrifuge	CF-01	Remove the bacterial cells from the effluent of the largest reactor	54.0L	Aluminum, Teflon Coating	$34,226.00	DJB Labcare	25 °C	1 bar
Seed Fermentor	F-01	Expand the initial inoculum of *E.coli* bacteria	7.5 L	Glass	$35,000.00	New Brunswick	37 °C	1 bar
Small Fermentor	F-02	Increase the total number of *E.coli* bacteria	65 L	Stainless Steel	$127,032.00	New Brunswick	37 °C	1 bar
Medium Fermentor	F-03	Increase the total number of *E.coli* bacteria	1000 L	Stainless Steel	$460,000.00	New Brunswick	37 °C	1 bar
Chemical Production Fermentor	F-04	Increase the total number of E.coli bacteria and convert glucose to MAA/MEK	8500 L	Stainless Steel	$27,569.50	Correlations	37 °C	1 bar
Agitator	AG-01	Mix the contents of the largest fermentor, F-04	2.24 kW	Stainless Steel	$3,616.03	Correlations	37 °C	1 bar
Pasteurizer	HX-01	Sterilize the molasses before it is fed into the fermentors	4569 BTU/hr	Stainless Steel	$3,455.94	Correlations	71.7 °C	1 bar
Pump 1	P-01	Move molasses from molasses storage tank (ST-01) to pasteurizer (HX-01)	0.015 kW	Stainless Steel	$799.00	eBay	37 °C	1 bar
Pump 2	P-02	Move water to mixing tank (MT-01)	0.45 kW	Stainless Steel	$1,000.00	eBay	37 °C	1 bar
Pump 3	P-03	Deliver fresh medium to all 4 fermentors during growth phase and to chemical production fermentor during production phase.	1.032 kW	Stainless Steel/ Rubber	$1,350.00	eBay	37 °C	1 bar
Pump 4	P-04	Move fermentation broth from seed fermentor (F-01) to small fermentor (F-02)	0.0008 kW	Acetal, Aluminum, Stainless Steel	$1,350.00	New Brunswick	37 °C	1 bar
Pump 5	P-05	Move fermentation broth from small fermentor (F-02) to medium fermentor (F-03)	0.0050 kW	PSF, Stainless Steel	$1,350.00	New Brunswick	37 °C	1 bar
Pump 6	P-06	Move fermentation broth from medium fermentor (F-03) to production fermentor (F-04)	0.0155 kW	Stainless Steel	$1,350.00	New Brunswick	37 °C	1 bar
Pump 7	P-07	Move fermentation broth containing MAA or MEK to centrifuge for cell separations.	1.1185 kW	Stainless Steel	$1,000.00	eBay	37 °C	1 bar
Pump 8	P-08	Move supernatant containing MAA or MEK and water to storage tank prior to further separations.	0.9694 kW	Stainless Steel	$1,000.00	eBay	37 °C	1 bar
Water/Molasses Mixing Tank	MT-01	Hold 2 months worth of molasses supply	67,380 L	316 Stainless Steel	$20,681.00	Correlations	37 °C	1 bar
Pasteurized Molasses Storage Tank	ST-01	Hold enough pasteurized molasses for three fermentation batches in case of a contaminated batch	4,921 L	316 Stainless Steel	$10,264.95	Correlations	37 °C	1 bar
Supernatant Storage Tank	ST-02	Hold 3 batches of supernatant from the largest fermentor before purification in case of a contaminated batch	24,605 L	316 Stainless Steel	$23,325.54	Correlations	25 °C	1 bar
Total					**$754,369.96**			

Centrifuge (CF-01)

Cells are separated from the fermentor effluent after the production phase by this continuous centrifuge. With a throughput of 14.26 gal/min, the centrifuge is able to separate the cells quickly enough for the process requirements. The use of a continuous centrifuge also reduces operator involvement, which is desirable given the potentially hazardous bioaerosols produced by a centrifuge.

Seed Fermentor (F-01)

Expansion of *E. coli* cells begins in this 5L seed fermentor. The fermentor is inoculated with enough inoculum from a lab scale culture for an initial cell density of $1x10^9$ cell/ml. The batch time for this unit is 16.5hrs, in which time the cells grow from a concentration of $1x10^9$ cell/ml to $5x10^{13}$ cells/ml. Glucose equivalent consumption within this reactor is 1.1 mol of glucose or 198 g, leaving 4.9 mol or 883 g of glucose equivalent within the reactor.

Small Fermentor (F-02)

After expansion of *E.coli* cells to optimal cell density in the seedfermentor, the 5L of fermentation broth containing the cells and remaining glucose equivalent are transferred from F01 to the 50L small fermentor (F02). The batch time for this unit is 4.44 hrs. Glucose equivalent consumption within this reactor is 10.7 mol or 1.93kg.

Medium Fermentor (F-03)

After expansion of *E.coli* cells to optimal cell density in the small fermentor, the 50L of fermentation broth containing the cells and remaining glucose are transferred from F02 to the 500L medium fermentor (F03). The batch time for this unit is 4.44 hrs. Glucose equivalent consumption within this reactor is 108 mol or 19.5kg. After the fermentation broth is emptied from this fermentor, there will be a CIP process lasting 5 hrs and a SIP

process lasting 2.5 hrs in order to clear the fermentor of any possible contaminants before future use.

Chemical Production Fermentor (F-04)

After expansion of *E.coli* cells to optimal cell density in the medium fermentor, the 500L of fermentation broth containing the cells and remaining glucose are transferred from F03 to the 8500L chemical production fermentor (F04). This fermentor is a fed-batch system in which both cell growth and product formation occur. After inoculation of the fermentor with 500L from the medium fermentor, the cells grow to optimal density. This growth phase requires 3.45hrs. Once the cells have reached optimal density, MEK and MAA product formation begin. The remaining glucose equivalent in the culture medium is converted first. One hour before complete consumption of this glucose equivalent, a fed batch phase begins. For the MEK process, a 1:4 dilution of molasses is fed at a rate of 312.15 L/hr. For the MAA process, a 1:6 dilution of molasses is fed at a rate of 468.22 L/hr. The total time for MAA or MEK production before available glucose equivalent is consumed is 11.67 hrs. For each batch, a total of 232.9kg of MEK or 366.8kg of MAA is produced.

After the fermentation broth is emptied from this fermentor, there will be a CIP process lasting 7 hrs and a SIP process lasting 5.5 hrs in order to clear the fermentor of any possible contaminants before future use.

Agitator (AG-01)

This agitator is a feature of the custom designed chemical production fermentor. It ensures that the fermentation broth is always well mixed so that cells have equal exposure to nutrients. It operates at 3HP.

Pasteurizer (HX-01)

The pasteurizer is a stainless steel heat exchanger specified for the elimination of microbial contamination from the molasses feed stream. By raising briefly raising the temperature of the molasses to 71.7°C, microbes within the molasses are killed. It is important to remove microbial contamination from the molasses so that the recombinant microorganism does not face competition for resources, as such would reduce its ability to grow and produce.

Pumps 1-3, 7-8 (P-01 – P-03, P-07 – P-08)

These pumps move liquid flows between various units within the process. Most of these pumps are pre-owned or refurbished in order to reduce costs, and thus some of them have flow rates that exceed the requirements of the process. Further details for each pump are provided in the specification sheets.

Pumps 4-6 (P-04 – P-06)

These pumps are included in the specifications for the fermentors and transfer fermentation broth from one fermentor to another. Further details for each pump are provided in the specification sheets.

Molasses Storage Tank (ST-01)

The 17,800 gal molasses storage tank ensures a continuous supply of molasses to the process. The partner facility which supplies molasses only operates for nine months out of the year; this storage tank has enough capacity to supply molasses for the plant during the two months wherein the plant is operation while molasses are unavailable.

Water/Molasses Mixing Tank (MT-01)

This 1,300 gal mixing tank holds medium for the fermentation process. It is large

enough to maintain a supply of medium for three fermentation batches. This buffer of supply is maintained so that the fermentation process always has a feed, even if there are one or two intermittent contaminated batches.

Supernatant Storage Tank (ST-02)

This 6,500 gal storage tank stores supernatant from the continuous centrifuge to maintain a steady supply for the downstream MAA and MEK separation processes. Like the water/molasses mixing tank (MT-01), this tank has capacity to store three batches so that the process can continue even when contaminated batches occur.

SPECIFICATION SHEETS

Seed Fermentor (MAA/MEK)			
Identification:	Item: 7.5 L Fermentor Item No. F-01 No. Req. 1		Date 04/05/11
Function:	Expand the initial inoculum of *E.coli* bacteria		
Operation:	Batch		
Materials Handled:			
	Inlet	**Outlet**	
Stream ID:	S04, S05	S07	
Quantity (kg/batch):	5.8396	5.8396	
Composition (kg/batch):			
Glucose	0.2246	0.8822	
Fructose	0.2567	0.0000	
Sucrose	0.5704	0.0000	
SiO2	0.0125	0.0125	
K2O	0.0624	0.0624	
CaO	0.0339	0.0339	
MgO	0.0018	0.0018	
P2O5	0.0036	0.0036	
Fe2O3	0.0071	0.0071	
Sulfates as SO3	0.0321	0.0321	
Cl	0.0071	0.0071	
Organic Non-Sugars	0.2495	0.2495	
Total Water	4.0708	3.8444	
CO2	0.0000	0.0000	
Micronutrients	0.3071	0.3071	
Kanamycin	1.250E-04	1.250E-04	
E.coli Cells	1.500E-06	0.0750	
MAA/MEK	0.0000	0.0000	
Design Data:			
Material	Glass		
Manufacturer	New Brunswick		
Model	BR310		
Unit Cost	$35,000		
Heat Duty	25,815	BTU/hr	
Utilities	6,376	lb steam/yr	
	19.13	$/yr	
Comments: Batch Time = 16.5 hrs			

Small Fermentor (MAA/MEK)			
Identification:	Item: 65 L Fermentor Item No. F-02 No. Req. 1		Date 4/5/2011
Function:	Increase the total number of *E.coli* bacteria		
Operation:	Batch		
Materials Handled:			
	Inlet	**Outlet**	
Stream ID:	S06, S07	S09	
Quantity (kg/batch):	58.3887	58.3887	
Composition (kg/batch):			
Glucose	2.9035	8.6791	
Fructose	2.3101	0.0000	
Sucrose	5.1335	0.0000	
SiO2	0.1248	0.1248	
K2O	0.6239	0.6239	
CaO	0.3387	0.3387	
MgO	0.0178	0.0178	
P2O5	0.0356	0.0356	
Fe2O3	0.0713	0.0713	
Sulfates as SO3	0.3208	0.3208	
Cl	0.0713	0.0713	
Organic Non-Sugars	2.4954	2.4954	
Water	40.8028	41.7886	
CO2	0.0000	0.0000	
Micronutrients	3.0705	3.0705	
Kanamycin	0.0013	0.0013	
E.coli Cells	0.0675	0.7496	
MAA/MEK	0.0000	0.0000	
Design Data:			
Material	Stainless Steel		
Manufacturer	New Brunswick		
Model	BR610		
Unit Cost	$127,032		
Heat Duty	115,815	BTU/hr	
Utilities	122,352	lb steam/yr	
	367	$/yr	
Comments: Batch Time = 4.44 hrs			

Medium Fermentor (MAA/MEK)			
Identification:	Item: 1000 L Fermentor Item No. F-03 No. Req. 1		Date 4/5/2011
Function:	Increase the total number of *E.coli* bacteria		
Operation:	Batch		
Materials Handled:		Inlet	Outlet
Stream ID:		S08,S09	S11
Quantity (kg/batch):		583.7859	583.7859
Composition (kg/batch):			
	Glucose	28.8921	85.4312
	Fructose	23.1006	0.0000
	Sucrose	51.3347	0.0000
	SiO2	1.2477	1.2477
	K2O	6.2386	6.2386
	CaO	3.3867	3.3867
	MgO	0.1782	0.1782
	P2O5	0.3565	0.3565
	Fe2O3	0.7130	0.7130
	Sulfates as SO3	3.2084	3.2084
	Cl	0.7130	0.7130
	Organic Non-Sugars	24.9544	24.9544
	Water	408.0700	419.1443
	CO2	0.0000	0.0000
	Micronutrients	30.7050	30.7050
	Kanamycin	0.0125	0.0125
	E.coli Cells	0.6746	7.4965
	MAA/MEK	0.0000	0.0000
Design Data:			
	Material	Stainless Steel	
	Manufacturer	New Brunswick	
	Model	BR-PRO	
	Unit Cost	$460,000	
	Heat Duty	518,732	BTU/hr
	Utilities	548,013	lb steam/yr
		1,644	$/yr
Comments:	Includes SIP/CIP systems. Batch Time = 4.44 hrs		

Chemical Production Fermentor (MAA)		
Identification:	Item: 8500 L Fermentor Item No. F-04 No. Req. 1	Date 4/5/2011

Function: Increase the total number of *E.coli* bacteria and convert glucose to MAA

Operation: Batch

Materials Handled:	Inlet	Outlet
Stream ID:	S10, S11, S12	S13
Quantity (kg/batch):	8357.5592	8357.5592
Composition (kg/batch):		
Glucose	329.4974	38.2521
Fructose	278.9328	0.0000
Sucrose	619.8507	0.0000
SiO2	14.8070	14.8070
K2O	74.0348	74.0348
CaO	40.1903	40.1903
MgO	2.1153	2.1153
P2O5	4.2306	4.2306
Fe2O3	8.4611	8.4611
Sulfates as SO3	38.0750	38.0750
Cl	8.4611	8.4611
Organic Non-Sugars	296.1390	296.1390
Water	6547.7696	6720.9098
CO2	0.0000	619.2089
Micronutrients	88.0564	88.0564
Kanamycin	0.1913	0.1913
E.coli Cells	6.7468	37.4900
MAA	0.0000	366.9366

Design Data:	Inner Shell	Outer Shell
Material:	Stainless Steel	Stainless Steel
Height (ft)	11.52	12
Diameter (ft)	5.76	6
Wall thickness (ft)	0.026	0.208
Weight (lb)	3734.894	33394.14
C_v	6773.146	7873.831
C_{PL}	7432.828	7884.927
C_P	24365.69	27569.5
Utilities	1,717	lb steam/hr
	3,856	$/yr

Comments: 133 batches: Growth = 3.45 hrs, Production = 16.67 hrs, Feed Time = 11 hrs, Working Volume = 7650 L

Chemical Production Fermentor (MEK)		
Identification:	Item: 8500 L Fermentor Item No. F-04 No. Req. 1	Date 4/5/2011

Function: Increase the total number of *E.coli* bacteria and convert glucose to MAA

Operation: Batch

Materials Handled:	Inlet	Outlet
Stream ID:	S10, S11, S12	S13
Quantity (kg/batch):	6632.02	6632.02
Composition (kg/batch):		
Glucose	329.4974	29.6681
Fructose	278.9328	0.0000
Sucrose	619.8507	0.0000
SiO2	14.8070	14.8070
K2O	74.0348	74.0348
CaO	40.1903	40.1903
MgO	2.1153	2.1153
P2O5	4.2306	4.2306
Fe2O3	8.4611	8.4611
Sulfates as SO3	38.0750	38.0750
Cl	8.4611	8.4611
Organic Non-Sugars	296.1390	296.1390
Water	4842.0361	5153.5158
CO2	0.0000	623.5010
Micronutrients	68.2959	68.2959
Kanamycin	0.1483	0.1483
E.coli Cells	6.7468	37.4900
MEK	0.0000	232.8890

Design Data:	Inner Shell	Outer Shell
Material:	Stainless Steel	Stainless Steel
Height (ft)	11.52	12
Diameter (ft)	5.76	6
Wall thickness (ft)	0.026	0.208
Weight (lb)	3734.894	33394.14
C_v	6773.146	7873.831
C_{PL}	7432.828	7884.927
C_P	24365.69	27569.5
Utilities	1,717	lb steam/hr
	3,856	$/yr

Comments: 84 batches: Growth = 3.45 hrs, Production = 16.67 hrs, Feed Time = 11 hrs, Working Volume = 5934 L

Agitator			
Identification:	Item:	Agitator	Date 4/5/2011
	Item No.	AG-01	
	No. Req.	1	
Function:	Mix the contents of the largest fermentor, F-04		
Operation:	Batch		

Design Data:

Material:	Stainless Steel	
Volume requiring mixing (gal)	2020	
Power (HP)	3	
Power (kW)	$	3,616.03
C_P	$	3,616.03

Comments:

Pasteurizer

Identification	Item:	Double Pipe Heat Exchanger	Date 4/5/2011
	Item No.	HX-01	
	No. Req.	1	

Function: Sterilize the molasses before it is fed into the fermentors

Operation: Continuous

Materials Handled:		Molasses		Steam	
		Inlet	Outlet	Inlet	Outlet
Stream ID:		S01	S02		
Temperature (C)		25	71.7	90	120
Temperature (F)		77	161.06	194	248
Quantity (kg/hr):		58.16	58.16	2.28	2.28
Composition (kg/hr):					
	Molasses	58.16	58.16	0.00	0.00
	Water	0.00	0.00	2.28	2.28

Design Data:

Heat Duty		4569	BTU/hr
Flow rate of steam		5.01	lb/hr
Log Mean Temp. Difference		101.23	degrees F
Heat Transfer Coefficient		26	BTU/(F-ft^2-hr)
Area		1.707	ft^2
Materials			
	Shell	Stainless Steel	
	Tube	Stainless Steel	
Unit Cost, C_p		$ 3,455.94	per unit
Utilities		$ 86.76	per year

Comments: Molasses raised to high temperature for 20 seconds for pasteurization.

Pump 1			
Identification:	Item:	Peristaltic Pump	Date 4/5/2011
	Item No.	P-01	
	No. Req.	1	
Function:	Move molasses from Molasses Storage Tank (ST-01) to Pasteurizer (HX-01)		
Operation:	Continuous		

Materials Handled:	Molasses to Pasteurizer
Stream ID:	S01
Quantity (kg/hr):	58.16
Flow Rate (L/hr)):	40.78

Design Data:

Material	Stainless Steel	
Manufacturer	Watson-Marlow	
Model	503U	
Pumping Capacity	2L/min (680 ml/min required)	
Unit Cost	$799	
Utilities	419,832	BTU electricity/yr
	7.38	$/yr

Comments:

Pump 2		
Identification:	Item: One Channel Peristaltic Pumps Item No. P-02 No. Req. 1	Date 4/5/2011
Function:	Move water to mixing tank (MT-01)	
Operation:	Batch	

Materials Handled:		
	Water	
Stream ID:	S03	
Quantity (kg/batch):	6167.02	max

Design Data:	
Material	Stainless Steel
Manufacturer	Ponndorf
Model	PDX 35
Pumping Capacity	18 gpm (6.6 gpm required)
Unit Cost	$1,000
Utilities	331,285 BTU electricity/yr
	5.83 $/yr

Comments:

Pump 3

Identification:	Item:	Peristaltic Hose Pump	Date	4/5/2011
	Item No.	P-03		
	No. Req.	1		

Function: To deliver fresh medium to all four fermentors during the growth phase and to the chemical production fermentor during the production phase.

Operation: Batch

Materials Handled:

	Inlet	Outlet
Stream ID:		-
Quantity (kg/batch):	Variable	Variable

Design Data:

Material		Stainless Steel/Rubber		
Manufacturer		Watson-Marlow Bredel	520,701	BTU/yr
Model		SPX-25	9.16	$/yr
Unit Cost		$1,350		

Utilities		Flow Rate (gpm)	Duration (hrs)	Power Requirement (BTU/batch)	
	Transfer 1	1.3	0.016667	3.53	
	Transfer 2	1.0	0.2	42.41	
	Transfer 3	2.6	0.75	318.05	
	Transfer 4	8.8	1	2035.55	
	Transfer 5	2.1	11	4664.79	
	Transfer 6	1.4	11	2332.40	
	Total			**1,222,752**	**BTU electricity/yr**
				21.50	**$/yr**

Comments: This pump has a controller to produce the various flow rates needed for the different transfers between the mixing/storage tank (ST-01) and the fermentors. Power requirements were estimated from the data for the pump given by Watson-Marlow. Transfer 1 = Medium to F-01. Transfer 2 = Medium to F-02. Transfer 3 = Medium to F-03. Transfer 4 = Medium to F-04. Transfer 5 = Feed stream to F-04 during MAA production phase. Transfer 6 = Feed stream to F-04 during MEK production phase.

Pump 4			
Identification:	Item:	One Channel Peristaltic Pumps	Date 4/5/2011
	Item No.	P-04	
	No. Req.	3	

Function: Move fermentation broth from seed fermentor (F-01) to small fermentor (F-02)

Operation: Batch

Materials Handled:

	Inlet	Outlet
Stream ID:	S07	S07
Quantity (kg/batch):	5.84	5.84
Composition (kg/batch):		
Glucose	0.8822	0.8822
Fructose	0.0000	0.0000
Sucrose	0.0000	0.0000
SiO2	0.0125	0.0125
K2O	0.0624	0.0624
CaO	0.0339	0.0339
MgO	0.0018	0.0018
P2O5	0.0036	0.0036
Fe2O3	0.0071	0.0071
Sulfates as SO3	0.0321	0.0321
Cl	0.0071	0.0071
Organic Non-Sugars	0.2495	0.2495
Water	4.1652	4.1652
CO2	0.0000	0.0000
Micronutrients	0.3071	0.3071
Kanamycin	0.0001	0.0001
E.coli Cells	0.0750	0.0750
MAA/MEK	0.0000	0.0000

Design Data:

Material	Acetal, Aluminum, Stainless Steel	
Manufacturer	Watson-Marlow	
Model	400F/B1 (two 12RPM and one 100RPM)	
Pumping Capacity	73.2 ml/min (69.4 ml/min required)	
Unit Cost	Cost is included in F-01 pricing	
Utilities	3.4	BTU electricity/batch
	Negligible	$/yr

Comments: These pumps are part of the seed fermentor unit (F-01). Operating time = 1.2 hrs per cycle

Pump 5				
Identification:	Item:	One Channel Peristaltic Pumps	Date	4/5/2011
	Item No.	P-05		
	No. Req.	3		
Function:	Move fermentation broth from small fermentor (F-02) to medium fermentor (F-03)			
Operation:	Batch			

Materials Handled:		Inlet	Outlet
Stream ID:		S09	S09
Quantity (kg/batch):		58.39	58.39
Composition (kg/batch):			
	Glucose	8.6791	8.6791
	Fructose	0.0000	0.0000
	Sucrose	0.0000	0.0000
	SiO_2	0.1248	0.1248
	K_2O	0.6239	0.6239
	CaO	0.3387	0.3387
	MgO	0.0178	0.0178
	P_2O_5	0.0356	0.0356
	Fe_2O_3	0.0713	0.0713
	Sulfates as SO_3	0.3208	0.3208
	Cl	0.0713	0.0713
	Organic Non-Sugars	2.4954	2.4954
	Water	41.7886	41.7886
	CO_2	0.0000	0.0000
	Micronutrients	3.0705	3.0705
	Kanamycin	0.0013	0.0013
	E.coli Cells	0.7496	0.7496
	MAA/MEK	0.0000	0.0000

Design Data:			
	Material	PSF, Stainless Steel	
	Manufacturer	Masterflex	
	Model	Easy-Load One channel pump (three 100RPM)	
	Pumping Capacity	840 ml/min (278 ml/min required)	
	Unit Cost	Cost is included in F-02 pricing	
	Utilities	50.9	BTU electricity/batch
		Negligible	$/yr

Comments: These pumps are part of the small fermentor unit (F-02). Operating time = 3 hrs per cycle

Pump 6			
Identification:	Item:	Fixed-Speed Peristaltic Pump	Date 4/5/2011
	Item No.	P-06	
	No. Req.	4	
Function:	Move fermentation broth from medium fermentor (F-03) to production fermentor (F-04)		
Operation:	Batch		

Materials Handled:	Inlet	Outlet
Stream ID:	S11	S11
Quantity (kg/batch):	583.79	583.79
Composition (kg/batch):		
Glucose	85.4312	85.4312
Fructose	0.0000	0.0000
Sucrose	0.0000	0.0000
SiO_2	1.2477	1.2477
K_2O	6.2386	6.2386
CaO	3.3867	3.3867
MgO	0.1782	0.1782
P_2O_5	0.3565	0.3565
Fe_2O_3	0.7130	0.7130
Sulfates as SO_3	3.2084	3.2084
Cl	0.7130	0.7130
Organic Non-Sugars	24.9544	24.9544
Water	419.1443	419.1443
CO_2	0.0000	0.0000
Micronutrients	30.7050	30.7050
Kanamycin	0.0125	0.0125
E.coli Cells	7.4965	7.4965
MAA/MEK	0.0000	0.0000

Design Data:		
Material	Stainless Steel	
Manufacturer	New Brunswick	
Model	four 100 RPM pumps	
Pumping Capacity	1.12 L/min (833 ml/min required)	
Unit Cost	Cost is included in F-03 pricing	
Utilities	53.1	BTU electricity/batch
	2.02	$/yr

Comments: Pumps are included with medium fermentor (F-03). Operating time = 10 hrs per cycle

Pump 7 - MAA Process			
Identification:	Item:	Peristaltic Hose Pump	Date 4/5/2011
	Item No.	P-07	
	No. Req.	1	

Function: Move fermentation broth containing MAA to centrifuge for cell separations.

Operation: Batch

Materials Handled:		
	Inlet	Outlet
Stream ID:	S13	S13
Quantity (kg/batch):	8357.56	8357.56
Composition (kg/batch):		
Glucose	38.2521	38.2521
Fructose	0.0000	0.0000
Sucrose	0.0000	0.0000
SiO_2	14.8070	14.8070
K_2O	74.0348	74.0348
CaO	40.1903	40.1903
MgO	2.1153	2.1153
P_2O_5	4.2306	4.2306
Fe_2O_3	8.4611	8.4611
Sulfates as SO_3	38.0750	38.0750
Cl	8.4611	8.4611
Organic Non-Sugars	296.1390	296.1390
Water	6720.9098	6720.9098
CO_2	619.2089	619.2089
Micronutrients	88.0564	88.0564
Kanamycin	0.1913	0.1913
E.coli Cells	37.4900	37.4900
MAA	366.9366	366.9366

Design Data:

Material	Stainless Steel	
Manufacturer	Ponndorf	
Model	PDX 35	
Pumping Capacity	18 gpm (12.25 gpm required)	
Unit Cost	$1,000	
Utilities	10,496	BTU electricity/batch
	15.5	$/yr

Comments: Operating Time = 2.75 hrs per cycle

Pump 7 - MEK Process			
Identification:	Item:	Peristaltic Hose Pump	Date 4/5/2011
	Item No.	P-07	
	No. Req.	1	

Function: Move fermentation broth containing MEK to centrifuge for cell separations.

Operation: Batch

Materials Handled:

	Inlet	Outlet
Stream ID:	S13	S13
Quantity (kg/batch):	6632.02	6632.02
Composition (kg/batch):		
Glucose	29.6681	29.6681
Fructose	0.0000	0.0000
Sucrose	0.0000	0.0000
SiO2	14.8070	14.8070
K2O	74.0348	74.0348
CaO	40.1903	40.1903
MgO	2.1153	2.1153
P2O5	4.2306	4.2306
Fe2O3	8.4611	8.4611
Sulfates as SO3	38.0750	38.0750
Cl	8.4611	8.4611
Organic Non-Sugars	296.1390	296.1390
Water	5153.5158	5153.5158
CO2	623.5010	623.5010
Micronutrients	68.2959	68.2959
Kanamycin	0.1483	0.1483
E.coli Cells	37.4900	37.4900
MEK	232.8890	232.8890

Design Data:

Material	Stainless Steel	
Manufacturer	Ponndorf	
Model	PDX 35	
Pumping Capacity	18 gpm (9.5 gpm required)	
Unit Cost	$1,000	
Utilities	6,997	BTU electricity/batch
	16.36	$/yr

Comments: Operating Time = 2.75 hrs per cycle

Pump 8 - MAA Process			
Identification:	Item:	Peristaltic Hose Pump	Date 4/5/2011
	Item No.	P-08	
	No. Req.	1	
Function:	Move supernatant containing MAA and water to storage tank prior to further separations.		
Operation:	Batch		

Materials Handled:	Inlet	Outlet
Stream ID:	S14	S14
Quantity (kg/batch):	8319.88	8319.88
Composition (kg/batch):		
Glucose	38.2521	38.2521
Fructose	0.0000	0.0000
Sucrose	0.0000	0.0000
SiO2	14.8070	14.8070
K2O	74.0348	74.0348
CaO	40.1903	40.1903
MgO	2.1153	2.1153
P2O5	4.2306	4.2306
Fe2O3	8.4611	8.4611
Sulfates as SO3	38.0750	38.0750
Cl	8.4611	8.4611
Organic Non-Sugars	296.1390	296.1390
Water	6720.9098	6720.9098
CO2	619.2089	619.2089
Micronutrients	88.0564	88.0564
Kanamycin	0.0000	0.0000
E.coli Cells	0.0000	0.0000
MEK	366.9366	366.9366

Design Data:		
Material	Stainless Steel	
Manufacturer	Ponndorf	
Model	PDX 35	
Pumping Capacity	18 gpm (9.5 gpm required)	
Unit Cost	$1,000	
Utilities	9,923	BTU electricity/batch
	14.66	$/yr

Comments: Operating Time = 3 hrs per cycle

Pump 8 - MEK Process			
Identification:	Item: Peristaltic Hose Pump Item No. P-08 No. Req. 1		Date 4/5/2011
Function:	Move supernatant containing MEK and water to storage tank prior to further separations.		
Operation:	Batch		
Materials Handled:			
		Inlet	Outlet
Stream ID:		S14	S14
Quantity (kg/batch):		6594.38	6594.38
Composition (kg/batch):			
	Glucose	29.6681	29.6681
	Fructose	0.0000	0.0000
	Sucrose	0.0000	0.0000
	SiO_2	14.8070	14.8070
	K_2O	74.0348	74.0348
	CaO	40.1903	40.1903
	MgO	2.1153	2.1153
	P_2O_5	4.2306	4.2306
	Fe_2O_3	8.4611	8.4611
	Sulfates as SO_3	38.0750	38.0750
	Cl	8.4611	8.4611
	Organic Non-Sugars	296.1390	296.1390
	Water	5153.5158	5153.5158
	CO_2	623.5010	623.5010
	Micronutrients	68.2959	68.2959
	Kanamycin	0.0000	0.0000
	E.coli Cells	0.0000	0.0000
	MEK	232.8890	232.8890
Design Data:			
	Material	Stainless Steel	
	Manufacturer	Ponndorf	
	Model	PDX 35	
	Pumping Capacity	18 gpm (9.5 gpm required)	
	Unit Cost	$1,000	
	Utilities	6,107	BTU electricity/batch
		14.28	$/yr
Comments: Operating Time = 3 hrs per cycle			

Molasses Storage Tank			
Identification:	Item:	Open Roof Storage Tank	Date 4/5/2011
	Item No.	ST-01	
	No. Req.	1	

Function: Hold 2 months supply of molasses so that production can run during the off-season that does not overlap with plant downtime

Operation: Continuous

Materials Handled:	Molasses to Pasteurizer
Stream ID:	S01
Temperature (C)	25
Flow Rate (gal/hr)	10.77

Design Data:

Material:	316 Stainless Steel	
Volume of Storage Tank	17,800	gal
	67,380	L
C_P	$ 20,681.16	

Comments: This large unit will be housed next to the pilot plant

Water/Molasses Mixing Tank				
Identification:	Item:	Cone Roof Storage Tank	Date	4/5/2011
	Item No.	MT-01		
	No. Req.	1		
Function:	Hold enough water/molasses medium to supply three fermentation batches			
Operation:	Continuous			

Materials Handled:	Molasses from Pasteurizer	Molasses to All Fermentors
Stream ID:	S02	
Temperature (C)	71.7	25.00
Flow Rate (gal/hr)	10.77	10.77

Design Data:

Material:	316 Stainless Steel		
Volume of Storage Tank	1300	gal	
	4921	L	
C_P	$ 10,264.95		

Comments:

Supernatant Storage Tank					
Identification:	Item:	Cone Roof Storage Tank	Date	4/5/2011	
	Item No.	ST-02			
	No. Req.	1			
Function:	Hold 3 batches of supernatant from the largest fermentor before purification				
Operation:	Continuous				
Materials Handled:		Supernatant from Centrifuge	Molasses to Separation Process		
Stream ID:		S14			
Temperature (C)		71.7	25.00		
Flow Rate (gal/hr)		174.09	174.09		
Composition (kg/hr)		711.42	711.42		
	WATER	650.26	650.26		
	METHA-01	0.51	0.51		
	N-BUT-01	4.02	4.02		
	Glucose	3.53	3.53		
	Fructose	0.00	0.00		
	Sucrose	0.00	0.00		
	SiO2	1.37	1.37		
	K2O	6.84	6.84		
	CaO	3.71	3.71		
	MgO	0.20	0.20		
	P2O5	0.39	0.39		
	Fe2O3	0.78	0.78		
	Sulfates as SO3	3.52	3.52		
	Cl	0.78	0.78		
	Organic Non-Sugars	27.36	27.36		
	Micronutrients	8.13	8.13		
	Kanamycin	0.02	0.02		
Design Data:					
	Material:	316 Stainless Steel			
	Volume of Storage Tank	6,500	gal		
		24,605	L		
	C_P	$ 23,325.54			
Comments: This is a very large unit so it will be housed next to the pilot plant					

Centrifuge

Identification:	Item:	Centrifuge	Date 4/5/2011
	Item No.	CF-01	
	No. Req.	1	
Function:	Remove the bacterial cells from the effluent of the largest reactor		
Operation:	Continuous		
Materials Handled:	Effluent from largest reactor (see F-04)		

Design Data:

Manufacturer	Sorvall	
Model	C40	
Maximum Flow rate	14.26	gal/hr
Speed	17000	rpm
Centripetal Force	25040	x gravity
Sediment Capacity	400 mL	
Rotor Material	Aluminum, Teflon coating	
Utilities	$ 3136.32/yr	
Cost of Centrifuge	$ 13,844.00	
Cost of Rotor	$ 20,382.00	
Total Cost	$ 34,226.00	

Comments: This model was chosen to eliminate the need for one incredibly large centrifuge or several smaller batch centrifuges. This model reduces the need for an operator because it operates continuously. This model also exceeds the required flow rate, which is 10.77 gal/hr

PART III: Continuous Processes

MAA and MEK Separations

MAA SEPARATIONS

One production goal of this pilot plant is to be able to produce 30M kg/year of methacrylic acid (MAA). As mentioned previously this process will run separately from the MEK process. In this way the equipment and design will not be responsible for separating mixture of MEK and MAA in water. As with MEK, once the initial startup time has passed the process will be run discretely.

CONCEPT STAGE

PROCESS SELECTION AND PRELIMINARY PROCESS SYNTHESIS

Distillation vs. Liquid-liquid Extraction (LLE) and Distillation

Two separations process possibilities were examined to produce the required purity of MAA desired for later polymerization reactions. The first model that was considered consisted of only distillation. This model however, was not able to achieve sufficient recovery of MAA. To increase the purity and recovery of MAA, a liquid-liquid extraction step was introduced before any distillation steps. This design was inspired by a patent which described the separation for 1,3-propanediol (PDO). The patent explicitly states several entrainers common for PDO separation. Figure X and Table Y show the results obtained from the various entrainers examined. Of the four listed, benzene, p-xylene, dodecane and n-butyl acetate, NBA was

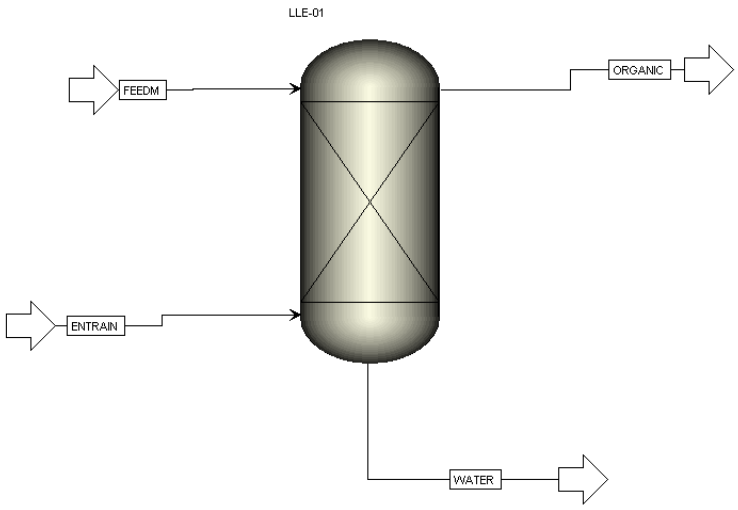


Figure 12. Liquid-liquid Extraction Setup. The setup above was used to try various entrainers in the separation of MAA from water.

able to completely strip MAA into the organic phase, with minimal water cross-over. Ternary diagram shows why for all of the entrainers offered as possibilities, n-butyl acetate (NBA) proved the most promising. The ternary diagram for the WATER-MAA-NBA mixture is depicted below.

ENTRAINER		Mass Flow (kg/hr)			
	Species	FEEDM	ENTRAIN	ORGANIC	WATER
Dodecane	Water	620.90	0.00	0.23	620.67
	MAA	33.90	0.00	18.78	15.12
	Dodecane	0.00	250.00	250.00	0.00
P-Xylene	Water	620.90	0.00	0.29	620.62
	MAA	33.90	0.00	17.53	16.37
	P-Xylene	0.00	250.00	249.81	0.19
Benzene	Water	620.90	0.00	0.56	620.34
	MAA	33.90	0.00	26.45	7.45
	Benzene	0.00	250.00	248.78	1.22
N-Butyl Acetate	Water	620.90	0.00	14.52	606.39
	MAA	33.90	0.00	33.90	0.00
	NBA	0.00	250.00	246.39	3.61

Table 3: Flow Rates of MAA and water after being treated with a organic solvent. Figure X shows the Aspen configuration that was used. N-butyl acetate is able to extract all of the MAA with only minimal water cross-over. The resulting mixture could then be distilled to produce MAA of the desired purity.

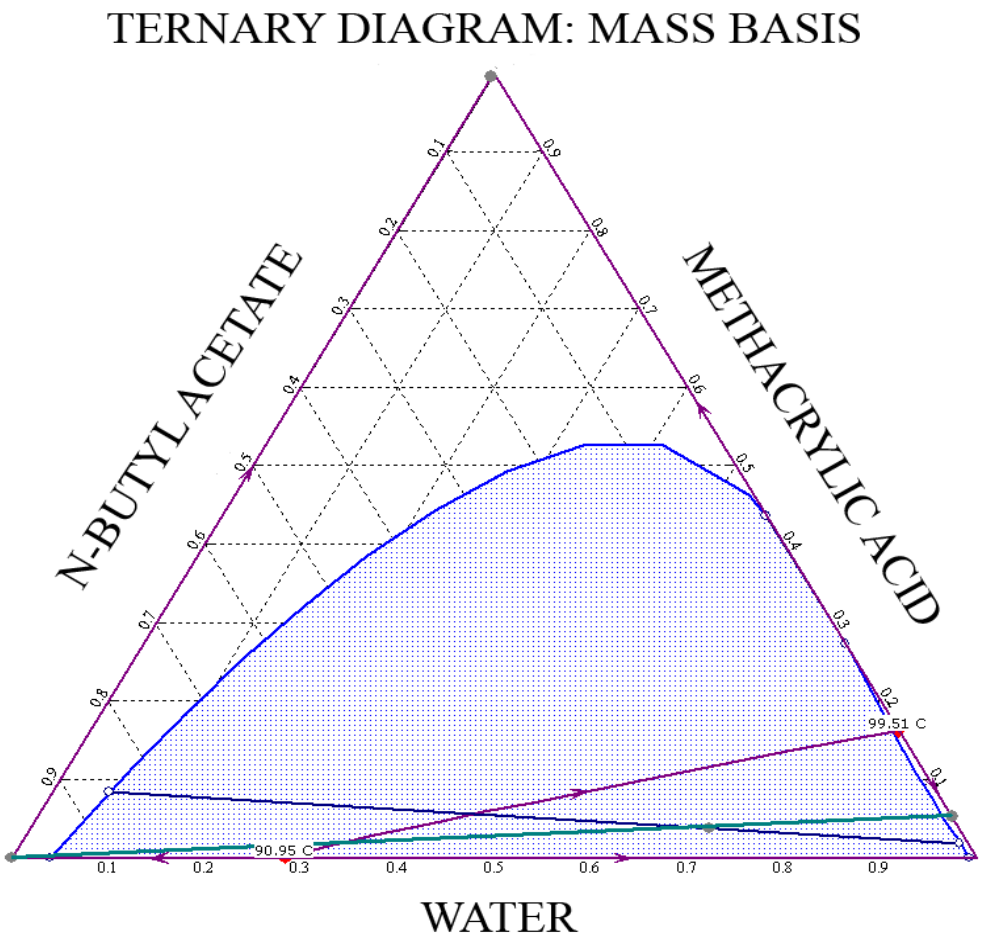


Figure 13. Ternary Phase diagram of MAA/NBA/H_2O.

The ternary diagram shows the feeds to the liquid-liquid extractor. The MAA-water feed is on the incorrect side of the azeotrope to proceed with distillation. Adding in n-butyl acetate generates an immiscible mixture, which separates along the tie line depicted in the diagram. One of the compositions coming out of the extractor is now on the side of the azeotrope where 100% MAA is achievable. This mixture is sent to the distillation column.

Membranes were not considered for this process because the desired separation was achievable using a combination of distillation and liquid-liquid extraction. Also, due to the general scalability and cost concerns associated with operating a membrane unit, especially

within the context of a full scale commercial plant, the technology was not considered as a financially viable option.

It is important to note that there are no heat exchangers in the process other than the condenser and reboiler for the distillation columns. Due to the scale of the process and the fact that all hot streams are recycled, the cost for construction and maintenance of heat exchangers was deemed unnecessary for this process. The only stream that needs cooling is the pure MAA product for reasons to be discussed in the *Safety* section of this paper. The only heat exchangers in the plant are for the MEK process requires one to maintain sufficient membrane flux.

Off-Site Experiments

Many times in the design of novel separations processes, it becomes necessary to go to an outside, off-site vendor to run a larger scale test of the pilot plant. The current drawbacks to pilot plants is that they fail to give reliable operating conditions (HETP, number of trays, tray efficiency, etc…) do to the size of the column diameters and the random packing used in those columns. It is planned that these vendors will take any of the left over product, that not given away as free samples, and run it through a column more analogous to what would be used in a commercial process, such as a tray tower. In these off-site experiments, the purified MAA will simply be remixed with water and run through the column again to obtain more information. The columns in the pilot plant in this design are large enough that this approach is probably unnecessary. However, if the column size or efficiency is drastically different from what was predicted in ASPEN, performing off-site experiments is always an option.

Inhibitor

Monomethyl-ether-hydroquinone (MEHQ), or p-methoxy-phenol, is a common inhibitor used to prevent free radical auto-polymerization that can in occur in many high purity monomer products. It will be added at the end of the process, as even small concentrations of MEHQ created problems within the ASPEN model due to irregularities in the thermodynamics of the mixture. It is worth investigating during the actual pilot runs whether the simulation problems encountered by ASPEN are legitimate or if the relevant amounts of MEHQ can be successfully carried through the process from the feed to the product stream. Whether the inhibitor is added at the beginning is also dependent on whether the residence time of the MAA in COL-01 is such that polymerization can occur in the column. If polymerization in COL-01 is not a problem, then the MEHQ can simply be added at the end as stated above. It is unlikely that polymerization would occur because it must be initiated by the presence of free radicals, which come from peroxides or radiation. It is also important to note that the other suggested inhibitor, MMA, cannot be used as its boiling point is almost identical to the boiling point of water. This means that it will distill off with water and be removed from the MAA. The final MEHQ concentration will be 250 ppm.

FEASIBILITY, DEVELOPMENT, MANUFACTURING STAGES

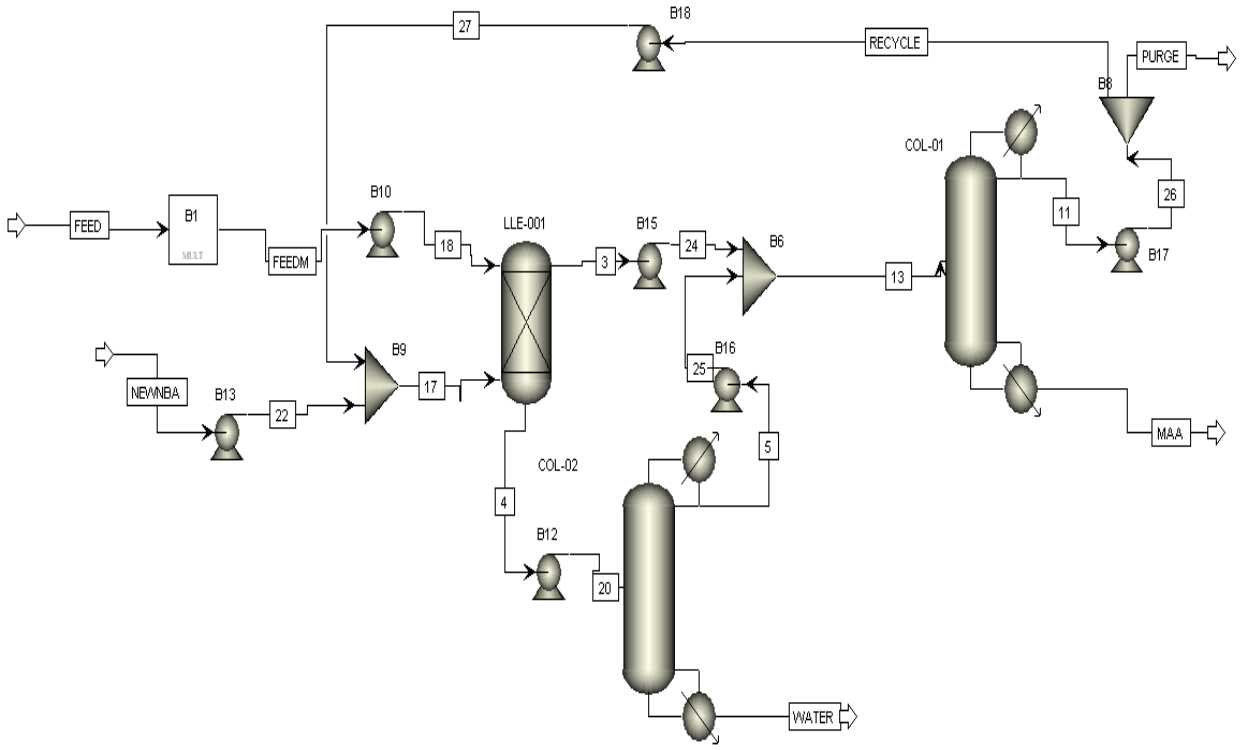


Figure 14: Process Flow Diagram for Fermentation Steps

SAFETY

MAA Handling Considerations

Methacrylic acid carries the most safety concerns out of all the chemicals to be used in this pilot plant. MAA has a vapor pressure of 0.67mmHg in air at room temperature. This is rather low when compared to more volatile substances such as ethanol which has a vapor pressure of 59 mmHg at room temperature. MAA is flammable in air in the range of 1.6-8.3% MAA in air, which is below the vapor pressure at room temperature, so auto-ignition is not of great concern. The main problem with MAA is that it can auto-polymerize, especially during long periods of storage without sufficient ventilation or mixing. To avoid

MAA is generally product MAA is mixed with a polymerization inhibitor, either methyl methacrylate MMA or MEHQ. MAA should never be stored in an oxygen free atmosphere as dissolved O_2 is needed for MEHQ to work effectively (5-21% O_2 atmosphere is desired). According to various MSDSs, the optimal MEHQ concentration is 250 ppm. MAA is also photochemically active, in that sunlight will cause the auto-polymerization to occur. The above properties are a strong function of temperature, so it is advised that MAA be stored at 18˚C, slightly above its freezing point, 15˚C. Storage below MAA's freezing is not advised as the freezing/thawing cycle will create areas of low inhibitor concentration (and thus lead to runaway polymerization) if the container is not well mixed. For these reasons it is recommended that the pure MAA product be kept in a dark, cool, and well-ventilated place. The heat of this reaction is extremely exothermic (786 kJ/kg), so barrels containing MAA will explode if not properly treated and stored. Finally, metal ions formed from the corrosion of pipes can trigger polymerization. In order to avoid significant corrosion, it is advised that stainless steel be used over carbon steel. For storage, it has been advised that MAA be kept in polyethylene drums.

Since some MAA will be discharged in the waste water stream, it is important to adhere to current environmental standards. However, it likely that no further treatment will be necessary, as ASPEN predicts an MAA concentration of ~3 ppb, which is well below current environmental standards. In the case of higher contamination of the waste water stream, money will have to be set aside for waste removal and treatment by some third party.

N-Butyl Acetate Handling Concerns

During the MAA separation process, n-butyl acetate (NBA) will be used as an entrainer for liquid-liquid extraction. As such, some of the NBA will need to be purged and replaced

with fresh entrainer to avoid accumulation of contaiminants in the system. NBA is not nearly as reactive as MAA, but does exhibit more handling hazards to the environment and workers. NBA is much more volatile than MAA with a vapor pressure of 11.7 mmHg at room temperature, so any containers containing NBA should be tightly sealed. Exposure to NBA at levels 200 ppm STEL (toxicity limit) will cause eye and respiratory tract irritation, drowsiness, and dizziness. OSHA levels for exposure to NBA are 150 ppm TWA. The explosion limits are 1.3-7.6 NBA in air, but NBA is still very flammable. Provided that no external impurities are present, there is no concern for NBA to react violently. For these reasons it is advised that NBA be kept in a cool, well-ventilated area. It is currently unknown if special containers are needed for NBA storage as with MAA due to corrosion concerns. However, as corrosion was primarily a concern for MAA as the metal could catalyze auto-polymerization, it is believe that stainless steel vessels and drums will be sufficient.

However, if any is purged as waste, NBA will decompose to carbon monoxide, carbon dioxide, and water after a period of 1-10 days in an open environment. If the waste is in the vapor form, NBA will photochemically decompose in the atmosphere in 6 days. If pure NBA is to be purge as waste, lower limit of environmental toxicity is 18g/L, so any stream should meet this standard to avoid any significant environmental concerns. These should not be problems for this plant because the NBA will be returned to the sugar refinery and combusted in a furnace.

Monomethyl-Ether-Hydroquinone

Monomethyl ether hydroquinone will be used as an inhibitor in the MAA and MEK to prevent auto-polymerization. Unlike the other chemicals used in this process, it is only

volatile at elevated temperatures, having a vapor pressure of essentially zero at room temperature. Like NBA, MEHQ carries more health than reactivity concerns, but it is being handled in such small amounts that it will be easy to handle safely.

FLOWSHEET DIAGRAM AND MASS BALANCE

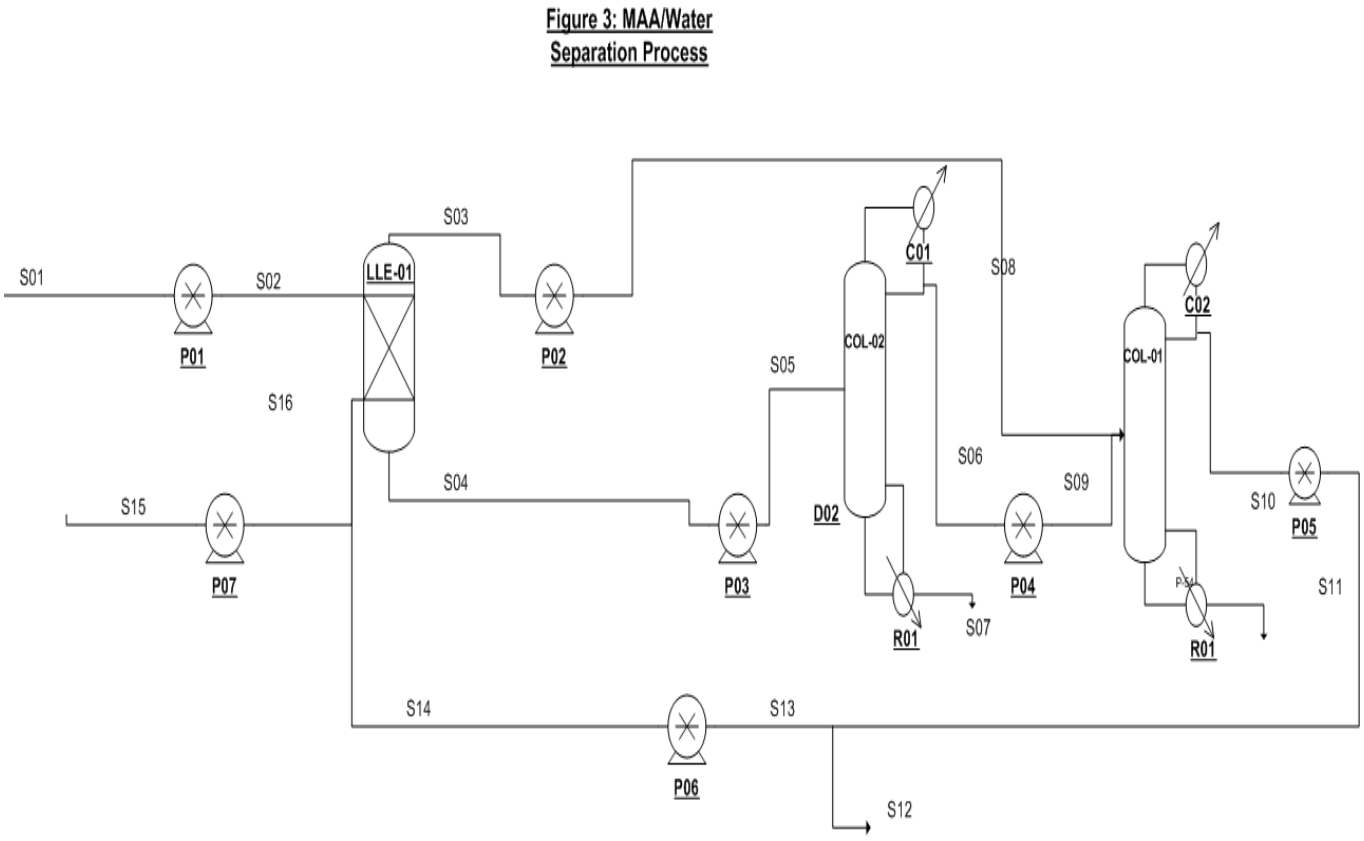


Figure 15: Process Flow Diagram for Fermentation Steps

Figure 16: ASPEN Mass Balance for Process Flow Diagram in Figure 15.

		Inlets		Recycles	Outlets		
STREAM ID		FEEDM	NEWNBA	RECYCLE	WATER	PURGE	MAA
FROM :		B1	----	SPLIT01	COL-02	SPLIT01	COL-01
TO :		PUMP02	PUMP01	PUMP03	----	----	B3
SUBSTREAM:							
PHASE:		LIQUID	LIQUID	LIQUID	LIQUID	LIQUID	LIQUID
COMPONENTS:	KMOL/HR						
WATER		34.4654	0.000	2.365	34.387	7.8190-02	1.4847-09
METHA-01		0.3938	0.000	7.1157-03	3.4378-03	2.3523-04	0.3901
N-BUT-01		0	6.8870-02	2.035	7.9459-15	6.7256-02	1.4499-03
P-MET-01		0	0.000	0.000	0.000	0.000	0
METHY-01		0	0.000	0.000	0.000	0.000	0
COMPONENTS:	MOLE FRAC						
WATER		0.9887	0.000	0.537	1.000	0.537	3.7919-09
METHA-01		1.1296-02	0.000	1.6147-03	9.9962-05	1.6147-03	0.9963
N-BUT-01		0	1.000	0.462	2.3105-16	0.462	3.7031-03
P-MET-01		0	0.000	0.000	0.000	0.000	0
METHY-01		0	0.000	0.000	0.000	0.000	0
COMPONENTS:	KG/HR						
WATER		620.9045	0.000	42.611	619.494	1.409	2.6748-08
METHA-01		33.899	0.000	0.613	0.296	2.0251-02	33.5833
N-BUT-01		0	8.000	236.328	9.2300-13	7.813	0.1684
P-MET-01		0	0.000	0.000	0.000	0.000	0
METHY-01		0	0.000	0.000	0.000	0.000	0
COMPONENTS:	MASS FRAC						
WATER		0.9482	0.000	0.152	1.000	0.152	7.9248-10
METHA-01		5.1770-02	0.000	2.1914-03	4.7751-04	2.1914-03	0.995
N-BUT-01		0	1.000	0.845	1.4892-15	0.845	4.9900-03
P-MET-01		0	0.000	0.000	0.000	0.000	0
METHY-01		0	0.000	0.000	0.000	0.000	0

TOTAL FLOW:						
KMOL/HR	34.8592	6.8870-02	4.407	34.391	0.146	0.3915
KG/HR	654.8035	8.000	279.552	619.790	9.241	33.7518
L/MIN	10.9782	0.152	5.523	11.357	0.183	0.6567
STATE VARIABLES						
TEMP K	295.9278	295.928	361.609	381.292	361.609	444.8262
PRES ATM	1.1568	1.157	1.157	1.327	1.157	1.3575
VFRAC	0	0.000	0.000	0.000	0.000	0
LFRAC	1	1.000	1.000	1.000	1.000	1
SFRAC	0	0.000	0.000	0.000	0.000	0
ENTHALPY:						
CAL/MOL	-2.861	3.734	-5.270	-2.683	-5.270	-5.2621
CAL/GM	-3652.51	-1089.710	-1461.332	-3708.151	-1461.332	-1074.46
CAL/SEC	-1.6435	-2421.577	3.865	-1.384	-3751.311	2.9926
ENTROPY:						
CAL/MOL-K	-39.4488	-165.763	-89.926	-34.683	-89.926	-73.4371
CAL/GM-K	-2.1001	-1.427	-1.418	-1.925	-1.418	-0.8519
DENSITY:						
MOL/CC	5.2922-02	7.5598-03	1.3298-02	5.0470-02	1.3298-02	9.9378-03
GM/CC	0.9941	0.878	0.844	0.910	0.844	0.8567
AVG MW	18.7842	116.160	63.435	18.022	63.435	86.2018

EQUIPMENT LIST: UNIT DESCRIPTIONS AND COSTS

Liquid-Liquid Extraction Tower (LLE-001)

The column will be a liquid-liquid extraction tower that will contact the feed MAA and water mixture with fresh NBA and recycle (which is approximately 85% NBA with a balance of water). The NBA and MAA leave through the top as one phase and the water exits through the bottom as another. All remaining salts and sugars that were in the molasses mixture are assumed to remain in the aqueous phase and come out the bottom of the LLE column. The column consists of theoretical 8 stages and has a pressure drop of 7 psi from the bottom to the top. Due to the small scale of our plant, Professor Fabiano suggested using a Karr column. The Karr column allows for the same separation to occur within an even smaller tower. The principle idea behind the column's design is that there is a piston running through the column attached to which are perforated trays. As the pistons move, they agitate the solution providing better separation through enhanced contact and agitation between the two liquid phases. Koch Modular Process Systems, LLC (KMPS) suggested using a Scheibel column for this extraction step. Based on our flow rates and theoretical stages, KMPS specified and priced a 14-inch diameter, 50-agitated stage Scheibel column constructed from 316 stainless steel.

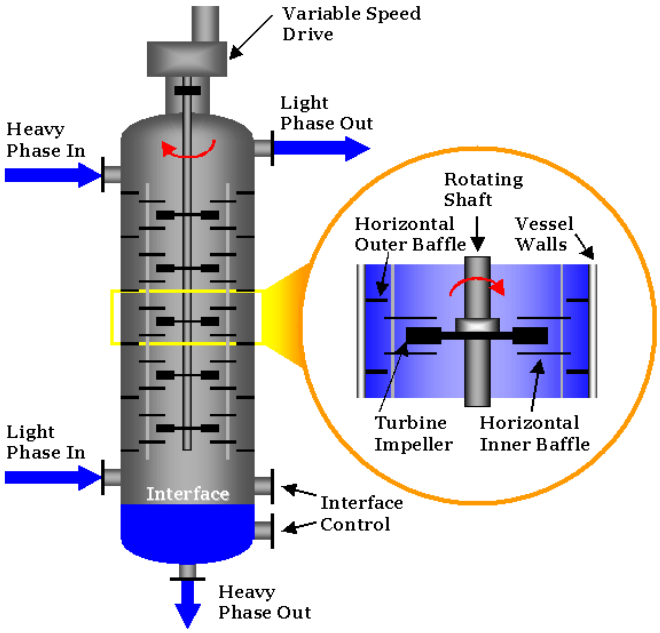


Figure 17. Schematic diagram of Scheibel Column. The Scheibel column has a central rotor attached to which are baffles. A motor turns the central shaft creating agitation with the impeller allowing for better mixing in a smaller column.

This column is similar to a rotating disc contactor but it has baffles that direct flow and increase efficiency.

Water Purification Column (COL-02)

This column will distill the water coming off of the bottom of the LL extractor. The distillate will be fed to another column that will have pure MAA as a product. The bottoms will contain most of the H_2O in the feed with a little MAA and trace NBA. This design layout allows for all of the salts to come out the bottom in an almost pure water stream (no salt or residual sugar is assumed to be carried in the vapor). The bottoms will simply go to waste, as the MAA limit is below EPA standards. NBA is determined to be trace by ASPEN (< 1 PPB) and the salts came from organic matter to begin with. Depending on how this waste is treated, this column may not be necessary. If all waste water from the pilot plant is going to be sent off-site for waste disposal, this column is unnecessary, as negligible amounts of MAA are lost and the fresh NBA feed can simply be increased. But if the water is to be dumped to the environment it must be treated on site to meet rigorous EPA COD and BOD standards.

Figure 18. Detailed Overview of Process Units in MAA Separation.

MAA Separation Equipment								
Unit Name	**Unit No.**	**Function**	**Size/ Capacity**	**Construction Material**	**Estimated Cost**	**Source of Cost Estimate**	**Operating Temp.**	**Operating Pressure**
Scheibel column	LLE-01	Separate MAA from water using NBA entrainer	50 agitated stages HETP = 2 ft Theoretical Stages=8	-	$ 350,000.00	KMPS LLC	Adiabatic	-
Water Purification Column	COL-02	Remove organic impurities from water before dumping	Diameter=12 in HETP =2 ft Theoretical stages = 12 3.5-in Flexiring Packing	Stainless Steel	$ 38,532.58	Correlations	Reboiler-108 °C Condenser-95 °C	17-20 psia
MAA Production Column	COL-01	Produce the 99.5 wt% MAA product stream	Diameter=12 in HETP =2 ft Theoretical stages = 15 3.5-in Flexiring Packing	Stainless Steel	$ 19,441.38	Correlations	Reboiler-172 °C Condenser-89 °C	17-20 psia
Pump 1	P-01	Pump in feed	10.978 L/min	PVC	$ 2,500.00	Watson-Marlow Pumps Group	Isothermal	Max 25 psia
Pump 2	P-02	Pump LLE top stream to COL-01	5.257 L/min	PVC	$ 2,500.00	Watson-Marlow Pumps Group	Isothermal	Max 25 psia
Pump 3	P-03	Pump LLE bottom stream to COL-02	11.168 L/min	PVC	$ 2,500.00	Watson-Marlow Pumps Group	Isothermal	Max 25 psia
Pump 4	P-04	Pump COL-02 Distillate to COL-01	0.0642 L/min	PVC	$ 2,500.00	Watson-Marlow Pumps Group	Isothermal	Max 25 psia
Pump 5	P-05	Pump NBA recycle back to LLE-01	5.706 L/min	PVC	$ 2,500.00	Watson-Marlow Pumps Group	Isothermal	Max 25 psia
Pump 6	P-06	Pump in fresh solvent	5.524 L/min	PVC	$ 2,500.00	Watson-Marlow Pumps Group	Isothermal	Max 25 psia
Pump 7	P-07	Pump in fresh solvent	0.152 L/min	PVC	$ 2,500.00	Watson-Marlow Pumps Group	Isothermal	Max 25 psia
Chiller 1	CH-01	Chill MAA product stream for storage	Area = 1.483 ft^2	Stainless Steel	$ 3,379.11	Correlations	17 °C	17 psia
Reboiler 1	RB-01	Vaporize Liquid in the bottom of COL-01	Area = 48.181 ft^2	Stainless Steel-Stanless Steel	$ 7,077.02	Correlations	Inlet-94.0 °C Outlet-123.8 °C	20 psia
Reboiler 2	RB-02	Vaporize Liquid in the bottom of COL-02	Area = 32.149 ft^2	Stainless Steel-Stanless Steel	$ 6,633.39	Correlations	Inlet-123.8 °C Outlet-89 °C	20 psia
Condneser 1	CON-01	Condense Vapor in Overhead of COL-01	Area = 33.449 ft^2	Stainless Steel-Stanless Steel	$ 6,675.61	Correlations	Inlet-123.8 °C Outlet-94.0 °C	17 psia
Condneser 2	CON-02	Condense Vapor in Overhead of COL-02	Area = 13.806 ft^2	Stainless Steel-Stanless Steel	$ 5,794.32	Correlations	Inlet-99.4 °C Outlet-95.0 °C	17 psia
Reflux accumulator 1	RA-01	Collect Condensate from CON-01	Diameter = 1.97 ft Length = 3.95 ft	Stainless Steel	$ 460.84	Correlations	94 °C	17 psia
Reflux accumulator 2	RA-02	Collect Condensate from CON-02	Diameter = 0.98 ft Length = 1.97 ft	Stainless Steel	$ 102.25	Correlations	95 °C	17 psia
Total					**$ 455,596.49**			

PROCESS UNIT DESCRIPTIONS

LLE-01

The liquid-liquid extraction tower will take the fermentation feed (**S01**) and contact it with a mixed recycle and fresh NBA stream (**S16**). The tower consists of 8 stages with an estimated HETP of two feet. The tower contains 3.5 inch Flexiring random packing. The tower operates with a pressure gradient of 25 psia at the bottom and 17 psia at the top. The organic top stream (**S03)** is 9.8wt% MAA and 4.0wt% H_2O with a balance of NBA solvent. The stream recovers 99.9 wt% of the MAA in **S01** and is sent to COL-01. The bottoms stream (**S04**) is 99.4wt% H_2O with a balance of NBA and trace MAA and is sent to COL-02. The column also operates adiabatically.

Note: In Aspen, the reboiler and condenser are counted as stages. The condenser, being at the top of the column, is always stage 1 and the reboiler is always the last stage.

COL-01

The distillation tower takes stream **S03** (top stream form **LLE-01**) mixed with stream **S05** (distillate from **COL-02**) as a feed. The tower has 15 stages with the inlet being fed onto stage 9. The tower is filled with 3.5 inch Flexiring random packing with an assumed HETP of 2 ft. The tower operates with a 0.15 psia drop per theoretical stage of packing, a 1 psia drop for the condenser, and condenser operating pressure of 17 psia. The column contains 15 stages with inlet being fed onto stage 9. The bottoms, S17 is the product of the process and it contains 99.5 wt% MAA with the balance being NBA. This stream recovers better than 99.8% fed to the separations process. The distillate, S10, is 94.8 wt% NBA with

the balance being water and trace MAA. . The reboiler operates at 114°C requiring XX Btu/hr of heating. The condenser operates at 93°C requiring XX BTU/hr of cooling.

COL-02

The distillation column takes stream, **S04**, as a feed to purify the wastewater. The tower has 12 stages with the inlet being fed onto stage 6. The tower is filled with 3.5 inch Flexiring random packing with an assumed HETP of 2 ft. The tower operates with a 0.15 psia drop per theoretical stage of packing, a 1 psia drop for the condenser, and condenser operating pressure of 17 psia. The bottoms, **S07**, is 99.99 wt% water with a balance of MAA and trace NBA. The stream also carries any salt impurities not filtered out prior to separation. The distillate, **S05**, has a composition of 41.6 wt% water, 58.3 wt% NBA with MAA as the balance. The reboiler operates at 108°C requiring XX Btu/hr of heating. The condenser operates at 95°C requiring XX BTU/hr of cooling.

Effect of Inhibitor on MAA Purity

At the end of the process, MEHQ is added as an inhibitor to concentration of 250 ppm. Adding this will subsequently lower the purity of the final product, so the bottoms of composition of COL-01 must be slightly greater to allow for the addition of MEHQ. The Design Spec in ASPEN was set to 0.99501 to account for this. The table below shows how this new specification meets the purity requirements even after the MEHQ is added.

Inhibitor Effect of Mass Purity					
Molar	kmol/hr		Mass	kg/hr	
MEHQ	0.000097925		MEHQ	1.21564E-05	
METHA-01	0.3900937		METHA-01	33.5833382	
N-BUT-01	0.0014499		N-BUT-01	0.16842137	
total Moles	0.391641525		Total Mass	33.75177173	
Mole Fraction			Mass Fraction		
MEHQ	0.000250037	>250 ppm	MEHQ	0.0000003602	<40 ppb
METHA-01	0.996047853		METHA-01	0.995009639	>99.5 wt%
N-BUT-01	0.00370211		N-BUT-01	0.004990001	
Total MEHQ Needed			0.037	kg/year	

Table 4. Result of Adding MEHQ to the MAA Product Stream. The table shows how the addition of the inhibitor does not cause the purity of MAA to fall below industry standards. The table also shows the total consumption of MEHQ a year.

Condenser (MAA)					
Identification	Item: Double Pipe Heat Exchanger Item No. CON-01 No. Req. 1				Date
Function: Condense the vapor effluent of distillation column D-01					
Operation: Continuous					
Materials Handled:		Hot Side		Cooling Water	
		Inlet	Outlet	Inlet	Outlet
Stream ID:					
Temperature (C)		109.01	90.46	32.22	48.89
Temperature (F)		228.218	194.828	90	120
Quantity (kg/hr):		598.0791	598.0791	8097.21	8097.21
Composition (kg/hr):					
	WATER	31.3094	31.3094	8097.21	8097.21
	METHA-01	0.0703	0.0703	0	0
	N-BUT-01	566.6994	566.6994	0	0

Design Data:

Heat Duty	534,416	BTU/hr
Flow rate of cooling water	2138.52	gal/hr
Log Mean Temp. Difference	106.514009	degrees F
Heat Transfer Coefficient	150	BTU/(F-ft^2-hr)
Area	33.4488708	ft^2
Materials		
Shell	Stainless Steel	
Tube	Stainless Steel	
Unit Cost, C_p	$ 6,675.61	per unit
Utilities	$ 176.43	per year

Comments:

Condenser (MAA)

Identification		Date
	Item: Double Pipe Heat Exchanger Item No. CON-02 No. Req. 1	3/28/2011

Function: Condense the vapor effluent of distillation column D-02

Operation: Continuous

Materials Handled:	Hot Side		Cooling Water	
	Inlet	Outlet	Inlet	Outlet
Stream ID:				
Temperature (C)	105.19	95.07	32.22	48.89
Temperature (F)	221.342	203.126	90	120
Quantity (kg/hr):	113.75	113.75	3361.42	3361.42
Composition (kg/hr):				
WATER	99.99087	99.99086955	3361.42	3361.42
METHA-01	0.70247	0.702469788	0	0
N-BUT-01	13.05666	13.05666079	0	0

Design Data:

Heat Duty	221,854	BTU/hr
Flow rate of cooling water	887.77	gal/hrr
Log Mean Temp. Difference	107.1260005	degrees F
Heat Transfer Coefficient	150	BTU/(F-ft^2-hr)
Area	13.80642104	ft^2
Materials		
Shell	Stainless Steel	
Tube	Stainless Steel	
Unit Cost, C_p	$ 5,794.32	per unit
Utilities	$ 73.24	per year

Comments:

Reboiler (MAA)				
Identification	Item: Double Pipe Heat Exchanger Item No. RB-01 No. Req. 1			Date
Function: To revaporize the liquid collected from the bottom of the distillation column D-01				
Operation: Continuous				
Materials Handled:	Cool Side		Steam	
	Inlet	Outlet	Inlet	Outlet
Stream ID:				
Temperature (C)	171.68	171.68	196.67	196.67
Temperature (F)	341.024	341.024	386.006	386.006
Quantity (kg/hr):	965.3681	965.368054	850.15	850.15
Composition (kg/hr):				
WATER	0	0	850.15	850.15
METHA-01	960.5277	960.527722	0	0
N-BUT-01	4.840332	4.840332	0	0

Design Data:		
Molar Boilup Ratio	43.73	
Heat Duty	578177	BTU/hr
Flow rate of steam	482.22	lb/hr
Grade of Steam	450	psi
Heat Flux	12,000	BTU/(ft^2-hr)
Area	48.1814167	ft^2
Materials		
Shell	Stainless Steel	
Tube	Stainless Steel	
Unit Cost, C_p	$ 7,077.02	per unit
Utilities	$ 3,209.17	per year

Comments:

Reboiler (MAA)					
Identification	Item:	Double Pipe Heat Exchanger		Date	3/28/2011
	Item No.	RB-02			
	No. Req.	1			
Function: To revaporize the liquid collected from the bottom of the distillation column D-01					
Operation: Continuous					

Materials Handled:	Cool Side		Steam	
	Inlet	Outlet	Inlet	Outlet
Stream ID:				
Temperature (C)	108.15	108.15	133.14	133.14
Temperature (F)	226.67	226.67	271.652	271.652
Quantity (kg/hr):	712.4829	712.48286	434.6769	434.6769
Composition (kg/hr):				
WATER	712.4153	712.41534	434.6769	434.6769
METHA-01	0.067524	0.067524	0	0
N-BUT-01	0	0	0	0

Design Data:

Molar Biolup Ratio	0.292	
Heat Duty	385782	BTU/hr
Flow rate of steam	621.35	lb/hr
Grade of Steam	50	psi
Heat Transfer Coefficient	12000	BTU/(ft^2-hr)
Area	32.1485	ft^2
Materials		
Shell	Stainless Steel	
Tube	Stainless Steel	
Unit Cost, C_p	$6,633.39	per unit
Utilities	$1,879.58	per year

Comments:

Distillation Column (MAA)				
Identification	Item:	RADFRAC Distillation Column	Date	3/28/2011
	Item No.	D-01		
	No. Req.	1		
Function:	To separate the MAA from residual solvents and achieve 99.5% purity			
Operation:	Continuous			

Materials Handled:		Inlet	Tops	Bottoms
Stream ID:				
Temperature (C)		35	88.5	171.7
Quantity (kg/hr):		322.545	288.793	33.752
Composition (kg/hr):				
	WATER	44.019	44.019	0
	METHA-01	34.216	0.633	33.583
	N-BUT-01	244.309	244.141	0.168

Design Data:

Material:	316 Stainless Steel	
Theoretical Trays	13	
Packing Type	FLEXIRING (plastic)	
HETP	2	ft
Functional Height	26	ft
Inner Diameter	20	in
Pressure	17	psi
Pressure drop/stage	0.15	psi
Feed Stage	9	(on stage)
Wall Thickness	0.25	in
Weight (lb)	1479.248353	lb
Cost of Pressure Vessel	$ 35,356.08	
Cost of Packing	$ 3,176.50	
Total Production Cost	$ 38,532.58	
Condenser	See specification sheet	
Reboiler	See specification sheet	

Comments:

Distillation Column (MAA)			
Identification	Item:	RADFRAC Distillation Column	Date ########
	Item No.	D-02	
	No. Req.	1	

Function: To recover MAA and n-butyl acetate from the aqueous phace of the LLE

Operation: Continuous

Materials Handled:				
		Inlet	Tops	Bottoms
Stream ID:				
Temperature (C)		41.4	95.1	108.1
Quantity (kg/hr):		654.79	35	619.79
Composition (kg/hr):				
	WATER	650.261	30.766	619.494
	METHA-01	0.512	0.216	0.296
	N-BUT-01	4.017	4.017	0

Design Data:

Material:	316 Stainless Steel	
Theoretical Trays	10	
Packing Type	FLEXIRING (plastic)	
HETP	2	ft
Functional Height	20	ft
Inner Diameter	12	in
Pressure	17	psi
Pressure drop/stage	0.15	psi
Feed Stage	6	(on stage)
Wall Thickness	0.25	in
Weight (lb)	680.96	lb
Cost of Pressure Vessel	$ 23,411.34	
Cost of Packing	$ 879.65	
Total Production Cost	$ 24,290.98	
Condenser	See specification sheet	
Reboiler	See specification sheet	

Comments: This column is shared between both the MEK and MAA processes. A separate specification sheet is included for when the column is running MEK, but the price is only listed once

Liquid-Liquid Extractor (MAA)

Identification	Item:	EXTRACT liquid-liquid contactor	Date	3/28/2011
	Item No.	L-01		
	No. Req.	1		

Function: To break the MAA-water azeotrope by contacting with a third solvent

Operation: Continuous

Materials Handled:

		Feed	Solvent	Tops	Bottoms
Stream ID:					
Temperature (C)		22.8	86.9	22.9	41.4
Quantity (kg/hr):		654.804	287.552	287.565	654.79
Flow Rate (cum/hr)		0.659	0.34	0.315	0.67
Composition (kg/hr):					
	WATER	620.904	42.611	13.255	650.261
	METHA-01	33.899	0.613	34	0.512
	N-BUT-01	0	244.328	240.311	4.017

Design Data:

Material:	316 Stainless Steel	
Theoretical Stages	8	
Agitation Stages	50	
HETP	2 ft	
Functional Height	16 ft	
Inner Diameter	14 in	
Feed Stage	1	
Solvent Stage	8	
Feed Flow Rate	23.272585	cuft/hr
Solvent Flow Rate	12.0071	cuft/hr
Cost*	$ 350,000	

Comments: Cost is a price estimate given to us by Koch Modular Process Systems LLC

Reflux Accumulator (MAA)			
Identification	Item:	Reflux Accumulator	Date ########
	Item No.	RA-01	
	No. Req.	1	

Function: Hold 10 min of reflux for distillation column D-01

Operation: Continuous

Materials Handled:		From Stage 2	Distillate
Stream ID:			
Temperature (C)		88.50	88.50
Quantity (kg/hr):		866.38	288.79
Volume (cum/hr)		1.03	0.34
Volume (cuft/min)		0.60	0.20
Composition (kg/hr):			
	WATER	132.06	44.02
	METHA-01	1.90	0.63
	N-BUT-01	732.42	244.14

Design Data:

Material:	316 Stainless Steel	
Reflux Ratio	2	
Condensate Flow Rate	0.603887	cuft/min
Volume	12.07773	cuft
Volume	90.3535	gal
Diameter	1.97	ft
Length	3.95	ft
Thickness	0.25	in
Weight	353.51	lb
Cost	$ 460.84	

Comments: The reflux accumulator will hold ten minutes worth of condensate when half full.

Reflux Accumulator (MAA)				
Identification	Item:	Reflux Accumulator	Date	########
	Item No.	RA-02		
	No. Req.	1		
Function:	Hold 10 min of reflux for distillation column D-02			
Operation:	Continuous			

Materials Handled:		From Stage 2	Distillate
Stream ID:			
Temperature (C)		95.10	95.10
Quantity (kg/hr):		113.75	35.00
Volume (cum/hr)		0.13	0.04
Volume (cuft/min)		0.07	0.02
Composition (kg/hr):			
	WATER	99.99	30.77
	METHA-01	0.70	0.22
	N-BUT-01	13.06	4.02

Design Data:

Material:	316 Stainless Steel	
Reflux Ratio	2.25	
Condensate Flow Rate	0.074603	cuft/min
Volume	1.492059	cuft
Volume	11.16209	gal
Diameter	0.98	ft
Length	1.97	ft
Thickness	0.25	in
Weight	88.61	lb
Cost	$ 102.25	

Comments: This reflux accumulator is small enough that a stainless steel drum
Could be used to hold the condensate

MEK SEPARATIONS

The second production goal is to create 30 M kg/year of MEK. This process will run during the second half of the year once MAA production has been completed. One unit in the MAA process is shared with the MEK process to reduce plant costs.

CONCEPT STAGE

PRELIMINARY PROCESS SYNTHESIS

Methyl ethyl ketone is not normally separated from water in industrial processes, so many options were explored for the design of this pilot plant. MEK is normally produced from the oxidation of 2-butanol in the gas phase on solid metal catalysts. Any unreacted 2-butanol is recycled to extinction so there are very few separation issues. The main problem encountered when separating MEK from water is the azeotrope that forms at 87 mass % MEK. A variety of solutions exist to break this azeotrope including azeotropic distillation, pressure swing distillation, and pervaporation. The method that was settled upon for this pilot plant was distillation up to the azeotropic composition followed by pervaporation to the required purity.

Azeotropic Distillation via Entrainers

As table 5 shows several other common entrainers for liquid-liquid extraction were modeled using ASPEN before settling on pervaporation. The process model used was identical to the one used for MAA but the flows were adjusted to account for differences in

the fermentation feed. Despite showing promise, each of the entrainers proved to make separation more difficult and were not considered to be as effective as pervaporation.

ENTRAINER		Mass Flow (kg/hr)			
	Species	FEEDM	ENTRAIN	ORGANIC	WATER
Dodecane	Water	475.08	0.00	0.26	474.82
	MEK	21.51	0.00	21.51	0.01
	Dodecane	0.00	250.00	250.00	0.00
P-Xylene	Water	475.08	0.00	0.30	474.78
	MEK	21.51	0.00	21.51	0.00
	P-Xylene	0.00	250.00	249.89	0.11
Benzene	Water	475.08	0.00	0.47	474.61
	MEK	21.51	0.00	21.51	0.00
	Benzene	0.00	250.00	249.11	0.89
N-Butyl Acetate	Water	475.08	0.00	8.21	466.87
	MEK	21.51	0.00	21.51	0.00
	NBA	0.00	250.00	247.21	2.79

Table 5. Aspen Results for MEK/Water Separation. Of the four entrainers used dodecane and p-xylene were equally effective on removing the MEK from the water, while allowing minimal water cross-over.

The first entrainer tested for the extraction step was n-butyl acetate (NBA) because it was also used in the MAA separation process. NBA was able to sufficiently recover the MEK in the organic solutions, but also managed to carry a lot of water into the NBA phase. The amount of water that ends up in the top product of the LLE column presented a problem due to the azeotrope with MEK and H_2O as discussed earlier. The second entrainer that was tested was benzene. One patent (Harney, 1948) suggests using a benzene entrainer to strip the MEK from the water before distillation. This method is not a viable separation option because benzene forms an additional azeotrope with MEK. The minimum-boiling azeotrope is hard to break as their boiling points differ by only 1˚C, which meant that their volatilities are not significantly different for separation by distillation to be effective. This resulted in a significant amount of benzene contaminant in our final MEK

product. It is very easy to see from the ternary diagram that in order to get into the high purity MEK corner of the diagram, only small amounts of benzene can be added. This results in significant amount of water being sent into the subsequent distillation column.

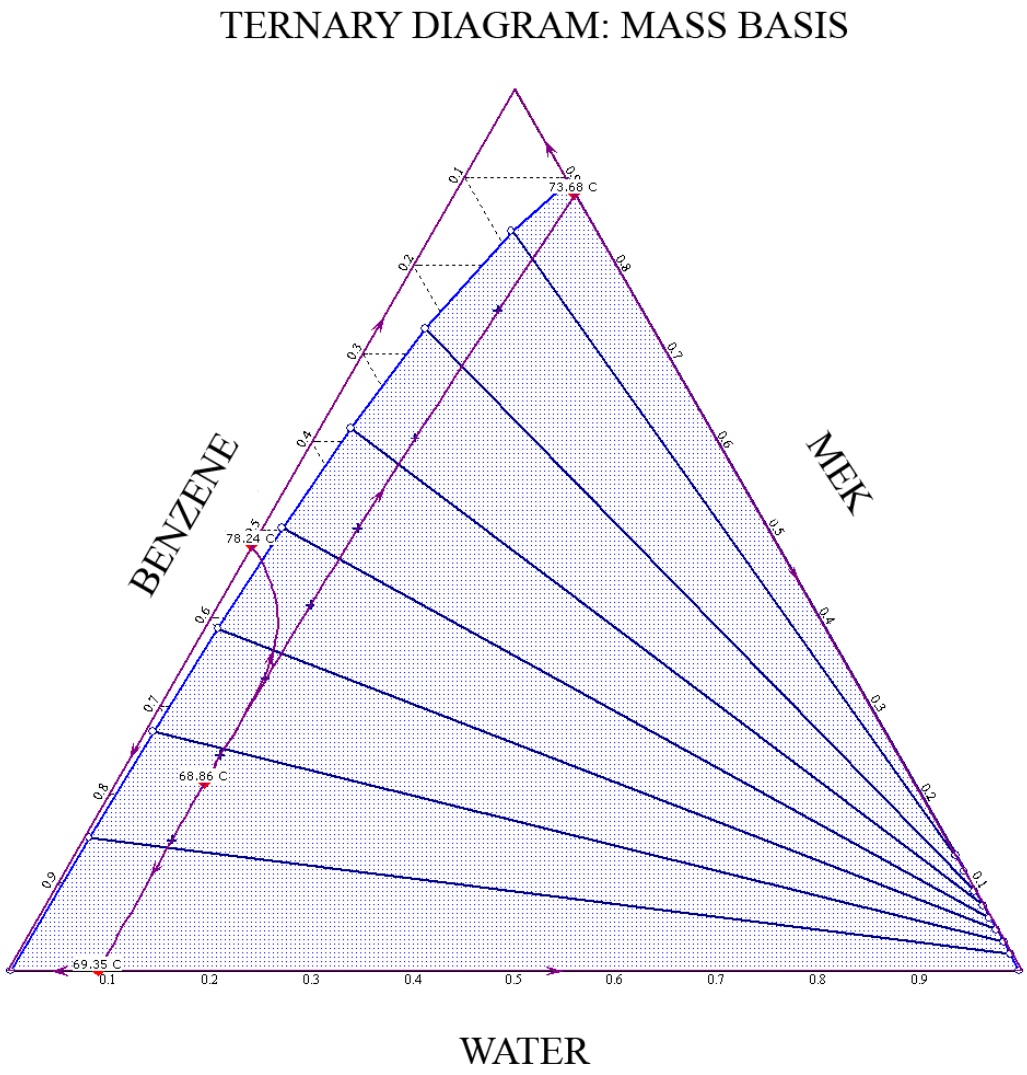


Figure 19. Ternary Phase Diagram of MEK/Dodecane/H_2O.

The final two entrainers examined were p-xylene and dodecane. P-xylene was investigated because it is similar to benzene, but has a higher boiling point than benzene so should be much easier to separate from MEK. Both of these components managed to

extract almost all of the MEK into the organic solvent and did not form an azeotrope with MEK. However, it still proved impossible to get the desired purity of MEK if further treated with traditional distillation. This is due to the solubility of water in MEK; the polarity of MEK seems to interact positively with water. Since both solvents are extremely hydrophobic further distillation would yield a distillate of MEK and water with trace solvent and a bottoms product of almost pure solvent. This distillate requires further distillation to meet purity standards, where the azeotrope again appears. The azeotrope is such that the recovery does not meet the 99% recovery required for a commercial scale plant. Therefore, all azeotropic distillation via entrainers was ruled out.

Pressure Swing Distillation

Pressure swing distillation was another option explored for separation of the water-MEK mixture. The azeotropic composition of this water-MEK mixture is very sensitive to pressure. The azeotrope occurs at 87% mass MEK at atmospheric pressure. When the pressure is increased to 10 atmospheres, the azeotrope falls to 80% mass of MEK. The shift in the azeotrope is depicted in the figure below.

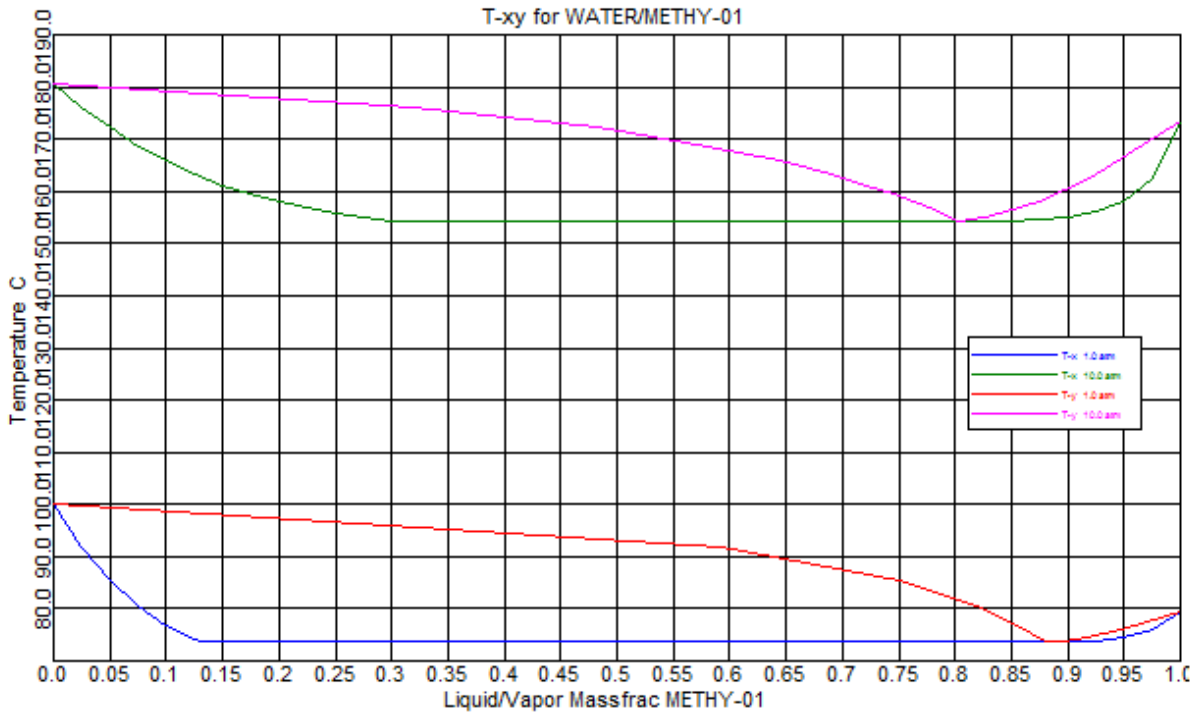


Figure 20: T-xy Diagrams for Pressure Swing Distillation. The T-xy equilibrium lines show how a change in pressure affects the azeotropic composition of a MEK/water mixture. By increasing the pressure it is theoretically possible to distill from the feed composition to pure MEK.

Pressure swing distillation takes advantage of this shift using a simple two-column set up. The first column runs at atmospheric pressure, and the bottoms consists of pure water while the distillate consists of the azeotropic mixture. This mixture is now fed into the second

column at elevated pressure. The distillate of the column now contains the azeotropic mixture while the retentate is pure MEK. The distillate can be fed back into the first column.

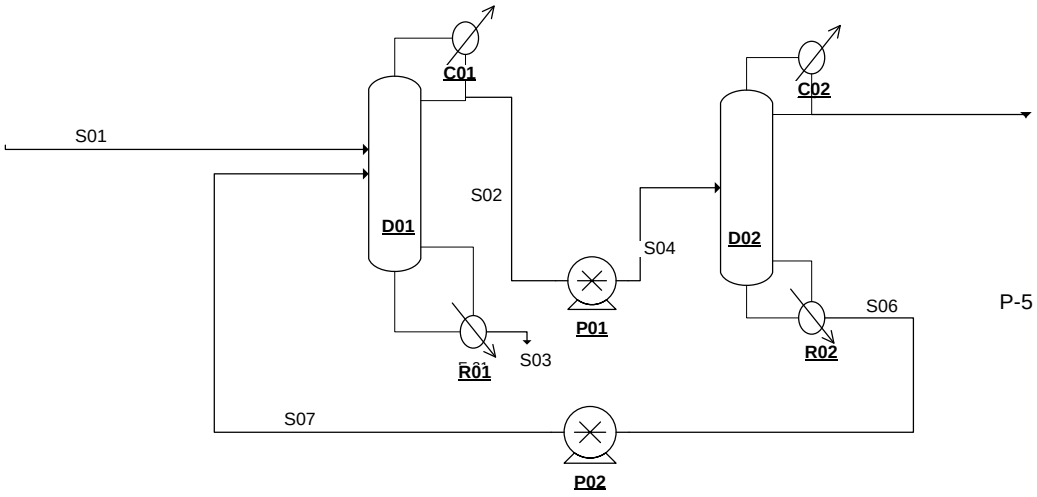


FIGURE 21: Flowsheet illustrating azeotropic distillation for separation of MEK.

This design was implemented in ASPEN with some difficulty. The water-MEK mixture forms two liquid phases, which must be taken into account when modeling the system in RADFRAC columns. Getting a single column to generate reasonable data was troublesome, as the column was very sensitive to minute changes in the distillate rate and reflux ratio. Therefore, when the second column and a recycle stream were added, ASPEN was not properly converging and the answers generated by the simulation were unreliable. The pressure swing model was therefore rejected.

Pervaporation

The third method investigated was pervaporation. In this technique a water and methyl ethyl ketone mixture can be separated by partial vaporization through a nonporous, permeate-selective membrane. In this process, the liquid feed mixture circulates while in contact with the active, non-porous side of the membrane. A vacuum is applied on the other side of the membrane (Figure 3). A phase change of the membrane-selective

permeate, methyl ethyl ketone, allows it to diffuse through the membrane and desorb on the posterior side of the membrane. The energy required to pass water through the membrane is much greater so most of the water stays on the retentate side of the membrane. The vacuum present on the posterior side of the membrane causes the MEK to evaporate and pulled away from the membrane. This flux generates the driving force that allows new MEK to proceed through the membrane. The collected MEK is then sent into a condenser outside the membrane, where it is collected with a purity of about 99.8% according to PervaTech. PervaTech is the company that builds these membranes and consulted with this design team on the design of the pervaporation modules. The figure below shows a possible setup with two pervaporators in series to achieve the required purity.

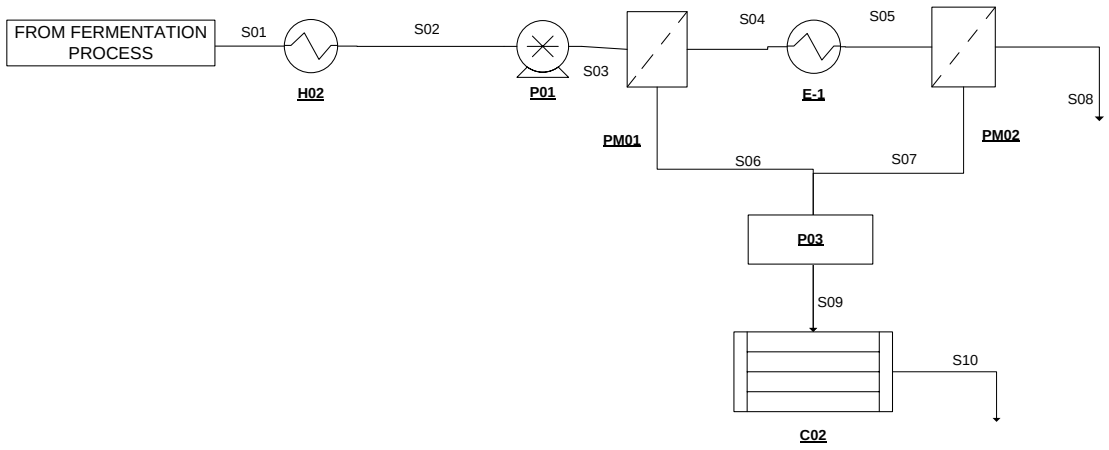


FIGURE 22. Flowsheet for a single pervaporation process to separate MEK from water.

Pervaporation has several advantages over traditional distillation. It offers reduced energy utilization because only the fraction of the liquid that needs to be separated is vaporized. Second, the process requires fewer process units since only a small vacuum pump is needed to create a driving force for the permeate to flow through the membrane.

Initially, it would have been ideal for this pilot plant to just use pervaporation modules, without a distillation column, because of the low flow rates that are achieved in the plant, and the high efficiency of the pervaporation process. Using a single pervaporation step would lower energy and equipment costs of the pilot plant. For comparison purposes amongst other reasons, a hybrid process of coupling distillation and pervaporation was also investigated. Some publications (Uwe HoÈmmerich, 1998) claim that the combination of pervaporation with distillation can offer great economic advantages. Due to the low attainable fluxes in pervaporation modules, which decrease significantly with decreasing feed concentrations, a pervaporation operates optimally when the amount being separated is as small as possible. The need for a more concentrated feed to the pervaporation module justifies the use of a distillation step before the pervaporator; the more concentrated feed increases the driving force and therefore the flux across the membrane. The feed stream coming off of the fermentation process is only 4% MEK by mass, so it would be useful to concentrate this stream before feeding it into the pervaporation units. A distillation-pervaporation setup is depicted below, in the process flow diagram.

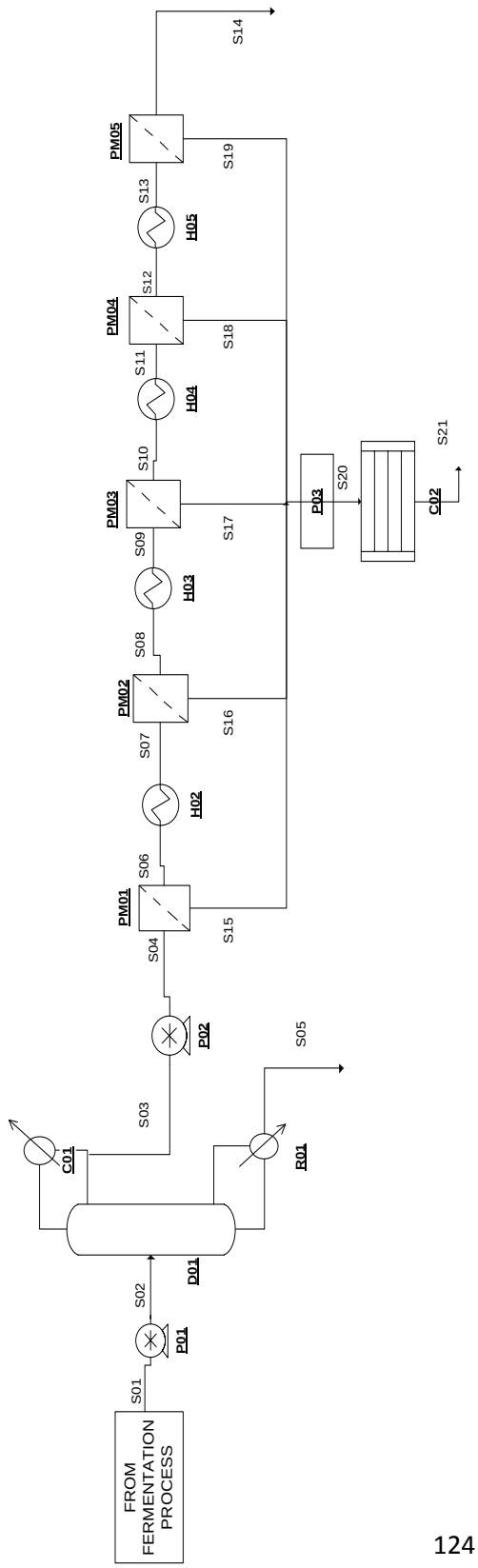
The main reason to consider distillation-pervaporation over single step pervaporation is because it is a more scalable process. The less water there is in the feed streams to the pervaporation units, the less membrane area required for separation. This would greatly drive down costs in a full scale plant. Furthermore, in a commercial plant the necessary vacuum equipment and the plate-and-frame module system, which are still state-of-the-art for most commercial membranes, make pervaporation and vapor permeation expensive if used by its own with very high flow rates. Using the single membrane separation step would also present a problem when the electrolytes, salts and inorganic waste from the

fermentor contact the membrane. Introducing a distillation column before the pervaporator creates a way to dispose of these electrolytes which may otherwise damage the pervaporation membranes. These salts are flushed out with the bottoms product, which is pure water. This will increase the lifetime of the membrane while reducing cleaning costs.

FEASIBILITY, DEVELOPMENT, MANUFACTURING STAGES

PROCESS FLOW DIAGRAM

Figure 23. Process Flow Diagram for Pervaporation/Distillation Separation of MEK and Water



Stream Label	Stream Name	Stream Label	Stream Name	Equipmer Label	Equipment Name
S01	Distillation Column feed	S12	Perv. Feed 5	C01	Condenser 1
S02	Distillation Column feed	S13	Perv. Feed 5	C02	Condenser 2
S03	Distillate	S14	Retentate	D01	Distillation Column
S04	Bottoms	S15	Permeate 1	H01	Heater 1
S05	Perv. Feed 1	S16	Permeate 2	H03	Heater 2
S06	Perv Feed 2	S17	Permeate 3	MT01	Mixing Tank
S07	Perv Feed 2	S18	Permeate 4	P01	Pump 1
S08	Perv. Feed 3	S19	Permeate 5	P02	Pump 2
S09	Perv. Feed 3	S20	Vapor Permeate	P03	Vacuum Pump
S10	Perv. Feed 4	S21	Condensed Permeate (Pure MEK)	PM01-05	Pervaporation Membranes
S11	Perv. Feed 4				

TABLE 6A: Stream report for the hybrid distillation-pervaporation process.

	S01	S02	S03	S04	S05
Temperature C	37.00	38.00	77.77	77.77	108.34
F	98.60	100.40	171.99	171.98	227.01
Pressure bar	1.01	2.01	1.17	4.00	1.34
Psi	14.69	29.19	17.00	58.00	19.49
Vapor Frac	0.00	0.00	0.00	0.00	0.00
Mole Flow kmol/hr	28.76	28.76	0.50	0.50	28.26
Mass Flow kg/hr	548.17	548.17	26.32	26.32	521.86
Volume Flow cum/hr	0.78	0.78	0.04	0.05	0.79
Enthalpy kJ/hr	-8.90E+06	-8.90E+06	-1.36E+05	-1.36E+05	-8.90E+06
Mass Flow kg/hr					
WATER	509.00	509.00	3.28	3.28	505.73
METHY-01	23.04	23.04	23.04	23.04	0.00
CALCI-01	3.96	3.96	0.00	0.00	3.96
HEMAT-01	0.83	0.83	0.00	0.00	0.83
CALCI-02	4.00	4.00	0.00	0.00	4.00
POTAS-01	7.34	7.34	0.00	0.00	7.34
Mass Frac					
WATER	0.93	0.93	0.12	0.12	0.97
METHY-01	0.04	0.04	0.88	0.88	0.00
CALCI-01	0.01	0.01	0.00	0.00	0.01
HEMAT-01	0.00	0.00	0.00	0.00	0.00
CALCI-02	0.01	0.01	0.00	0.00	0.01
POTAS-01	0.01	0.01	0.00	0.00	0.01
Mole Flow kmol/hr					
WATER	28.25	28.25	0.18	0.18	28.07
METHY-01	0.32	0.32	0.32	0.32	0.00
CALCI-01	0.03	0.03	0.00	0.00	0.03
HEMAT-01	0.01	0.01	0.00	0.00	0.01
CALCI-02	0.07	0.07	0.00	0.00	0.07
POTAS-01	0.08	0.08	0.00	0.00	0.08
Mole Frac					
WATER	0.98	0.98	0.36	0.36	0.99
METHY-01	0.01	0.01	0.64	0.64	0.00
CALCI-01	0.00	0.00	0.00	0.00	0.00
HEMAT-01	0.00	0.00	0.00	0.00	0.00
CALCI-02	0.00	0.00	0.00	0.00	0.00
POTAS-01	0.00	0.00	0.00	0.00	0.00

	S06	S07	S08	S09	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21
Temperature C	45.00	80.00	20.70	80.00	36.00	90.00	36.00	100.00	30.00	45.00	20.70	36.00	36.00	36.00	80.00	25.00
F	113.00	176.00	69.26	176.00	69.26	194.00	96.80	212.00	86.00	113.00	69.26	96.80	96.80	96.80	176.00	77.00
Pressure bar	3.50	3.50	3.00	3.00	2.50	2.50	2.00	2.00	1.50	0.02	0.02	0.02	0.02	0.02	0.02	1.03
Psi	50.75	50.75	43.50	43.50	36.25	36.73	29.00	29.00	22.04	0.30	0.30	0.30	0.30	0.30	0.30	14.97
Vapor Frac	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
Mole Flow kmol/hr	0.34	0.34	0.18	0.16	0.18	0.16	0.16	0.16	0.18		0.16	0.16	0.16	0.16	0.32	0.32
Mass Flow kg/hr	-1.35	-1.35	-5.98	-5.98	-10.61	-10.61	-15.24	-15.24	3.17	4.63	4.63	4.63	4.63	4.63	23.15	23.15
Volume Flow cum/hr				0.02		0.02	0.02	0.02		0.02	0.02	0.02	0.02	0.02	590.81	0.41
Enthalpy kJ/hr	-2.67E+06	-2.67E+06	-2.70E+06	-2.70E+06	-2.76E+06	-2.76E+06	-2.83E+06	-2.83E+06	-2.00E+03	-1.50E+04	-1.50E+04	-1.50E+04	-1.50E+04	-1.50E+04	-7.56E+04	-7.56E+04
Mass Flow kg/hr																
WATER	3.25	3.25	3.23	3.23	3.21	3.21	3.19	3.19	3.17	0.02	0.02	0.02	0.02	0.02	0.11	0.11
METHY-01	-4.61	-4.61	-9.22	-9.22	-13.82	-13.82	-18.43	-18.43	0.00	4.61	4.61	4.61	4.61	4.61	23.04	23.04
Mass Frac																
WATER	0.22	0.22	1.00	0.00	1.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
METHY-01	0.78	0.78	0.00	1.00	0.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mole Flow kmol/hr																
WATER	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.00	0.00	0.00	0.00	0.00	0.01	0.01
METHY-01	-0.06	-0.06	-0.13	-0.13	-0.19	-0.19	-0.26	-0.26	0.00	0.06	0.06	0.06	0.06	0.06	0.32	0.32
Mole Frac																
WATER	0.53	0.53	1.00	0.01	1.00	0.01	0.01	0.01		0.01	0.01	0.01	0.01	0.01	0.01	0.01
METHY-01	0.47	0.47	0.00	0.99	0.00	0.99	0.99	0.99		0.99	0.99	0.99	0.99	0.99	0.99	0.99

TABLE 6B: Stream report for the hybrid distillation-pervaporation process.

PROCESS DESCRIPTION

Distillation-Pervaporation

Process 1, as illustrated in figure 23, contains one distillation tower coupled with five pervaporation modules in series, four heaters, one positive displacement pump, one vacuum pump and a condenser at the posterior end of the pervaporators. In the first distillation column, **D01**, the feed stream comes in from the storage tank in the fermentation process via stream **S01,** which is pumped into the distillation tower via stream **S02**. The greater mass flow of water and other unwanted fermentation products all go out of the distillation column via the bottoms stream **S05**. A mass recovery of 100% methyl ethyl ketone, MEK, is obtained in the distillate steam **S03**, in which the MEK/water mixture comes out at its azeotropic composition (mass fraction of ~87.6 wt% MEK, with water making up the balance (see Table 6)). The distillate stream, **S03,** is then pressurized by pump **P01** as it flows through **S04**, which feeds the first pervaporator module PM01. In PM01, a fifth of the MEK in the distillate is separated from water in the permeate stream, **S15**, before it is heated up again and passed on to the next module, PM02. This sequence goes to the last module, PM05 where pure water is obtained as a retentate stream **S14**. The five permeate streams (**S15-S19**) will pass through the vacuum pump **P03** and combine as **S20**. Finally, the vapor will be condensed in the condenser **C02**, passing out in the product stream **S21.**

Separation in the Pervaporation Modules

The pervaporation modules were operated using the inlet stream parameters specified in Table 6 for feed streams S04, S07, S09. and S13 entering into the five modules. The total area for the membranes 0.5m^2 was split into five 0.1m^2 membranes operated in series so as

to increase the efficiency of the membrane (Dutta, D. K., 1997). Operating in series will allow water to be recyled and act as a solvent to carry on the energy needed for the MEK to vaporize. This will result in lower temperature drops across the membranes. This is important because the membranes operate more efficiently at higher temperatures.

The streams entering into all membranes are pressurized so as to counter the pressure drop across each unit during operation. The pervaporation model operates such that the heat of vaporization of MEK will be obtained from the energy of the heated water-MEK solution (from the distillation column stream **S03**). When the heated stream encounters the pressure drop generated by the vacuum pump, it will vaporize. This leads to a significant temperature drop across the pervaporation membrane as shown in figure 24 below. This temperature drop also correlates directly with the membrane performance, which will be discussed in the *Approximations* section.

In order to ensure than the membrane performance still matched the one specified by (Pervatech BV, 2005), the feed temperature and pressure were high enough such that the temperature and pressure drops would not significantly affect the predicted membrane performance by Pervatech BV. (2005). Figure 24 shows how the performance varies across the membranes PM01-05. The average temperature of the streams matched the specified feed stream temperature which validated our estimates.

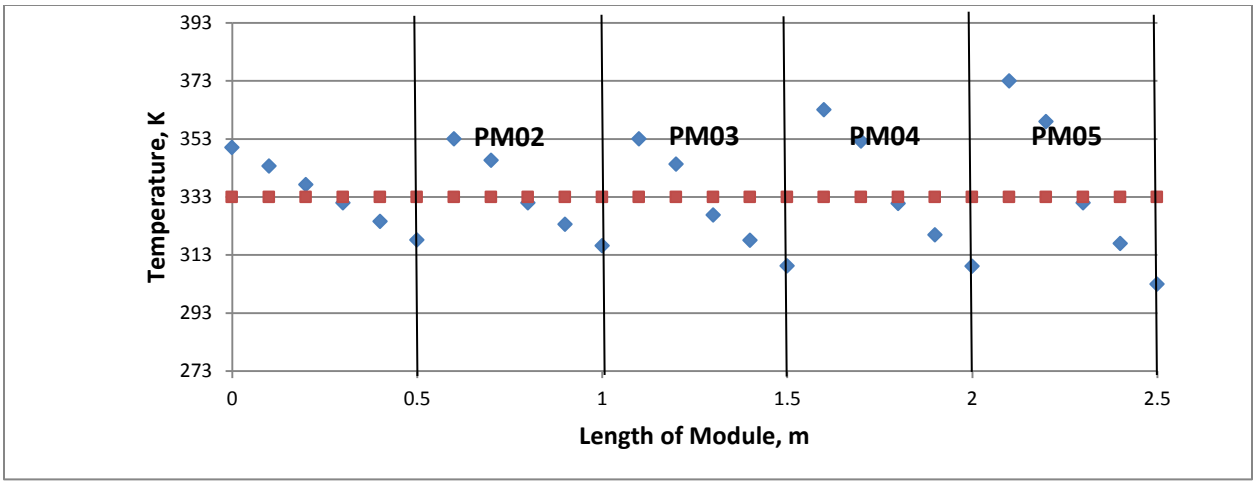


FIGURE 24. The temperature drop across the two membranes represents the performance of the membrane across the module.

EQUIPMENT LIST (REFER TO FIGURE 23)

Figure 25. Detailed Overview of Process Units in Pervaporation/Distillation Separation of MEK and water.

MEK Separation Equipment:								
Unit Name	Unit No.	Function	Size/ Capacity	Construction Material	Estimated Cost	Source of Cost Estimate	Operating Temp.	Operating Pressure
Distillation Column	D01	Separate excess water and waste from MEK	see MAA		see MAA			
Condenser	C01	Condense Distillate	8 ft^2	Glass	4,420.95	Correlations	37 °C	1 bar
Condenser	C02	Condense Permeate from PM01-05	1.64 ft	Stainless Steel	501.00	Fischer Scientific	37 °C	1 bar
Reboiler	R01	Reboil the bottoms	7.61	Stainless Steel	4,389.65	Correlations	37 °C	1 bar
Pervaporation Modules	PM01-PM05	To separate MEK/water mixture	0.1m2 * 5	Stainless Steel	46,761.00	Pervatech	37 °C	1 bar
Pump	P01	Move liquid to distillation tower	0.5 HP	Stainless Steel	see MAA	Novatech US	37	1
Pump	P02	Pressurize pervaporation feed	0.5 HP	Stainless Steel	1,817.43	Novatech US	77	4
Vacuum Pump	P03	Vaporize MEK	4569 BTU/hr	Stainless Steel	1,906.51	Fischer Scientific	25	21 mbar
Heaters	H01-H04	Heat up cooled retentate streams to feed them into next membrane.	1706 BTU/hr	Silicone Rubber	796.16	Fischer Scientific	80-100 °C	0.5 kW
Total					**60,592.70**			

Distillation Column - D01

This unit is a non-forming, 316 Stainless Steel that uses FLEXIRING plastic packing. The feed (S01) has a mass flow of 548.2 kg/hr comes from the storage tank from the fermentation process at a temperature of 37°C. The distillate **S03**, which has a flow rate of 23.3 kg/hr (87.6 wt% MEK) is discharged to the pervaporation steps, comes out at 77.7°C The bottoms product, comes out at 108°C with 97wt% water with a flow rate of 505.7 kg/hr. This unit separates out MEK from water up to the azeotropic composition of 87.6 wt% MEK. The diameter of this unit is 12 inches, and the column has 8 tray stages plus the reboiler and condenser, which makes a total of ten theoretical stages. The second distillation column from the MAA, **D02** was used in order to cut costs since the two chemicals, MEK and MAA are going to be produced at different times in the year. The only difference between these two columns are the operating parameters and utilities.

Pervaporation Membranes: PM01-PM05

These units consist of 10-ceramic tube modules (type PVM-045), with a length of 50 cm. The pervaporation tubes are coated with a ceramic HybSi membrane material. The total membrane surface area of 0.5m^2 is split equally among the five modules. Maximum process temperature for the membrane is 150°C. Standard enclosures with O-ring EPDM-PC has a maximum temperature of 130°C. The heat duty for each of these units is +1895 BTU/hr and this unit costs $9,613.50.

PM01

The inlet process temperature (**S04**) is 77 °C, and pressure of 4 bars (58 psi). This unit separates 4.6 kg/hr of MEK into the permeate stream (S15) from an inlet stream of 3.25 kg/hr water and 23.04 kg/hr MEK giving a 99.8% purity of MEK. The remainder of the MEK

and water in the retentate is then heated up and fed into PM02 (S07). The outlet stream has a temperature of 45^{o}C which indicates a temperature drop of 32^{o}C (see **figure 23**) and also a pressure of 3.5bar indicating a pressure drop of 0.5 bar across the membrane (PERVATECH, 2011).

PM02

The inlet process temperature (**S07**) is 80 ^{o}C, and pressure of 3.5 bars (50.8 psi). This unit separates 4.6 kg/hr of MEK into the permeate stream (S16) from an inlet stream of 3.23 kg/hr water and 18.4 kg/hr MEK giving a 99.8% purity of MEK. The remainder of the MEK and water in the retentate is then heated up and fed into PM03.

PM03

The inlet process temperature (**S09**) is 80 ^{o}C, and pressure of 3 bars (43.5 psi). This unit separates 4.6 kg/hr of MEK into the permeate stream (S17) from an inlet stream of 3.21 kg/hr water and 13.8 kg/hr MEK giving a 99.8% purity of MEK. The remainder of the MEK and water in the retentate is then heated up and fed into PM04.

PM04

The inlet process temperature (**S11**) is 90 ^{o}C, and pressure of 2.5 bars (36.25 psi). This unit separates 4.6 kg/hr of MEK into the permeate stream (S18) from an inlet stream of 3.18 kg/hr water and 9.2 kg/hr MEK giving a 99.8% purity of MEK. The remainder of the MEK and water in the retentate is then heated up and fed into PM05. The flux in this module is lower due to the lower MEK wt%, therefore a higher operating temperature is required in order to supply for the extra heat duty per unit mass.

PM05

The inlet process temperature (**S13**) is 100 ^{o}C, and pressure of 2.0 bars (29 psi). This unit separates 4.6 kg/hr of MEK into the permeate stream (S19) from an inlet stream of 3.16 kg/hr water and 4.6 kg/hr MEK giving a 99.8% purity of MEK. Since this is the final module, the remaining water will be recovered in the retentate stream **S14**.

The description of smaller units, pumps (P01-P03), the reboiler (R01), condensers (C01-C02) and heaters (H01 - H02) can be found in section containing the specification sheets.

NECESSARY APPROXIMATIONS

Constant Flux Assumption

Difficulties arose in calculating the change of the permeate flux and the driving force as a function of temperature as the equations were not explicit with all the parameters. HoÈmmerich Uwe et al. states that the permeate flux varies with the temperature drop in a membrane according to the following equation:

$$m_p(T) = m_p(T_0) * \exp\left(\frac{E_{MEK}}{R}\left(\frac{1}{T} - \frac{1}{T_0}\right)\right) \qquad \text{Eqn. 1}$$

m_p (T) = permeate flux at temperature, T.

T = operating temperature of the membrane at a given point.

T_o = temperature of feed solution.

E_{MEK} = activation energy of permeation.

This relationship suggests that if the difference of the inverses of the temperatures is very small, then the permeate flux essentially remains constant throughout the separation process. In estimating the performance of the pervaporator for this process, it was assumed that the difference between the inlet and outlet temperature inverses ($1/T_{in} - 1/T_{out}$) is negligible, such that the permeate flux does not change significantly. In order to validate this assumption, the membrane was modeled at an inlet temperature of 373 K instead of the specified 333 K. After the MEK evaporates and the outlet stream cools, the average temperature in the system would be 333K as specified (see **figure 23)**. At 373K, the MEK/water mixture will be in its gaseous phase, so a pump will be used to pressurize the solution to keep the mixture in its liquid form. This pressure could also increase the flux of the membrane, as (Dutta, 1997) states that flux is a function of the pressure gradient

across the membrane. Pressure gradients were not considered in this design, so the membrane efficiencies are slightly underestimated. The membranes are therefore slightly oversized, which is good in case the separation efficiency is not as high as it is expected to be.

It was also assumed that the area of the membranes applicable for this project is small enough to estimate the inlet flux to be the overall average flux for the rest of the membrane. In order to account for any drop in permeate flux, it is recommended membranes with an area that is 20% more than our calculated value, so as to account for any variations, and thus maximize performance. These assumptions also meant that the driving force of the permeate stream would also change insignificantly throughout this process.

Energy Balance Estimation

The energy balance for the pervaporation was very hard to calculate and incorporate into the rest of the ASPEN generated stream table. An assumption was made that the energy is in the retentate is going to be dominantly the negative of the latent heat of vaporization of MEK in each pervaporator. In the case, ASPEN generated results for the incoming stream's enthalpy when the vacuum was modeled. These results took into account the non-standard conditions the vacuum presented, so these values were used to determine the total permeate stream enthalpy change.

SPECIFICATION SHEETS

Pervaporation Modules			
Identification	Item:	1-tube module type PVM-035, including	Date
	Material	1 ceramic HybSi pervaporation membrane.	########
	Item No.	PM01-05	
	No. Req.	5	
	Total Cost ($)=	48,067.50	
Function: To separate Methyl Ethyl Ketone from water			
Operation: Continuous			

Materials Handled:	Inlet	Retentate	Permeate
Stream ID:	S06	S07	S16
Quantity (kg/hr):	26.316	21.6944	4.6216
Composition:			
WATER	3.276	3.2544	0.0216
METHA-01	23.04	18.44	4.6
Temperature (^{o}F):	212	113	211.4
Pressure (psi)	79.8	79.8	26.83
Vapor fraction	0	0	1

	Inlet	Retentate	Permeate
Stream ID:	S08	S09	S17
Quantity (kg/hr):	21.6944	17.0944	4.6216
Composition:			
WATER	3.2544	3.2544	0.0216
METHA-01	18.44	13.84	4.6
Temperature (^{o}C):	257	69.62	211.46
Pressure (bar)	73	65.3	26.83
Vapor fraction	0	0	1

	Inlet	Retentate	Permeate
Stream ID:	S10	S11	S18
Quantity (kg/hr):	3.2544	-1.3456	4.6216
Composition:			
WATER	0	0	0.0216
METHA-01	3.2544	-1.3456	4.6
Temperature (^{o}C):	257	69.62	211.46

Pressure (bar)	73	65.3	26.83
Vapor fraction	0	0	1
	Inlet	Retentate	Permeate
Stream ID:	S12	S13	S19
Quantity (kg/hr):	0	-4.6	4.6216
Composition:			
WATER	0	0	0.0216
METHA-01	0	-4.6	4.6
Temperature (^{o}C):	257	69.62	211.46
Pressure (bar)	73	65.3	26.83
Vapor fraction	0	0	1
	Inlet	Retentate	Permeate
Stream ID:	S14	S15	S20
Quantity (kg/hr):	0	-4.6	4.6216
Composition:			
WATER	0	0	0.0216
METHA-01	0	-4.6	4.6
Temperature (^{o}C):	257	69.62	211.46
Pressure (bar)	73	65.3	26.83
Vapor fraction	0	0	1

Design Data:

Module Type:	Tubular
: Materials:	Ceramic Membrane
Membrane Area:	0.5 m2 total
Heat Duty:	9526 BTU/hr
Pressure drop:	max of 6.8 psi per stage

Comments: Due for servicing in 3 years.

Vacuum Pump			
Identification	Item:	Welch Standard-Duty Vacuum Pump	Date 3/28/2011
	Item No.	P03	
	No. Req.	1	
	Total Cost ($)=	1,906.51	

Function:	To create a vacuum on the posteria end of the pervaporation modules.			
Operation:	Continuous			
Materials Handled:				
		Inlet		Outlet
Stream ID:		**S16/17/18/19/20**		**S21**
Quantity (kg/hr):			23.256	23.256
Composition:				
	WATER		2.16E-01	2.16E-01
	MEK		23.04	23.04
Temperature (^{o}F):			99.7	92
Pressure (psi)			26.83	3.00E-04
Vapor fraction			0	1
Fluid HP			-0.3	
Brake HP			-0.18	
Pump efficiency			50%	
Design Data:				
	Motor Type:	1/3 hp motor		
:	Materials:	316 Stainless-Steel		
	Flowrate	air displacement 99L/min		
	Ultimate Vacuum:	< 0.0002psi		
Comments:	Quotation acquired from Fischer Scientific. http://www.fishersci.com			

Condenser			
Identification	Item:	Graham Condenser with Inner and Outer Joints	Date
	Material	Pyrex	3/28/2011
	Item No.	C02	
	No. Req.	1	
	Total Cost ($)=	501.39	
Function:	to condense MEK vapor permeate.		
Operation:	Continuous		
Materials Handled:			
		Inlet	Outlet
Stream ID:		**S21**	**S22**
Quantity (kg/hr):			
Composition:			
	WATER	0.11	0.11
	MEK	23.04	23.04
Temperature (F):		197.6	77
Pressure (psi)		0.0003	14.69
Vapor fraction		1	0
Design Data:			
	Heat Duty:	-12633 BTU/hr	
	Condenser Type:	Pyrex Brand Graham Condensers	
	Dimensions:	0.5 m	
Comments:	Quotation from Fischer Scientific. http://www.fishersci.com		

Heater			
Identification	Item:	Thermo Scientific BriskHeat Heating Blanket	Date
	Material	Silicone Rubber	3/28/2011
	Item No.	H01, H02 (MEK Process)	
	No. Req.	5	
	Total Cost ($)=	796.16	
Function: to condense MEK vapor permeate.			
Operation: Continuous			

Materials Handled:		
	Inlet	Outlet
Stream ID:	S08	S09
Quantity (kg/hr):		
Composition:		
WATER	3.23	3.23
METHA-01	13.84	13.84
Temperature (F):	96.8	176
Pressure (psi)	43.5	43.5
Vapor fraction	0	0

Materials Handled:		
	Inlet	Outlet
Stream ID:	S10	S11
Quantity (kg/hr):		
Composition:		
WATER	3.21	3.21
METHA-01	9.24	9.24
Temperature (F):	96.8	176
Pressure (psi)	36.25	36.25
Vapor fraction	0	0

Materials Handled:		
	Inlet	Outlet
Stream ID:	S12	S13
Quantity (kg/hr):		
Composition:		
WATER	3.19	3.19
METHA-01	4.64	4.64
Temperature (F):	86	176
Pressure (psi)	29	29
Vapor fraction	0	0

Total Utilities per year:		
Design Data:		
	Heat Duty:	1706 Btu/hr
	Wattage:	0.5 kW
Heater	Max Temperature:	358F
Comments:	Quotation from Fischer Scientific.	

Distillation Column (MEK)			
Identification	Item:	RADFRAC Distillation Column	Date
	Item No.	D-02	
	No. Req.	1	

Function: To take the water-MEK mixture to its azeotropic composition

Operation: Continuous

Materials Handled:			
	Inlet	Tops	Bottoms
Stream ID:			
Temperature (C)	37	77.8	108.5
Quantity (kg/hr):	558.128	26.3	531.828
Composition (kg/hr):			
WATER	509	3.3	505.7
METHY-01	23	23	0
CALCI-01	3.96	0	3.96
HEMAT-01	0.828	0	0.828
CALCI-02	13.996	0	13.996
POTAS-01	7.344	0	7.344

Design Data:

Material:	316 Stainless Steel	
Theoretical Trays	10	
Packing Type	FLEXIRING (plastic)	
HETP	2	ft
Functional Height	20	ft
Inner Diameter	8	in
Pressure	17	psi
Pressure drop/stage	0.15	psi
Feed Stage	6	(on stage)
Wall Thickness	0.25	in
Weight (lb)	452.7274997	lb
Cost of Pressure Vessel	$ -	
Cost of Packing	$ -	
Total Production Cost	$ -	
Condenser	See specification sheet	
Reboiler	See specification sheet	

Comments: This column is shared between both the MEK and MAA processes. A separate specification sheet is included for when the column is running MEK

Reboiler (MEK)				
Identification	Item: Double Pipe Heat Exchanger Item No. RB-02 No. Req. 1		Date	3/28/2011
Function: To revaporize the liquid collected from the bottom of the distillation column D-01				
Operation: Continuous				
Materials Handled:	Cool Side Inlet	Cool Side Outlet	Steam Inlet	Steam Outlet
Stream ID:				
Temperature (C)	108.53	108.53	133.5278	133.5278
Temperature (F)	227.354	227.354	272.35	272.35
Quantity (kg/hr):	188.26	188.26	414.8049	414.8049
Composition (kg/hr):				
WATER	188.26	188.26	414.8049	414.8049
METHY-01	0.0703	0.0703	0	0

Design Data:

Molar Biolup Ratio	0.30304	
Heat Duty	997934	BTU/hr
Flow rate of steam	414.8049	lb/hr
Grade of Steam	50 psi	
Heat Flux	12000	BTU/(ft^2-hr)
Area	83.1611667	ft^2
Materials		
Shell	Stainless Steel	
Tube	Stainless Steel	
Unit Cost, C_p	$ 7,722.84	per unit
Utilities	$ 1,971.80	per year

Comments:

Condenser (MEK)					
Identification	Item:	Double Pipe Heat Exchanger		Date	3/28/2011
	Item No.	CON-02			
	No. Req.	1			
Function:	Condense the vapor effluent of distillation column D-02				
Operation:	Continuous				
Materials Handled:	Hot Side		Cooling Water		
	Inlet	Outlet	Inlet	Outlet	
Stream ID:					
Temperature (C)	79.76	77.77	32.22	48.89	
Temperature (F)	175.568	171.986	90	120	
Quantity (kg/hr):	80.41	80.41	12851.0	12851.0	
Composition (kg/hr):					
WATER	10.01	10.01	12851.0	12851.0	
METHY-01	70.40	70.4	0	0	

Design Data:

Heat Duty	848,165	BTU/hr
Flow rate of cooling water	3394.02	gal/hr
Log Mean Temp. Difference	67.9228975	degrees F
Heat Transfer Coefficient	150	BTU/(F-ft^2-hr)
Area	83.2478228	ft^2
Materials		
Shell	Stainless Steel	
Tube	Stainless Steel	
Unit Cost, C_p	$ 7,724.12	per unit
Utilities	$ 2,231.40	per year

Comments:

Reflux Accumulator (MEK)				
Identification	Item:	Reflux Accumulator	Date	3/28/2011
	Item No.	RA-02		
	No. Req.	1		
Function:	Hold 10 min of reflux for distillation column D-02			
Operation:	Continuous			

Materials Handled:		From Stage 2	Distillate
Stream ID:			
Temperature (C)		77.80	77.80
Quantity (kg/hr):		1341.30	26.30
Volume (cum/hr)		1.73	0.03
Volume (cuft/min)		1.02	0.02
Composition (kg/hr):			
	WATER	168.30	3.30
	METHY-01	1173.00	23.00
	CALCI-01	0.00	0.00
	HEMAT-01	0.00	0.00
	CALCI-02	0.00	0.00

Design Data:		
Material:	316 Stainless Steel	
Reflux Ratio	50	
Condensate Flow Rate	1.02	cuft/min
Volume	20.41	cuft
Volume	152.70	gal
Diameter	2.35	ft
Length	4.70	ft
Thickness	0.25	in
Weight	500.73	lb
Cost	$ 672.42	

Comments:

Part IV: Economic Analyses

EQUIPMENT COSTS

As mentioned at the beginning of this paper under *Pilot Plant Considerations*, it is not necessary for the pilot plant to be profitable. The production scale is such that the amount of product will not be able to defray operating or construction cost. Also, it will be difficult to find a buyer for the small amount the plant further hampering any ability if the pilot plant to offset any costs. However, it will still be necessary to appropriately cost the total investment required to build and run the pilot plant for three years. The tables that follow summarize the costs required to purchase the equipment and run the facilities for the fermentation and MEK and MAA separation processes. In most case, processes units were capable of being priced using the correlation available in *Seider et al*. In those cases manufacturers and distributors were directly contacted to receive estimations for the equipment that met the design specifications and such values are noted. Appendix C contains sample calculations and formulas used to produce the values in the tables.

Equipment Description	**Purchase Cost**	**F_{BM}**	**C_{BM} ($)**
Fermentation Process			
Centrifuge CF01	$34,226		$109,865
Seed Fermentor F01	$35,000	3.05	$106,750
Small Fermenter F02	$127,032	3.05	$387,448
Medium Fermenter F03	$460,000	3.05	$1,403,000
Chemical Production Fermenter F04	$27,570	3.05	$84,087
Agitator AG 01	$3,616	2.03	$7,341
Pasteurizer HX01	$3,456	3.30	$11,405
Pump P01	$799	3.30	$2,637
Pump P02	$1,000	3.30	$3,300
Pump P03	$1,350	3.30	$4,455
Pump P04	$1,350	3.30	$4,455
Pump P05	$1,350	3.30	$4,455
Pump P06	$1,350	3.30	$4,455
Pump P07	$1,000	3.30	$3,300

Pump P08	$1,000	3.30	$3,300
Water/Molasses Mixing Tank MT01	$20,681	3.05	$63,077
Pasteurized Molasses Storage Tank ST01	$10,265	3.05	$31,308
Supernatant Storage Tank ST02	$23,326	3.05	$71,143
Subtotal			**$2,305,780**

MAA Separation Process

Scheibel Column LLE-01	$350,000	4.30	$1,505,000
Water Purification Column COL02	$38,533	4.30	$165,690
MAA Purification Column COL01	$19,441	4.30	$83,598
Pump P01	$2,500	3.30	$8,250
Pump P02	$2,500	3.30	$8,250
Pump P03	$2,500	3.30	$8,250
Pump P04	$2,500	3.30	$8,250
Pump P05	$2,500	3.30	$8,250
Pump P06	$2,500	3.30	$8,250
Pump P07	$2,500	3.30	$8,250
Chiller CH01	$3,379		$10,847
Reboiler RB01	$7,077	3.17	$22,434
Reboiler RB02	$6,633	3.17	$21,028
Condenser CON01	$6,676	3.17	$21,162
Reflux Accumulator RA01	$461	3.05	$1,406
Reflux Accumulator RA02	$102	3.05	$312
Subtotal			**$1,889,226**

MEK Separation Process

Condenser C02 (MEK)	$501	3.17	$1,588
Vacuum Pump (MEK)	$1,907	3.30	$6,291
Heater H01	$199	3.30	$657
Heater H02	$199	3	$657
Heater H03	$199	3	$657
Heater H04	$199	3	$657
Pervaporation Module PM01	$9,352	3	$29,927
Pervaporation Module PM02	$9,352	3	$29,927
Pervaporation Module PM03	$9,352	3	$29,927
Pervaporation Module PM04	$9,352	3	$29,927
Pervaporation Module PM05	$9,352	3	$29,927
Subtotal			**$160,142**
Grand Total			**$4,355,148**

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SENSITIVITY ANALYSIS: BARE MODULE FACTOR

The bare module factors used to calculate the equipment costs were obtained from Seider et. al (2009). However, the values given are mostly suitable for large scale plants. In this pilot plant some of the equipment will be assembled on site and will not cost as much as the bare module cost predicts. The following plot shows a sensitivity analysis of the change in the Bare Module cost and Capital Investment as a function of the bare module factor.

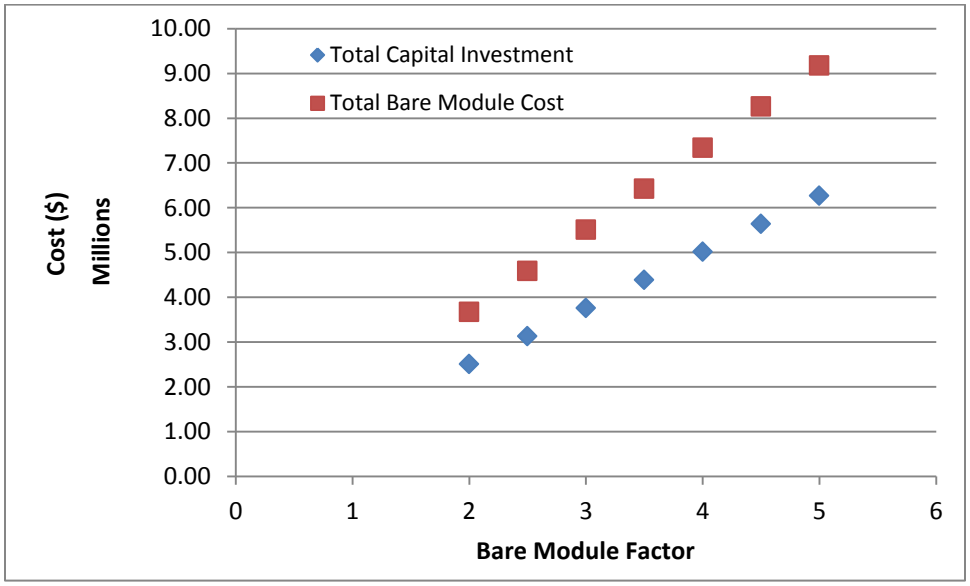

Figure 26: The bare module cost as well as the capital investment are highly sensitive to changes in the bare module factor.

UTILITY REQUIREMENTS

Total utility costs were calculated as described by Seider et. al 2007. The following section is arranged in order of the three main processes: fermentation, MAA separation and MEK separation.

Fermentation Process

Utilities:				
Low Pressure Steam (50 psig, $3/1000 lb)				
Equipment	**Unit**	**Flowrate (lb/hr)**	**Annual Consumption (lb/yr)**	**Annual Cost**
Seed Fermentor (MAA/MEK)	F-01	1.78	6376	$19.13
Small Fermentor (MAA/MEK)	F-02	126.99	122352	$367.00
Medium Fermentor (MAA/MEK)	F-03	568.79	548013	$1,644.04
Chemical Production Fermentor (MAA)	F-04	1716.77	497521	$1,492.56
Chemical Production Fermentor (MEK)	F-04	1716.77	787742	$2,363.23
Pasteurizer	HX-01	5.01	28920	$86.76
			TOTAL	**$5,972.72**
Electricity ($0.06/kWh)				
Equipment	**Unit**	**Power (kW)**	**Annual Consumption (kWh)**	**Annual Cost**
Agitator	AG-01	2.24	24.61	$1.23
Pump 1	P-01	0.02	123.04	$7.38
Pump 2	P-02	0.45	97.09	$5.83
Pump 3	P-03	1.0320	358.35	$21.50
Pump 4	P-04	0.0008	0.22	$0.01
Pump 5	P-05	0.0050	3.24	$0.19
Pump 6	P-06	0.0155	33.71	$2.02
Pump 7 (MAA)	P-07	1.1185	258.39	$15.50
Pump 7 (MEK)	P-07	0.7457	272.74	$16.36
Pump 8 (MAA)	P-08	0.9694	244.29	$14.66
Pump 8 (MEK)	P-08	0.5966	238.03	$14.28
Water/Molasses Mixing Tank	MT-01	9.6900	76,744.80	$4,604.69
			TOTAL	**$4,703.66**

Table 7: Outline of the utility costs for the fermentation process.

Fermentation Operating Costs				
Materials:				
	Annual Requirement		**Cost ($/gal or lb)**	**Annual Cost**
Molasses	85036.66	gal	0.83	$70,817.73
Water (WFI)	293206.8	gal	0.45	$133,189.01
Na_2HPO_4	18939.56	lb	11.14	$210,933.72
KH_2PO_4	9469.78	lb	9.95	$94,232.91
NaCl	1578.3	lb	9.95	$15,705.48
NH_4Cl	3156.59	lb	4.13	$13,038.31
$MgSO_4$	3156.59	lb	10.46	$33,017.46
$FeSO_4*7H_2O$	31.57	lb	45.63	$1,440.40
Kanamycin	78.91	lb	7.28	$574.87
			TOTAL	**572,949.89**

Table 8: The cost of the raw materials used in the fermentation process.

MAA Separation Process

Fermentation Operating Costs				
Materials:				
	Annual Requirement		**Cost ($/lb or g)**	**Annual Cost (USD)**
N-Butyl Acetate	16167.23	lb	0.590	9,538.67
MEHQ	0.037	kg	$20.40/100g	20.40
			TOTAL	9,559.07

Utilities:

Low Pressure Steam (50 psig, $3/1000lb)

Equipment	Unit	Flowrate (lb/hr)	Annual Consumption (lb/yr)	Annual Cost
Seed Fermentor (MAA/MEK)	F-01	1.78	6376	$19.13
Small Fermentor (MAA/MEK)	F-02	126.99	122352	$367.00
Medium Fermentor (MAA/MEK)	F-03	568.79	548013	$1,644.04
Chemical Production Fermentor (MAA)	F-04	1716.77	497521	$1,492.56
Chemical Production Fermentor (MEK)	F-04	1716.77	787742	$2,363.23
Pasteurizer	HX-01	5.01	28920	$86.76
			TOTAL	$5,972.72

Electricity ($0.06/kWh)

Equipment	Unit	Power (kW)	Annual Consumption (kWh)	Annual Cost
Agitator	AG-01	2.24	24.61	$1.23
Centrifuge	CF-01	6.60	52272.00	$3,136.32
Pump 1	P-01	0.02	123.04	$7.38
Pump 2	P-02	0.45	97.09	$5.83
Pump 3	P-03	1.0320	358.35	$21.50
Pump 4	P-04	0.0008	0.22	$0.01
Pump 5	P-05	0.0050	3.24	$0.19
Pump 6	P-06	0.0155	33.71	$2.02
Pump 7 (MAA)	P-07	1.1185	258.39	$15.50
Pump 7 (MEK)	P-07	0.7457	272.74	$16.36
Pump 8 (MAA)	P-08	0.9694	244.29	$14.66
Pump 8 (MEK)	P-08	0.5966	238.03	$14.28
Water/Molasses Mixing Tank	MT-01	9.6900	76,744.80	$4,604.69
			TOTAL	**$7,839.98**

Table 8. Detailed outline of the utilities for the MAA process.

MEK Separation Process

Electricity					
Unit ID	**Unit**	**kW**	**kW-hr/yr**	**Cost ($0.06/kwh)**	
-				-	
P01-P03	Pumps	0.13	152.75		9.165
H01-H04	Heaters	2	2350		141
Total					**150.165**
150 psig Pressure Steam					
Unit ID	**Unit**	**lb**	**lb/yr**	**Cost ($4.80/lb)**	
-				-	
R01	Reboiler	106.38	124996.5		599.9832
Total					**599.9832**

Table 9: The Utility costs in the MEK process.

ECONOMIC ANALYSIS

Raw Materials

	Raw Material:	Unit:	Required Ratio:		Cost of Raw Material:	
1	Molasses	gal	1.4172777	gal per kg of MEK and MAA	$0.830	per gal
2	NBA	lb	0.26945	lb per kg of MEK and MAA	$0.59	per lb
3	Na2HPO4	lb	0.3156593	lb per kg of MEK and MAA	$11.14	per lb
4	KH2PO4	lb	0.1578297	lb per kg of MEK and MAA	$9.95	per lb
5	NaCl	lb	0.026305	lb per kg of MEK and MAA	$9.95	per lb
6	NH4Cl	lb	0.0526098	lb per kg of MEK and MAA	$4.13	per lb
7	MgSO04	lb	0.0526098	lb per kg of MEK and MAA	$10.46	per lb
8	FeSO4*H2O	lb	0.0005262	lb per kg of MEK and MAA	$45.63	per lb
9	Kanamycin	lb	0.0013152	lb per kg of MEK and MAA	$7.28	per lb
10	Water	gal	4.88678	gal per kg of MEK and MAA	$0.45	per gal
	Total Weighted Average:				$9.684	per kg of MEK and MAA

Utilities

	Utility:	Unit:	Required Ratio		Utility Cost	
1	High Pressure Steam	lb	10.18725	lb per kg of MEK and MAA	$6.600E-03	per lb
2	Low Pressure Steam	lb	43.142211	lb per kg of MEK and MAA	$3.000E-03	per lb
3	Process Water	gal	4.88678	gal per kg of MEK and MAA	$1.800E-03	per gal
4	Cooling Water	gal	0	gal per kg of MEK and MAA	$0.075	per gal
5	Electricity	kWh	2.2674032	kWh per kg of MEK and MAA	$0.060	per kWh
6	Chilled Brine	ton-day	0.010259	ton-day per kg of MEK and MAA	$1.700	per ton-day
Total Weighted Average:					$0.359	per kg of MEK and MAA

Variable Costs

General Expenses:

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

Working Capital

Accounts Receivable	a	**0**	**Days**
Cash Reserves (excluding Raw Materials)	a	**0**	**Days**
Accounts Payable	a	**0**	**Days**
MEK and MAA Inventory	a	**0**	**Days**
Raw Materials	a	**0**	**Days**

Total Permanent Investment

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

Fixed Costs

Operations

Operators per Shift:	**3**	**(assuming 1 shifts)**
Direct Wages and Benefits:	**$35**	**/operator hour**
Direct Salaries and Benefits:	**15%**	**of Direct Wages and Benefits**
Operating Supplies and Services:	**6%**	**of Direct Wages and Benefits**
Technical Assistance to Manufacturing:	**$0.00**	**per year, for each Operator per Shift**
Control Laboratory:	**$0.00**	**per year, for each Operator per Shift**

Maintenance

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

Operating Overhead

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

Property Taxes and Insurance

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

Straight Line Depreciation

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

Other Annual Expenses

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

Depletion Allowance

Annual Depletion Allowance: **$0**

Variable Cost Summary

Variable Costs at 100% Capacity:

General Expenses

Selling / Transfer Expenses:			$ -
Direct Research:			$ -
Allocated Research:			$ -
Administrative Expense:			$ -
Management Incentive Compensation:			$ -
Total General Expenses			$ -
Raw Materials	$9.684113	per kg of MEK and MAA	$581,047
Byproducts	$0.000000	per kg of MEK and MAA	$0
Utilities	$0.358943	per kg of MEK and MAA	$21,537
Total Variable Costs			**$ 602,583**

Fixed Cost Summary

Operations

Direct Wages and Benefits	$ 218,400
Direct Salaries and Benefits	$ 32,760
Operating Supplies and Services	$ 13,104
Technical Assistance to	$ -

	Manufacturing Control Laboratory	$ -
	Total Operations	**$ 264,264**
Maintenance		
	Wages and Benefits	$ 254,384
	Salaries and Benefits	$ 63,596
	Materials and Services	$ 254,384
	Maintenance Overhead	$ 12,719
	Total Maintenance	**$ 585,084**
Operating Overhead		
	General Plant Overhead:	$ 40,409
	Mechanical Department Services:	$ 13,659
	Employee Relations Department:	$ 33,579
	Business Services:	$ 42,116
	Total Operating Overhead	**$ 129,764**
Property Taxes and Insurance		
	Property Taxes and Insurance:	$ 113,060
Other Annual Expenses		
	Rental Fees (Office and Laboratory Space):	$ -
	Licensing Fees:	$ -
	Miscellaneous:	$ -
	Total Other Annual Expenses	**$ -**
Total Fixed Costs		**$ 1,092,171**

Investment Summary

Bare Module Costs		
Fabricated Equipment	$ 4,267,041	
Process Machinery	$ 88,107	
Spares	$ -	
Storage	$ -	
Other Equipment	$ -	
Catalysts	$ -	
Computers, Software, Etc.	$ -	
Total Bare Module Costs:		**$ 4,355,148**
Direct Permanent Investment		
Cost of Site Preparations:	$ 217,757	
Cost of Service Facilities:	$ 217,757	
Allocated Costs for utility plants and related facilities:	$ -	
Direct Permanent Investment		**$ 4,790,662**
Total Depreciable Capital		
Cost of Contingencies & Contractor Fees	$ 862,319	
Total Depreciable Capital		**$ 5,652,982**
Total Permanent Investment		
Cost of Land:	$ 113,060	
Cost of Royalties:	$ -	
Cost of Plant Start-Up:	$ 565,298	
Total Permanent Investment - Unadjusted		$ 6,331,339
Site Factor		1.00
Total Permanent Investment		**$ 6,331,339**

Working Capital

	2012	2013	2014
Accounts Receivable	$ -	$ -	$ -
Cash Reserves	$ -	$ -	$ -
Accounts Payable	$ -	$ -	$ -
MEK and MAA Inventory	$ -	$ -	$ -
Raw Materials	$ -	$ -	$ -
Total	**$ -**	**$ -**	**$ -**
Present Value at 15%	*$ -*	*$ -*	*$ -*

Total Capital Investment **$ 6,331,339**

Profitability Measures

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

The Net Present Value (NPV) of this project in 2011 is $ (7,465,200)

ROI Analysis (Third Production Year)

Annual Sales	**-**
Annual Costs	**(1,694,755)**
Depreciation	**(506,507)**
Income Tax	**814,467**
Net Earnings	**(1,386,795)**
Total Capital Investment	**6,331,339**
ROI	**-21.90%**

Sensitivity Analyses

Note: The Sensitivity Analyses section below takes quite a bit of memory to update each time a cell is changed; therefore, automatic calculations are turned off. After making your axis selections, press "F9" to recalculate the IRR values. (These two lines may be deleted before printing.)

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

Variable Costs (columns); **Product Price** (rows)

	$301,292	$361,550	$421,808	$482,067	$542,325	***$602,583***	$662,842	$723,100	$783,358	$843,617	$903,875
.00%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
.00%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
.00%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
.00%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
.00%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
.00%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
.00%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
.00%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
.00%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
.00%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR
.00%	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR	Negative IRR

Cash Flow Summary

Capital Costs	Working Capital	Var Costs	Fixed Costs	Depreciation	Depletion Allowance	Taxible Income	Taxes	Net Earnings	Cash Flow	Cumulative Net Present Value at 15%
(6,331,300)	-	-	-	-	-	-	-	-	(6,331,300)	(6,331,300)
-	-	-	-	-	-	-	-	-	-	(6,331,300)
-	-	(602,600)	(1,092,200)	(1,130,600)	-	(2,825,400)	1,045,400	(1,780,000)	(649,400)	(6,822,400)
-	-	(602,600)	(1,092,200)	(1,809,000)	-	(3,503,700)	1,296,400	(2,207,300)	(398,400)	(7,084,300)
-	-	(602,600)	(1,092,200)	(1,085,400)	-	(2,780,100)	1,028,600	(1,751,500)	(666,100)	(7,465,200)

Figure 27. Net Present Cost of Running the pilot plant

The total investment necessary to construct and operate the proposed pilot plant will be $7.47 million. The initial capital investment is $6.33 million for the equipment with an annual operating cost of $600,000/year. This is a fairly reasonable investment for any corporate sponsor interested in commissioning the novel process for MEK and MAA production. Since the plant is so small it was assumed that the process would be operating a 100% capacity from the beginning.

PART V: Conclusions

The goal of this pilot plant was to produce 30 M kg/year of the monomers MEK and MAA from bacteria. Recent advances in biotechnology have engineered bacteria capable of producing these two chemicals during their normal growth cycle. The two organisms (one for each product) will be grown separately so that production of the MAA and MEK can be reasonably predicted and to simplify the separation process. The above investigation into the modern separations technologies lead to the recommendation of a distillation/liquid-liquid extraction process for MAA and a distillation/pervaporation process for MEK. The two models meet the minimum production goal, while achieving the purity necessary to be used in later polymer refining processes. Also, as a pilot plant, the process will be able to provide data for the separation of the desired compounds. This data can then be applied to more accurately scale up the separations technology to a commercial scale. The data gained from actually growing a culture of these recently bio-engineered microorganisms will also allow for more accurate modeling of any commercial plant based on this technology.

All equipment common between processes, like pumps, distillation columns, storage tanks, etc…, was used for both processes to reduced costs. All process units were optimized to reduce utility costs. All purge streams were treated so that the plants environmental impact was negligible, while all other waste streams were sent off-site for treatment. This plant will require a total capital investment of $6.33million with an annual utilities cost of approximately $600,000.

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Appendices

Appendix A: Problem Statement
Appendix B: Fermentation Calculations and Simulation
Appendix C: MAA Separations Calculations and Simulation
Appendix D: MEK Separations Calculations and Simulation
Appendix E: Material Data Safety Sheets
Appendix F: Correspondence

APPENDIX A: PROBLEM STATEMENT

7. Renewable Bio-Monomer Pilot Plant
(recommended by Stephen M. Tieri, DuPont)

Global climate change, dwindling petroleum resources, and a desire for energy independence, have driven significant research and investment in the last decade to develop technologies that reduce energy consumption, improve efficiency, and produce materials and fuels from renewable resources. Industry stakeholders, including corporate executives, employees, and customers, are now insisting on the development and incorporation of sustainably produced materials, in place of traditional petroleum sourced chemicals and derivatives. Recent increases in oil prices are assisting in the growing economic viability and commercial potential for conversion of renewable raw materials to basic chemicals and functional intermediates. Biomass derived sugars provide opportunities for new, and potentially low cost, routes to chemical intermediates. However, the disposition of agricultural resources and production to support both this transition from petroleum to renewable fuels and provide food to meet the demands of the increasing global population is a continuing source of controversy and significant discussion.

Your company, through its research efforts, has developed new and novel bio-based, biocatalyzed, routes to commercially important intermediates and basic chemicals. Among the most promising new technologies developed at laboratory scale are routes to the methacrylic acid and methylethylketone (MEK). Your company strongly believes biobased sugars will provide a price stable feedstock alternative, and will positively contribute to the overall process environmental life-cycle analysis. In addition to being cost competitive with conventional processes-derived materials, the bio-derived monomers must provide the same level of purity and reactivity for producing polymers, downstream intermediates, and polymeric derivatives. Based on initial laboratory results and testing, company leadership has confidence that the new bio-process will be economically competitive and attractive, with the additional development work that naturally comes with standard technology commercialization programs. Business leadership is eager to proceed with the next phase in commercializing these technologies, and has agreed to fund a new facility to conduct pilot testing for these products and processes. Your team's objective is to design the pilot facility to demonstrate the operation of the technology and develop critical process data for commercial design to produce methacrylic acid and MEK from cane sugar supplies. The primary function of this pilot plant is to generate data applicable to potential commercial scale processes, in current corporate business forecasts for Brazil. While considerable development resources have been spent on the biocatalyst (microorganism), less work has been completed with respect to the subsequent separation and purification of the individual materials. Therefore, your team will need to identify the most commercially viable separation technologies to include in the process design for each individual material. Each product will need to be separated from the fermentation broth and purified to meet or exceed standard commercial concentrations and quality measurements for polymer grade material.

The facility is expected to provide equal quantities of each material for market demonstration testing, initial customer qualification testing, as well as produce representative waste material for

treatment approval and potential agronomic acceptance (if a stream similar to the Vinasse from a sugar cane ethanol production mill is identified).
Although the goal for pilot operation is data generation, a minimum of 30 M kg/yr of each individual monomer product (60 M kg/yr total) is expected to be necessary to complete the market demonstration and qualification testing. As the facility is expected to explore the bounds of the process technologies, the business is willing to accept reasonable uptimes for this type of work in the first two years of pilot operation. While operation of the processes at the pilot scale is not expected to hit all economic targets for commercial viability, it is necessary to provide corporate leadership with an estimate of the overall pilot program costs, for an expected 5-year pilot development program. Monomer product within commercial specifications can be sold at current market pricing, without restriction, to help defray the pilot operational costs, as raw materials and utilities will be charged at current market pricing. It is also expected that successful pilot trials will support validation of commercial scale economic estimates.

Corporate leadership has identified a partner willing to host the proposed pilot plant, and supply sufficient molasses, cane juice, and utilities to support the development testing. The pilot facility will be located on the site of a current sugar and ethanol facility in the Sao Paolo region of Brazil. While the pilot plant will not require separate raw material storage facilities and utilities, the design will need to include the equipment reasonably necessary to transfer materials from the main plant's current storage and supply facilities. Based on the standard cane crushing season, it is expected that you will have access to a sugar supply from the plant's main storage tanks for 9 months/yr.

The pilot-plant equipment should allow for flexibility to investigate a wide range of operating conditions and to identify optimal process conditions for use in the future commercial facility, for each individual product.
Process parameters which are expected to be explored during the course of pilot trials include, but are not limited to, sugar feed concentration (molasses only, cane juice, or mixture), fermentation productivity, and separation process configuration.

The pilot facility is not expected to be a "showplace" facility, where external people are routinely given tours and used as a "sales device", but one where the technology and full process are demonstrated, studied, and optimized to produce a robust technology package for future commercialization. While corporate and business leadership understand that this facility is expected to handle the development work for several new technologies, explore optimal ranges of multiple processes, and handle hazardous materials, the budget is not unlimited, and there is an expectation that most (if not all) equipment will be shared between both products. However, due to the hazards present inherently with some of the products, it is critical that the pilot equipment safely handle all of the intended products and any isolated intermediates.

The pilot-plant design should be as environmentally-friendly as possible, and as required by state and federal emissions legislation. Recover and recycle process materials to the maximum economic extent. Also, energy consumption should be minimized, to the extent economically justified. The pilot-plant design must also be controllable and safe to operate. As the process technology integration and design team, you will be there for the start-up and will have to live with whatever design decisions you have made. You will need additional data beyond that given

here and listed in the references below. Cite any literature data used. If required, make reasonable assumptions, state them, and
state whether your design or economics are sensitive to the assumptions you have made.

References

• 20090275096 Microorganisms for the Production of Methacrylic Acid (US Patent Application)
• 20100184173 Microorganisms for the Production of Methyl Ethyl Ketone and 2-Butanol (U.S. Patent Application)
• 20080199926 Methods and Organisms for Growth-Coupled Production of 3-Hydroxypropionic Acid
• 20070111294 Methods and Organisms for the Growth-Coupled Production of Succinate
• http://www.genomatica.com
Web resource for sugar & sugar solution properties (including molasses), with references for items common to the sugar and ethanol industries.
http://www.sugartech.co.za/matlprop/index.php

APPENDIX B: FERMENTATION SAMPLE CALCULATIONS

MEK Process Sample Calculations

Cell Growth Parameters:

Glucose Uptake Rate = 0.01 mol/g-hr
MEK yield = 1 mol MEK/1 mol glucose
Growth Rate = $0.7hr^{-1}$
CDW/cell = $3*10^{-3}$ g/cell
Duration of lag phase = 1hr

Assume these initial conditions for the seed fermentor:

V = 5L (working volume)
Medium = 1:4 Molasses, 3:4 Water
Glucose Equivalent in Undiluted Molasses = 0.8653kg/L = 4.8 mol glucose equivalent/L
Initial Total Glucose = 5L*4.8mol glucose/L*(1/4) = 6 mol glucose
Growth Phase of Seed Fermentor:
Cell Concentration = $X = X_0 e^{-\mu(t-t_L)}$

where X is the cell concentration, X_0 is the initial cell concentration, μ is the growth rate (hr^{-1}), t is the time since inoculation of the culture, and t_L is the duration of the lag phase (hr)

Glucose Consumption in time interval Δt = $\Delta S = X * v * V * \Delta t$

where ΔS is the change in glucose substrate (mol), X is the cell concentration (cells/L), v is the glucose uptake rate (mol/cell-hr), V is the volume of the bioreactor (L), and Δt is the length of the time interval.

Cell Concentration and Glucose Consumption in Each Fermentor

Calculate the total glucose and cell concentration in 0.1hr increments. Sample results are presented on the next page for the first few hours of operation in the 5L fermentor.

Time	Cell Conc. (cells/L)	Total Glucose (mol)
0	1.00E+09	6.00E+00
0.1	1.00E+09	6.00E+00
0.2	1.00E+09	6.00E+00
0.3	1.00E+09	6.00E+00
0.4	1.00E+09	6.00E+00
0.5	1.00E+09	6.00E+00
0.6	1.00E+09	6.00E+00
0.7	1.00E+09	6.00E+00
0.8	1.00E+09	6.00E+00
0.9	1.00E+09	6.00E+00
1	1.00E+09	6.00E+00
1.1	1.07E+09	6.00E+00
1.2	1.15E+09	6.00E+00

1.3	1.23E+09	6.00E+00
1.4	1.32E+09	6.00E+00
1.5	1.42E+09	6.00E+00
1.6	1.52E+09	6.00E+00
1.7	1.52E+09	6.00E+00
1.8	1.52E+09	6.00E+00
1.9	1.88E+09	6.00E+00
2	2.01E+09	6.00E+00
2.1	2.16E+09	6.00E+00
2.2	2.32E+09	6.00E+00
2.3	2.48E+09	6.00E+00
2.4	2.66E+09	6.00E+00
2.5	2.66E+09	6.00E+00
2.6	2.66E+09	6.00E+00
2.7	3.29E+09	6.00E+00
2.8	3.53E+09	6.00E+00
2.9	3.78E+09	6.00E+00
3	4.06E+09	6.00E+00
3.1	4.35E+09	6.00E+00
3.2	4.66E+09	6.00E+00
3.3	4.66E+09	6.00E+00
3.4	4.66E+09	6.00E+00
3.5	5.75E+09	6.00E+00
3.6	6.17E+09	6.00E+00
3.7	6.62E+09	6.00E+00
3.8	7.10E+09	6.00E+00
3.9	7.61E+09	6.00E+00
4	8.17E+09	6.00E+00

After 16.45hrs, the cell concentration reaches the optimal level of $5x10^{13}$ cells/L and the remaining glucose present in the fermentor totals 4.9 mol.

When expanding to the second fermentor with a 50L working volume, the initial cell concentration is thus ($5x10^{13}$ cells/L*5L)/50L = $5x10^{12}$ cells/L. However, due to plasmid instability, the initial cell concentration for the second fermentor was determined as $5x10^{12}$*0.9 = 4.5cells/L.

In the 50L working volume, 45L is fresh molasses medium while 5L has partially consumed glucose. The initial glucose concentration for the subsequent fermentor is thus the following when using molasses diluted 1:4 -

(45L*1.2mol/L + 4.9mol)/50 = 1.178mol/L

This is a small difference from the initial concentration of 1.2mol/L, but in larger fermentors with higher glucose concentration, this calculation of initial concentration becomes non trivial.

Once these values are calculated, the cell concentration and total glucose in the 50L fermentor should be calculated in 0.1 hr increments again. A similar series of calculations should be calculated for the 500L fermentor and then the 2500L fermentor.

Fed-Batch Flow Rate

In the last fermentor, the number of cells should be used to determine the glucose conversion rate within the fermentor. In this case there is $2500L*5x10^{13}$cells/L*$3x10^{-16}$ kg/cell = 37.5 kg of cell mass available. Assuming the glucose uptake rate is equal to the conversion rate, the conversion rate is

37.5kg cell mass * 10 mol/kg cell-hr = 67.56 kg/hr

To calculate the fed batch rate in order to maintain a constant glucose concentration, the glucose concentration in the selected dilution of molasses should be divided by the glucose conversion rate. For instance, if the feed stream is a 1:4 dilution of molasses, which contains 0.2163kg glucose equivalent/L, the feed stream flow rate must be

67.56/0.2163 = 312 L/hr.

A fed batch time should then be determined in order to calculate the total amount of

Finally, the MEK production can be calculated by a simple stochiometric calculation. If, as is the case in the chemical production fermentor, a total of 582kg of glucose have been consumed, MEK production is equal to

582kg*(mol glucose/.18016 kg)*(1mol MEK/1 mol glucose)*(.07211kg/mol MEK) = 239.2 kg MEK/batch

The remaining glucose which is not converted to MEK is assumed to be converted to CO_2 and H_2O.

Heat Evolution

Finally, the heat evolution in each fermentor is calculated using the following parameters:

$\Delta H_{Glucose}$ = 15.57kJ/g

ΔH_{Cells} = 24.04 kJ/g cells

$Y_{X/S}$ = yield coefficient = Δcell mass / Δglucose

In the first 5L working volume fermentor.

$Y_{X/S}$ = 74.96/199.45 = 0.38 g cells / g glucose

$Y_H = Y_{X/S} / (\Delta H_{Glucose} - Y_{X/S} * \Delta H_{Cells})$

$Y_{X/S} / Y_H$ = 6.53 kJ released / g glucose consumed

So heat evolved in the seed fermentor is = glucose consumed * ($Y_{X/S} / Y_H$) = 1303.5 kJ

The batch time for the first fementor is 16.5hr, so the heat generated per time is equal to 1303.5 kJ/(16.5hr*3600s) = 0.0219 kJ/s

APPENDIX C: MAA SEPARATIONS: SAMPLE CALCULATIONS

Sample Calculations

The following sections outline the basic calculations used to find some of the various operating parameters necessary to effectively cost the five year operation of the plant. If multiple of the same unit was used only one calculation will be shown Unless otherwise stated, all calculations were done using equations in Product and Process Design Principles by Seider *et al*.

Utilities

Cooling Water Condensers in MAA COL-01

Heat Duty in COL-01 = -530.31*10^3 BTU/hr

Subcooled Heat Duty in COL-01 = -4.103*10^3 BTU/hr

Cooling Water In = 90˚F Cooling Water Out = 120˚F

C_P of water = 1 BTU/ lb-˚F

$$Q = M_{H_2O} C_P \Delta T$$

$$\frac{Q_{Total}}{C_P \Delta T} = \frac{530.31 * 10^3 + 4.103 * 10^3}{1 * (120 - 90)} = 17{,}813 \frac{lb}{hr} \; of \; H_2O = M_{H_2O}$$

Cost of Cooling Water = \$0.075/1,000 gal

Density of Water = 8.33 lb/gallon

$$Cost \; of \; CW = \frac{M_{H_2O}}{\rho_{H_2O}} * \frac{\$}{gal} = \frac{17{,}813}{8.33} * \frac{0.075}{1000} = \$0.16038/hr$$

Total Operating Time for MAA = 3080 hours

Total Operating Time for MEK = 4840 hours

Operating Parameter for 12 hours/day, 5 days/week = 2.8

$$\frac{Cost}{yr} = \frac{Cost}{hr} * \frac{total\ \#\ of\ hrs}{Operating\ Parameter} = \frac{\$0.00369}{hr} * (\frac{3080}{2.8}) =$$

Total Cooling Water Cost for Condensers = $176.43/year

Steam Reboilers in MAA COL-01

Operating Temperature = 341˚F

Heat Duty = 578.17*10^3 BTU/hr

Saturation Temperature of High Pressure Steam at 450 psig ≈ 450˚F.

Using a valve, lower steam pressure to 195.6 psig and temperature of 386˚F

ΔH_{Vap} for steam at these conditions = 1198.98 BTU/lb

$$Q = M_{Steam} * \Delta H_{Vap}$$

$$\frac{Q}{\Delta H_{Vap}} = 1198.98 = 482.22 \frac{lb}{hr}\ of\ steam = M_{Steam}$$

Cost of high pressure steam = $6.60.00/1,000 lb

Total Operating Time for MAA = 3080 hours

Operating Parameter of 11 hr/day, 5 days/week = 3.05454

Note : Heating Utilities will be turned off to shutdown for the day but not cooling utilities, as they are needed to condense the remaining vapor in the system.

$$\frac{Cost}{yr} = \frac{lb}{hr} * \frac{Cost}{lb} * total\#of\ hrs = 482.22 * \frac{6.63}{1000} * \frac{3080}{3.05454} =$$

Total Cost of Steam in COL-01 = $3,209.19/year

Chilled Brine Chiller for MAA Product

Need to chill MAA to 17 ˚C

Cannot used cooling water so use a chilled brine

Heat Duty to chill product = 8058 BTU/hr

Brine has 12,000 BTU/hr = 1 ton-day

Hours = 3080 hr, Operating Parameter = 3.36 (10 hr/day, 5 days/week producing MAA)

$$Ton - days\ needed = \frac{8058}{12{,}000} * \frac{3080}{3.36} = 615.54\ ton - days/year$$

Cost of Chilled Brine = $1.70/ton-day

Cost of Refrigeration = $1,046.42/year

Electricity Consumption for Pumps

Due to the low flow rates, the HP usage was set at the designated minimum possible for the pump selected

HP_{MIN} = 0.5

Conversion → 0.7457kW = 1HP

$$kWh = kW * Time\ in\ use = \frac{0.5 * 0.1457 * 3080}{2.8} = 410.14\ kWh/year$$

Cost – $0.06/kWh

Total Cost for year = $24.61/year

Equipment Sizing

Reflux Accumulator (COL-01)

Desired Design Specification – Length/Diamter=2

Accumulator should be half full

Accumulator should hold at least 10 minutes of solution

Distillate Mass Flow = 288.793 kg/hr Distillate Volumetric Flow = 0.2103 ft^3/min

$$R = \frac{Liquid\ Returned}{Liquid\ Drawn\ Off\ (Distillate)} = \frac{L}{D} = 2$$

$$Total\ Condensed\ Flow = 2 * D + D = .6309\ ft^3/min$$

So the reflux accumulator needs to hold 20 minutes or 12.618 ft^3

$$Volume = \frac{\pi d^2 L}{4} = \frac{\pi d^3}{2} \rightarrow d = \sqrt[3]{\frac{2V}{\pi}} = 2.00\ ft = Diameter$$

Diameter = 2ft

Length = 4 ft

Condenser (COL-01)

$$Q = UA\Delta T_{LM}$$

Q = 534.41*10^3 BTU/hr

U $\approx$ 150 BTU/(˚F-ft^2-hr) from Table 18.5

T_{CI} = 90˚F, T_{CO} =120 ˚F, T_{HI} = 254.75˚F, T_{HO} =201.11 ˚F

$$\Delta T_{LM} = \frac{(T_{HO} - T_{CI}) - (T_{HI} - T_{CO})}{\ln \frac{(T_{HO} - T_{CI})}{(T_{HI} - T_{CO})}} = \frac{(201.11 - 90) - (254.75 - 120)}{\ln \frac{(201.11 - 90)}{(254.75 - 120)}} = 122.55\ ^\circ F$$

$$A = \frac{Q}{U\Delta T_{LM}} = \frac{534{,}410}{150 * 122.55} = 31.24\ ft^2$$

Due to low area of condenser, use a double pipe heat exchanger

$$C_B = \exp(7.146 + 0.16[\ln(A)])\ , where\ A\ is\ the\ area\ in\ ft^2$$

$$C_P = F_p F_M C_B$$

F_P is a pressure factor, but since the condenser operates at 17 psia, it is assumed F_P = 1

F_M is a material factor which is 3 when both sides are stainless steel

$$C_P = 1 * 3 * \exp(7.146 + 0.16[\ln(31.24)])$$

C_P = $6,603.10 for the condenser on COL-01

Reboiler (COL-01)

Heat Duty = 578.17*10^3 BTU/hr

Flux ≈ 12,000 BTU/ft^2-hr

Mass of steam needed =482.22 lb/hr of high pressure steam

$$A = \frac{Q}{Flux} = \frac{578{,}170}{12{,}000} = 48.18\ ft^2$$

Due to low area of reboiler, use a double pipe heat exchanger

$$C_B = \exp(7.146 + 0.16[\ln(A)])\ , where\ A\ is\ the\ area\ in\ ft^2$$

$$C_P = F_p F_M C_B$$

F_P is a pressure factor, but since the reboiler operates at 195 psia, it is assumed $F_P = 1$

F_M is a material factor which is 3 when both sides are stainless steel

$$C_P = 1 * 3 * \exp(7.146 + 0.16[\ln(48.18)])$$

C_P = $7,077.02 for the reboiler on COL-01

COL-01 with Packing

Using Pressure Vessels estimates in Seider, as the range of sizes were closer and more appropriate to our needs

Diameter =20 in = 1.667 ft – Predicted by ASPEN and rounded up to the closest nominal pipe size

Height = #stages*HETP, where HETP is assumed to be 2 ft for Flexiring random packing

Height =13*2=26 ft

Thickness = 0.25 inches, the feed is non-corrosive and the vessel is not operating at elevated pressures

Density of steel = 490 lb/ft^3

$$Weight, W = \pi(D_i + t_s)(L + 0.8D_i)t_s\rho$$

$$Weight, W = \frac{\pi\left(\frac{20}{12} + \frac{0.25}{12}\right)\left(26 + 0.8\frac{20}{12}\right)0.25}{12}490 = 1479.25\ lb\ of\ steel$$

Estimate cost based on weight

$$C_P = F_M C_v + C_{PL}$$

$$C_v = \exp\{7.0132 + 0.1255[\ln(W)] + 0.02297[\ln(W)]^2\}$$

$$C_v = \exp\{7.0132 + 0.1255[\ln(1479.25)] + 0.02297[\ln(1479.25)]^2\} = \$14{,}321.50$$

F_M = 2.1 for 316 Stainless Steel

$$C_{PL} = 361.8(D_i)^{0.7396}(L)^{0.70684}$$

$$C_{PL} = 361.8(\frac{20}{12})^{0.7396}(26)^{0.70684} = \$5{,}280.90$$

$$C_P = 2.1 * 14{,}321.50 + 5{,}280.90 = \$35{,}356.08$$

Packing

Estimated Cost of Packing =$\$56/ft^3$ of column

$$Volume = \frac{\pi D^2}{4} H = \pi \left(\frac{20}{12}\right)^2 * \frac{26}{4} = 56.723 ft^3$$

$$Cost\ of\ Packing = 56.723 * 56 = \$3{,}176.50$$

Total cost of tower with packing = $38,532.58

LLE-01

This piece of equipment was not estimated. A quote was received from Koch Module Process Systems LLC for a Scheibel column.

APPENDIX D: MEK SEPARATIONS CALCULATIONS AND SIMULATION

Pervaporation

The calculations for the pervaporation step in the MEK separation process were based on data from Pervatech (ref appendix). The results of these calculations are summarized in Table 1. A number of parameters had to be specified for the in order for our system to work. The effectiveness of a polymeric membrane in separating a liquid mixture is characterized by two parameters – flux and separation factor. The flux is expressed as the amount of permeate collected per unit time per unit membrane area ($\frac{kg}{m^2 h}$) whilst the *separation factor, process enrichment,* are represented by α and β respectively.

$$Separation\ factor, \alpha = \frac{x_{permeate,MEK}/(x_{permeate,water})}{x_{feed,MEK}/(x_{feed,water})}$$ Dutta, D. K. (1997). (1)

$$Process\ Enrichment, \beta = \frac{x_{permeate,MEK}}{x_{feed,MEK}}$$ (Smetana, 1995) (2)

where, $x_{permeate,MEK}$ – the mass fraction of MEK in the permeate stream.

$x_{feed,MEK}$ -- the mass fraction of MEK in the feed stream.

$x_{permeate,water}$ – the mass fraction of water in the permeate stream.

$x_{feed,water}$ -- the mass fraction of water in the feed stream.

We used the feed and permeate compositions of water and MEK to establish the process selectivity, α and the process enrichment, β for the membranes we going to use for the given conditions (appendix). Smetana et al. indicated that the permeate flux would change proportionally with the feed mass flow rate of MEK and this was confirmed by Liu et al. So we estimated that for a fixed feed flow-rate, the permeate flux would scale up

proportionally with the mass fraction of MEK, in the feed stream, $x_{MEKfeed}$ in order to get a good idea of how much flux. After this it was assumed that the mass fraction of the MEK in the permeate stream would remain constant at 99.8 % purity as specified by the manufacturer of the pervaporation modules, Pervatech. Below is a sample calculation of how the permeate flux of MEK was obtained for the first pervaporation membrane (see figure 2) in line two of table 1:

$$\frac{x_{feed}}{x_{feed,ref}} m_{p,ref} = m_p \ (3)$$

$$\frac{0.875}{0.114} * 5.24 \frac{kg}{m^2 h} = 40.20 \ \frac{kg}{m^2 h} \ (4)$$

Liquid System [WATER/MEK]	Membrane	Temp/°C	$P_{permeate}$ (mbar)	P_{feed} (bar)	$X_{feed,MEK}$	$X_{permeate,MEK}$	Process Selectivity, α	Process Enrichment, β	m_p [kg/m2h]	$m_{p,MEK}$ [kg/m2h]	$m_{p,H2O}$ [kg/m2h]
Pervatech Data	MF0179	60	21	1	0.114	0.998	4139	8.75	6.24	6.22	0.01
PM01	MF0179	60	21	5	0.875	0.998	71.55	1.14	48.05	47.95	0.10

Table 8: The summary of the pervaporation process calculations, including the permeate fluxes, the feed and permeate compositions of MEK.

Area Calculation

In order to calculate the area of the membrane required to separate out the MEK from water, the following equation was utilized:

$$\frac{permeate\ flowrate\ (\frac{kg}{hr})}{permeate\ flux\ (\frac{kg}{m^2 hr})} = Area\ (m^2) \ (5)$$

In this paper, for a total permeate flow-rate of 23.04 kg/hr, with a composition of 87.5 wt% MEK the membrane area was determined as follows:

$$\frac{23.04\ kg/hr}{48.05\ kg/hr} = \ 0.48 m^2$$

Membrane Performance Calculations

Equation 1 establishes a relationship between permeate flux and temperature, which shows a direct dependence of flux on temperature.

$m_p(T) = m_p(T_o)\exp(\frac{E_{MEK}}{R}\left(\frac{1}{T_o} - \frac{1}{T}\right))$ (HoÈmmerich Uwe, 1998) **Equation 1**

The energy balance was estimated by assuming the entrance stream to each pervaporator to be the reference point. The retentate stream experienced an exothermic change in enthalpy and the permeate an endothermic change due to the vaporization of MEK.

The enthalpy change of the permeate stream was calculated by this equation:

$$\Delta H_v = \ \Delta H_p = \ C_{vap} * m_p$$

Where, ΔH_v – Enthalpy heat of vaporization.

Q – Enthalpy heat of permeation

C_{vap} - The specific heat of vaporization

m_p – Permeate flow rate

In order to calculate the temperature change along the pervaporator Enthalpy heat of permeation, Q was divided into 5 equal portions corresponding to equally spaced segments in the membrane. Temperature was then obtained via the following equation:

$$T_{x/l} = T_o - \frac{Q_{x/l}}{(c_{p,MEK} + \ C_{p,water})}$$

Where,

$T_{\frac{x}{l}}$ - Temperature a location x of a module with length l.

T_o - Temperature at the inlet.

$Q_{\frac{x}{l}}$ - Heat of vaporization at location x of the module with length l .

$c_{p,MEK}$ - specific heat capacity of MEK.

$C_{p,water}$ – specific heat capacity of water.

Table 2 below shows the results of the calculations for temperature change along the pervaporator.

	Membrane Perfomance				
length	x	Q(L/x)	M(water)	M(MEK)	T(L/(x)
m		kJ/hr	kg/hr	kg/hr	°C
0.000	0	0	3.276	23.00077	350.0
0.100	1	399.3	3.276	23.00077	343.6
0.200	2	798.7	3.276	23.00077	337.3
0.300	3	1198.0	3.276	23.00077	330.9
0.400	4	1597.4	3.276	23.00077	324.6
0.500	5	1996.7	3.276	23.00077	318.2

Table 9: The pervaporator performance as a function of length along the module for PM01.

APPENDIX E: MATERIAL DATA SAFETY SHEETS

MSDS Number: **B6184** * * * * * *Effective Date:* ***09/16/09*** * * * * * *Supercedes:* ***08/02/07***

MSDS Material Safety Data Sheet

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

Mallinckrodt CHEMICALS | J.T.Baker

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

National Response in Canada
CANUTEC: 613-996-6666

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

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

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

N-BUTYL ACETATE

1. Product Identification

Synonyms: 1-Butyl acetate; butyl ethanoate; acetic acid, n-butyl ester
CAS No.: 123-86-4
Molecular Weight: 116.16
Chemical Formula: C6H12O2
Product Codes:
J.T. Baker: 5365, 5681, 9159, 9161, 9164, 9171, 9173, 9191, D683
Mallinckrodt: 1820, H984

2. Composition/Information on Ingredients

Ingredient	CAS No	Percent	Hazardous
n-Butyl Acetate	123-86-4	90 - 100%	Yes

3. Hazards Identification

Emergency Overview

WARNING! FLAMMABLE LIQUID AND VAPOR. HARMFUL IF SWALLOWED OR INHALED. CAUSES SEVERE IRRITATION TO EYES. CAUSES IRRITATION TO SKIN AND RESPIRATORY TRACT. AFFECTS CENTRAL NERVOUS SYSTEM.

SAF-T-DATA(tm) Ratings (Provided here for your convenience)

Health Rating: 2 - Moderate (Life)
Flammability Rating: 2 - Moderate
Reactivity Rating: 1 - Slight
Contact Rating: 3 - Severe (Life)
Lab Protective Equip: GOGGLES & SHIELD; LAB COAT & APRON; VENT HOOD; PROPER GLOVES; CLASS B EXTINGUISHER
Storage Color Code: Red (Flammable)

Potential Health Effects

Inhalation:
Causes irritation to the respiratory tract. Symptoms may include coughing, shortness of breath. High concentrations have a narcotic effect.
Ingestion:
Irritant to tissues. Sore throat, abdominal pain, nausea, vomiting, diarrhea are the symptoms. Expected to have a narcotic effect. One ounce may produce severe poisoning.
Skin Contact:
This material degreases the skin. Irritation and discoloration of the skin are symptoms. Skin allergy occasionally develops. Persons who have become allergic can develop rash upon future exposure to low levels.
Eye Contact:
Vapors cause eye irritation. Splashes cause severe irritation, possible corneal burns and eye damage.
Chronic Exposure:
Repeated or prolonged skin contact may defat the skin and produce irritation and dermatitis. Kidney and liver damage are reported in animals.
Aggravation of Pre-existing Conditions:
Persons with pre-existing skin disorders or eye problems, or impaired liver, kidney or respiratory function may be more susceptible to the effects of the substance.

---

4. First Aid Measures

Inhalation:
Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.
Ingestion:
Induce vomiting immediately as directed by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention.
Skin Contact:
Immediately flush skin with plenty of soap and water for at least 15 minutes. Remove contaminated clothing and shoes. Get medical attention. Wash clothing before reuse. Thoroughly clean shoes before reuse.
Eye Contact:
Immediately flush eyes with plenty of water for at least 15 minutes, lifting lower and upper eyelids occasionally. Get medical attention immediately.

---

5. Fire Fighting Measures

Fire:
Flash point: 26C (79F) CC
Autoignition temperature: 425C (797F)
Flammable limits in air % by volume:
lel: 1.7; uel: 7.6
Explosion:
Above flash point, vapor-air mixtures are explosive within flammable limits noted above. Vapors can flow along surfaces to distant ignition source and flash back. Sensitive to static discharge.
Fire Extinguishing Media:
Dry chemical, alcohol foam or carbon dioxide. Water may be ineffective. Water spray may be used to keep fire exposed containers cool.
Special Information:
In the event of a fire, wear full protective clothing and NIOSH-approved self-contained breathing apparatus with full facepiece operated in the pressure demand or other positive pressure mode.

---

6. Accidental Release Measures

Ventilate area of leak or spill. Remove all sources of ignition. Wear appropriate personal protective equipment as specified in Section 8. Isolate hazard area. Keep unnecessary and unprotected personnel from entering. Contain and recover liquid when possible. Use non-sparking tools and equipment. Collect liquid in an appropriate container or absorb with an inert material (e. g., vermiculite, dry sand, earth), and place in a chemical waste container. Do not use combustible materials, such as saw dust. Do not flush to sewer! US Regulations (CERCLA) require reporting spills and releases to soil, water and air in excess of reportable quantities. The toll free number for the US Coast Guard National Response Center is (800) 424-8802. If a leak or spill has not ignited, use water spray to disperse the vapors, to protect personnel attempting to stop leak, and to flush spills away from exposures.

J. T. Baker SOLUSORB® solvent adsorbent is recommended for spills of this product.

---

7. Handling and Storage

Protect against physical damage. Store in a cool, dry well-ventilated location, away from any area where the fire hazard may be acute. Outside or detached storage is preferred. Separate from incompatibles. Containers should be bonded and grounded for transfers to avoid static sparks. Storage and use areas should be No Smoking areas. Use non-sparking type tools and equipment, including explosion proof ventilation. Do Not attempt to clean empty containers since residue is difficult to remove. Do not pressurize, cut, weld, braze, solder, drill, grind or expose such containers to heat, sparks, flame, static electricity or other sources of ignition: they may explode and cause injury or death. Containers of this material may be hazardous when empty since they retain product residues (vapors, liquid); observe all warnings and precautions listed for the product.

---

8. Exposure Controls/Personal Protection

Airborne Exposure Limits:
-OSHA Permissible Exposure Limit (PEL):
150 ppm (TWA)
- ACGIH Threshold Limit Value (TLV)
150 ppm (TWA), 200 ppm (STEL)
Ventilation System:
A system of local and/or general exhaust is recommended to keep employee exposures below the Airborne Exposure Limits. Local exhaust ventilation is generally preferred because it can control the emissions of the contaminant at its source, preventing dispersion of it into the general work area. Please refer to the ACGIH document, *Industrial Ventilation, A Manual of Recommended Practices*, most recent edition, for details. Use explosion-proof equipment.
Personal Respirators (NIOSH Approved):
If the exposure limit is exceeded, a half-face organic vapor respirator may be worn for up to ten times the exposure limit or the maximum use concentration specified by the appropriate regulatory agency or respirator supplier, whichever is lowest. A full-face piece organic vapor respirator may be worn up to 50 times the exposure limit or the maximum use concentration specified by the appropriate regulatory agency or respirator supplier, whichever is lowest. For emergencies or instances where the exposure levels are not known, use a full-face piece positive-pressure, air-supplied respirator. WARNING: Air-purifying respirators do not protect workers in oxygen-deficient atmospheres.
Skin Protection:
Wear impervious protective clothing, including boots, gloves, lab coat, apron or coveralls, as appropriate, to prevent skin contact.
Eye Protection:

Maintain eye wash fountain and quick-drench facilities in work area. Use chemical safety goggles and/or full face shield where dusting or splashing of solutions is possible.

---

9. Physical and Chemical Properties

Appearance:
Clear, colorless liquid.
Odor:
Mild, fruity odor.
Solubility:
Slightly soluble in water (ca. 0.7% @ 20C)
Specific Gravity:
0.8822 at 20C/20C
pH:
No information found.
% Volatiles by volume @ 21C (70F):
100
Boiling Point:
126C (259F)
Melting Point:
-77C (-107F)
Vapor Density (Air=1):
4.0
Vapor Pressure (mm Hg):
15 @ 25C (77F)
Evaporation Rate (BuAc=1):
1

---

10. Stability and Reactivity

Stability:
Stable under ordinary conditions of use and storage.
Hazardous Decomposition Products:
Carbon dioxide and carbon monoxide may form when heated to decomposition.
Hazardous Polymerization:
Will not occur.
Incompatibilities:
Dangerous when exposed to heat or flame; can react with oxidizing materials, strong alkalis, acids, nitrates and potassium-tert-butoxide.
Conditions to Avoid:
Heat, flames, ignition sources and incompatibles.

---

11. Toxicological Information

Toxicological Data:
Oral rat LD50: 10.8 g/kg;
inhalation rat LC50: 390 ppm/4H
Skin rabbit LD50: >17,600 mg/kg;
Irritant, skin rabbit (Std. Draize): 500 mg/24H, moderate. Irritant, eye rabbit: 100 mg moderate.
Investigated as a reproductive effector.
Reproductive Toxicity:
Has shown teratogenic effects in laboratory animals.

```
  --------\Cancer Lists\-----------------------------------------------------
-
                                          ---NTP Carcinogen---
  Ingredient                              Known    Anticipated    IARC
Category
  ----------------------------------   -----    -----------    ------------
-
  n-Butyl Acetate (123-86-4)             No          No            None
```

12. Ecological Information

Environmental Fate:
When released into the soil, this material is expected to readily biodegrade. When released into the soil, this material may leach into groundwater. When released into the soil, this material is expected to have a half-life of less than 1 day. When released into water, this material is expected to readily biodegrade. When released into the water, this material is expected to have a half-life between 1 and 10 days. This material has an estimated bioconcentration factor (BCF) of less than 100. When released into the air, this material may be moderately degraded by reaction with photochemically produced hydroxyl radicals.
Environmental Toxicity:
96 Hr LC50 fathead minnow: 18 mg/L (flow-through);
96 Hr LC50 bluegill: 100 mg/L (Static);
96 Hr EC50 freshwater algae (Scenedesmus subspicatus): 320 mg/L;
48 Hr EC50 water flea: 44 mg/L.

13. Disposal Considerations

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

14. Transport Information

Domestic (Land, D.O.T.)

Proper Shipping Name: BUTYL ACETATES
Hazard Class: 3
UN/NA: UN1123
Packing Group: III
Information reported for product/size: 400LB

International (Water, I.M.O.)

Proper Shipping Name: BUTYL ACETATES
Hazard Class: 3
UN/NA: UN1123
Packing Group: III
Information reported for product/size: 400LB

---

15. Regulatory Information

--------\Chemical Inventory Status - Part 1\----------

Ingredient	TSCA	EC	Japan	Australia
n-Butyl Acetate (123-86-4)	Yes	Yes	Yes	Yes

--------\Chemical Inventory Status - Part 2\----------

Ingredient	Korea	--Canada-- DSL	NDSL	Phil.
n-Butyl Acetate (123-86-4)	Yes	Yes	No	Yes

--------\Federal, State & International Regulations - Part 1\----------

Ingredient	-SARA 302- RQ	TPQ	------SARA 313---- List	Chemical Catg.
n-Butyl Acetate (123-86-4)	No	No	No	No

--------\Federal, State & International Regulations - Part 2\----------

Ingredient	CERCLA	-RCRA- 261.33	-TSCA- 8(d)

n-Butyl Acetate (123-86-4)	5000	No	No

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

Australian Hazchem Code: 3[Y]E
Poison Schedule: None allocated.
WHMIS:
This MSDS has been prepared according to the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all of the information required by the CPR.

---

16. Other Information

NFPA Ratings: Health: **2** Flammability: **3** Reactivity: **0**
Label Hazard Warning:
WARNING! FLAMMABLE LIQUID AND VAPOR. HARMFUL IF SWALLOWED OR INHALED. CAUSES SEVERE IRRITATION TO EYES. CAUSES IRRITATION TO SKIN AND RESPIRATORY TRACT. AFFECTS CENTRAL NERVOUS SYSTEM.
Label Precautions:
Keep away from heat, sparks and flame.
Keep container closed.
Use only with adequate ventilation.
Avoid contact with eyes, skin and clothing.
Wash thoroughly after handling.
Avoid breathing vapor.
Label First Aid:
In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes. Remove contaminated clothing and shoes. Wash clothing before reuse. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. If swallowed, induce vomiting immediately as directed by medical personnel. Never give anything by mouth to an unconscious person. In all cases, get medical attention.
Product Use:
Laboratory Reagent.
Revision Information:
No Changes.
Disclaimer:
****
Mallinckrodt Baker, Inc. provides the information contained herein in good faith but makes no representation as to its comprehensiveness or accuracy. This document is intended only as a guide to the appropriate precautionary handling of the material by a properly trained person using this product. Individuals receiving the information must

exercise their independent judgment in determining its appropriateness for a particular purpose. MALLINCKRODT BAKER, INC. MAKES NO REPRESENTATIONS OR WARRANTIES, EITHER EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION ANY WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE WITH RESPECT TO THE INFORMATION SET FORTH HEREIN OR THE PRODUCT TO WHICH THE INFORMATION REFERS. ACCORDINGLY, MALLINCKRODT BAKER, INC. WILL NOT BE RESPONSIBLE FOR DAMAGES RESULTING FROM USE OF OR RELIANCE UPON THIS INFORMATION.

****

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

MSDS Number: **M2548** * * * * * *Effective Date:* ***05/17/07*** * * * * * *Supercedes:* ***05/07/07***

MSDS Material Safety Data Sheet

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

J.T.Baker

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

National Response in Canada
CANUTEC: 613-996-6666

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

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

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

P-METHOXYPHENOL

1. Product Identification

Synonyms: Phenol, p-methoxy; hydroquinone monomethyl ether; p-Hydroxyanisole
CAS No.: 150-76-5
Molecular Weight: 124.14
Chemical Formula: C7H8O2
Product Codes: P886

2. Composition/Information on Ingredients

Ingredient	CAS No	Percent	Hazardous
4-Methoxyphenol	150-76-5	100%	Yes

3. Hazards Identification

Emergency Overview

WARNING! HARMFUL IF SWALLOWED, INHALED OR ABSORBED THROUGH SKIN. CAUSES IRRITATION TO SKIN, EYES AND RESPIRATORY TRACT. MAY AFFECT CENTRAL NERVOUS SYSTEM.

SAF-T-DATA(tm) Ratings (Provided here for your convenience)

Health Rating: 2 - Moderate (Life)
Flammability Rating: 1 - Slight
Reactivity Rating: 0 - None
Contact Rating: 3 - Severe
Lab Protective Equip: GOGGLES & SHIELD; LAB COAT & APRON; VENT HOOD; PROPER GLOVES
Storage Color Code: Green (General Storage)

Potential Health Effects

Inhalation:
Inhalation can cause irritation of mucous membranes and upper respiratory tract. Symptoms may include burning sensation, coughing, wheezing, laryngitis, shortness of breath, sore throat, bronchitis, pneumonitis, and possible pulmonary edema.
Ingestion:
Moderately toxic by ingestion. Causes irritation to the gastrointestinal tract. Symptoms may include nausea, vomiting, diarrhea, lowered blood pressure and cardiac arrhythmias. May cause central nervous system depression and possibly coma. Ingesting a > 5% solution may cause oral burns; ingesting 1g can cause death.
Skin Contact:
Causes irritation. Symptoms include redness, itching and pain. Skin may become ulcerated. Prolonged skin contact can cause burns, dermatitis and discoloration of the skin. May be absorbed through the skin. Symptoms may parallel those from ingestion exposure.
Eye Contact:
Causes irritation and may cause lesions that can lead to corneal erosion, partial or total vision loss. May also cause keratitis and discoloration of the conjunctiva.
Chronic Exposure:
No information found.
Aggravation of Pre-existing Conditions:
Persons with pre-existing skin or eye disorders or impaired respiratory function may be more susceptible to the effects of this substance.

4. First Aid Measures

Inhalation:
Remove to fresh air. Get medical attention for any breathing difficulty.
Ingestion:
If swallowed, DO NOT INDUCE VOMITING. Get immediate medical attention. Never give anything by mouth to an unconscious person. Avoid dilution since this may enhance phenol absorption.
Skin Contact:
Immediately flush skin with plenty of soap and water for at least 15 minutes while removing contaminated clothing and shoes. Get medical attention. Wash clothing before reuse. Thoroughly clean shoes before reuse.
Eye Contact:
Immediately flush eyes with plenty of water for at least 15 minutes, lifting lower and upper eyelids occasionally. Get medical attention immediately.

5. Fire Fighting Measures

Fire:
Flash point: 124C (255F) CC
Autoignition temperature: 421C (790F)
May pose a fire hazard when exposed to heat, flame, or oxidizing agents.
Explosion:
Not considered to be an explosion hazard.
Fire Extinguishing Media:
Water, carbon dioxide, or dry chemical.
Special Information:
Wear full protective clothing and breathing equipment for high-intensity fire or potential explosion conditions.

6. Accidental Release Measures

Remove all sources of ignition. Ventilate area of leak or spill. Wear appropriate personal protective equipment as specified in Section 8. Spills: Clean up spills in a manner that does not disperse dust into the air. Use non-sparking tools and equipment. Reduce airborne dust and prevent scattering by moistening with water. Pick up spill for recovery or disposal and place in a closed container.

7. Handling and Storage

Keep in a tightly closed container, stored in a cool, dry, ventilated area. Protect against physical damage. Isolate from any source of heat or ignition. Isolate from oxidizing materials. Protect from direct sunlight. Protect against moisture and air. Store at temperatures not exceeding 230C (110F) for any extended time since sintering (heat-induced cohering without melting) can occur.

Containers of this material may be hazardous when empty since they retain product residues (dust, solids); observe all warnings and precautions listed for the product.

8. Exposure Controls/Personal Protection

Airborne Exposure Limits:
- ACGIH Threshold Limit Value (TLV): 5 mg/m3 (TWA)

Ventilation System:
A system of local and/or general exhaust is recommended to keep employee exposures below the Airborne Exposure Limits. Local exhaust ventilation is generally preferred because it can control the emissions of the contaminant at its source, preventing dispersion of it into the general work area. Please refer to the ACGIH document, *Industrial Ventilation, A Manual of Recommended Practices*, most recent edition, for details.

Personal Respirators (NIOSH Approved):
If the exposure limit is exceeded and engineering controls are not feasible, a full facepiece particulate respirator (NIOSH type N100 filters) may be worn for up to 50 times the exposure limit or the maximum use concentration specified by the appropriate regulatory agency or respirator supplier, whichever is lowest. If oil particles (e.g. lubricants, cutting fluids. glycerine, etc.) are present, use a NIOSH type R or P filter. For emergencies or instances where the exposure levels are not known, use a full-facepiece positive-pressure, air-supplied respirator. WARNING: Air-purifying respirators do not protect workers in oxygen-deficient atmospheres.

Skin Protection:
Wear impervious protective clothing, including boots, gloves, lab coat, apron or coveralls, as appropriate, to prevent skin contact.

Eye Protection:
Use chemical safety goggles and/or full face shield where dusting or splashing of solutions is possible. Maintain eye wash fountain and quick-drench facilities in work area.

9. Physical and Chemical Properties

Appearance:
White waxy solid.
Odor:
No information found.
Solubility:
Soluble in water.
Specific Gravity:
1.55 @ 20C
pH:
No information found.
% Volatiles by volume @ 21C (70F):
0
Boiling Point:
243C (469F)
Melting Point:
57C (135F)
Vapor Density (Air=1):
4.29
Vapor Pressure (mm Hg):
19 @ 140C (284F)
Evaporation Rate (BuAc=1):
No information found.

---

10. Stability and Reactivity

Stability:
Stable under ordinary conditions of use and storage.
Hazardous Decomposition Products:
Carbon monoxide.
Hazardous Polymerization:
Will not occur.
Incompatibilities:
Sodium hydroxide and oxidizing agents.
Conditions to Avoid:
Heat, flames, ignition sources and incompatibles.

---

11. Toxicological Information

No LD50/LC50 information found relating to normal routes of occupational exposure. Irritation skin rabbit (std Draize): 6 gm/12D-I, mild. Investigated as a mutagen.

--------\Cancer Lists\----------

Ingredient	---NTP Carcinogen--- Known	Anticipated	IARC Category
4-Methoxyphenol (150-76-5)	No	No	None

12. Ecological Information

Environmental Fate:
No information found.
Environmental Toxicity:
No information found.

13. Disposal Considerations

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

14. Transport Information

Not regulated.

15. Regulatory Information

--------\Chemical Inventory Status - Part 1\----------

Ingredient	TSCA	EC	Japan	Australia

```
  4-Methoxyphenol (150-76-5)                          Yes  Yes   Yes      Yes

  --------\Chemical Inventory Status - Part 2\-------------------------------
--
                                                                --Canada--
  Ingredient                                        Korea  DSL   NDSL  Phil.
  ------------------------------------------------  -----  ---   ----  -----
  4-Methoxyphenol (150-76-5)                         Yes   Yes   No     Yes

  --------\Federal, State & International Regulations - Part 1\--------------
--
                                                -SARA 302-    ------SARA 313----
--
  Ingredient                                    RQ    TPQ     List  Chemical
Catg.
  ----------------------------------------  ---   -----   ----  ------------
--
  4-Methoxyphenol (150-76-5)                No    No      No         No

  --------\Federal, State & International Regulations - Part 2\--------------
--
                                                          -RCRA-    -TSCA-
  Ingredient                                CERCLA      261.33     8(d)
  ----------------------------------------  ------      ------    ------
  4-Methoxyphenol (150-76-5)                No          No         Yes


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

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

---

16. Other Information

NFPA Ratings: Health: **1** Flammability: **1** Reactivity: **0**
Label Hazard Warning:
WARNING! HARMFUL IF SWALLOWED, INHALED OR ABSORBED THROUGH SKIN. CAUSES IRRITATION TO SKIN, EYES AND RESPIRATORY TRACT. MAY AFFECT CENTRAL NERVOUS SYSTEM.
Label Precautions:
Avoid breathing dust or vapors.
Avoid contact with eyes, skin and clothing.
Keep container closed.

Use only with adequate ventilation.
Wash thoroughly after handling.

Label First Aid:

If swallowed, DO NOT INDUCE VOMITING. Give large quantities of water. Never give anything by mouth to an unconscious person. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. In case of contact, immediately flush eyes or skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. In all cases, get medical attention.

Product Use:

Laboratory Reagent.

Revision Information:

MSDS Section(s) changed since last revision of document include: 3.

Disclaimer:

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****

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

Material Safety Data Sheet

2-Butanone (Methyl Ethyl Ketone)

ACC# 14460

Section 1 - Chemical Product and Company Identification

MSDS Name: 2-Butanone
Catalog Numbers: AC149670000, AC149670025, AC149670051, AC149670250, AC149670251, AC213010000, AC213015000, AC327910000, AC327910010, AC327911000, AC389430000, AC389430010, AC389430025, AC389570000, AC389570010, AC389570025, 14967-0010, M208-1, M208-20, M208-4, M209-1, M209-20, M209-200, M209-4, M209-500, M209FB115, M209FB19, M209FB200, M209FB50, M209RB-115, M209RS19, M209RS200, M209RS28, M209RS50, M209S-4, M209SS115, M209SS200, M209SS28, M209SS50
Synonyms: Ethyl methyl ketone; Methyl ethyl ketone; MEK.
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
78-93-3	2-Butanone	99+	201-159-0

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: clear, colorless liquid. Flash Point: -7 deg C.
Danger! Flammable liquid and vapor. Breathing vapors may cause drowsiness and dizziness. Causes eye and skin irritation. Repeated exposure may cause skin dryness or cracking. May cause respiratory tract irritation. Aspiration hazard if swallowed. Can enter lungs and cause damage.
Target Organs: Kidneys, central nervous system, liver, eyes, skin.

Potential Health Effects

Eye: Causes eye irritation. Animal evidence suggests that MEK is a moderate to severe eye irritant.
Skin: Causes skin irritation. May be harmful if absorbed through the skin. Repeated or prolonged exposure may cause drying and cracking of the skin. Only one human case of skin sensitization was located. Negative results were obtained in an animal test; MEK did not produce skin sensitization in the mouse ear thickness test.
Ingestion: Aspiration hazard. May cause irritation of the digestive tract. May be harmful if swallowed. May cause lung damage. Animal evidence suggests that MEK can be aspirated (inhaled) into the lungs during ingestion or vomiting.
Inhalation: May cause respiratory tract irritation. May be harmful if inhaled. Inhalation of vapors may cause drowsiness and dizziness. Neurobehavioural effects of exposure to MEK (200 ppm for 4 hrs) were studied with 137 volunteers. There were no statistically significant effects observed in biochemical, psychomotor, sensorimotor and psychological tests.
Chronic: May cause liver and kidney damage. Adverse reproductive effects have been reported in animals.

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.
Skin: Get medical aid. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes.
Ingestion: Potential for aspiration if swallowed. Get medical aid immediately. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If vomiting occurs naturally, have victim lean forward.
Inhalation: Remove from exposure and move to fresh air immediately. If breathing is difficult, give oxygen. Get medical aid. Possible aspiration hazard. Do not use mouth-to-mouth resuscitation if victim ingested or inhaled the substance; induce artificial respiration with the aid of a pocket mask equipped with a one-way valve or other proper respiratory medical device.
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. Vapors may form an explosive mixture with air. Vapors can travel to a source of ignition and flash back. Will burn if involved in a fire. Containers may explode in the heat of a fire. Flammable liquid and vapor.
Extinguishing Media: Use water spray, dry chemical, carbon dioxide, or chemical foam.
Flash Point: -7 deg C (19.40 deg F)
Autoignition Temperature: 404 deg C (759.20 deg F)
Explosion Limits, Lower:1.4 vol %
Upper: 11.5 vol %
NFPA Rating: (estimated) Health: 1; Flammability: 3; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Wear a self contained breathing apparatus and appropriate personal protection. (See Exposure Controls, Personal Protection section). Remove all sources of ignition. Use a spark-proof tool. Do not let this chemical enter the environment.

Section 7 - Handling and Storage

Handling: Use spark-proof tools and explosion proof equipment. Do not get in eyes, on skin, or on clothing. Take precautionary measures against static discharges. Keep away from heat, sparks and flame. Do not ingest or inhale. Use only in a chemical fume hood.
Storage: Keep away from sources of ignition. Store in a cool, dry place. Store in a tightly closed container. Flammables-area.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Use explosion-proof ventilation equipment. Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use only under a chemical fume hood.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs

2-Butanone	200 ppm TWA; 300 ppm STEL	200 ppm TWA; 590 mg/m3 TWA 3000 ppm IDLH	200 ppm TWA; 590 mg/m3 TWA

OSHA Vacated PELs: 2-Butanone: 200 ppm TWA; 590 mg/m3 TWA
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: 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: Liquid
Appearance: clear, colorless
Odor: sweetish odor - characteristic odor
pH: Not available.
Vapor Pressure: 105 mbar @ 20 deg C
Vapor Density: 2.41 (air=1)
Evaporation Rate:3.7 (nBuAc=1)
Viscosity: 0.42 mPa @ 15 deg C
Boiling Point: 80 deg C @ 760 mmHg
Freezing/Melting Point:-87 deg C
Decomposition Temperature: Not available.
Solubility: Soluble.
Specific Gravity/Density:0.806
Molecular Formula:C4H8O
Molecular Weight:72.11

Section 10 - Stability and Reactivity

Chemical Stability: Stable under normal temperatures and pressures.
Conditions to Avoid: Incompatible materials, ignition sources, excess heat.
Incompatibilities with Other Materials: Ammonia, copper, nitric acid, sulfuric acid, isocyanates, hydrogen peroxide, amines, caustics, chlorosulfonic acid.
Hazardous Decomposition Products: Carbon monoxide, carbon dioxide.
Hazardous Polymerization: Will not occur.

Section 11 - Toxicological Information

RTECS#:
CAS# 78-93-3: EL6475000
LD50/LC50:
CAS# 78-93-3:
Draize test, rabbit, eye: 80 mg;
Draize test, rabbit, skin: 500 mg/24H Moderate;
Draize test, rabbit, skin: 402 mg/24H Mild;
Inhalation, mouse: LC50 = 32 gm/m3/4H;
Inhalation, rat: LC50 = 23500 mg/m3/8H;
Oral, mouse: LD50 = 3000 mg/kg;
Oral, rat: LD50 = 2737 mg/kg;
Skin, rabbit: LD50 = 6480 mg/kg;
.

Carcinogenicity:
CAS# 78-93-3: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: No information available.
Teratogenicity: Teratogenic effects have occurred in experimental animals.
Reproductive Effects: Adverse reproductive effects have occurred in experimental animals.
Mutagenicity: Mutation in microorganisms:See actual entry in RTECS for complete information.
Neurotoxicity: No information found
Other Studies:

Section 12 - Ecological Information

Ecotoxicity: Fish: Fathead Minnow: LC50 = 3220 mg/L; 96 Hr; UnspecifiedFish: Bluegill/Sunfish: LC50 = 1690 mg/L; 96 Hr; UnspecifiedBacteria: Phytobacterium phosphoreum: EC50 = 51.9 mg/L; 25 min; Microtox testBacteria: Phytobacterium phosphoreum: EC50 =3373 mg/L; 30 min; Microtox test No data available.
Environmental: Substance evaporates in water with T1/2= 3D (rivers) to 12D (lakes). Substance is not expected to bioconcentrate in aquatic organisms.
Physical: Substance photodegrades in air with T1/2 = 2.3 days. Oxidizes rapidly by photo-chemical reactions in air. Readily biodegradable meeting the 10 day window criterion. Not expected to bioaccumulate significantly.
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:
CAS# 78-93-3: waste number U159 (Ignitable waste, Toxic waste).

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	ETHYL METHYL KETONE	ETHYL METHYL KETONE
Hazard Class:	3	3
UN Number:	UN1193	UN1193
Packing Group:	II	II

Section 15 - Regulatory Information

US FEDERAL

TSCA
CAS# 78-93-3 is listed on the TSCA inventory.
Health & Safety Reporting List
CAS# 78-93-3: Effective 10/4/82, Sunset 10/4/92
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# 78-93-3: 5000 lb final RQ; 2270 kg final RQ

SARA Section 302 Extremely Hazardous Substances

None of the chemicals in this product have a TPQ.

SARA Codes

CAS # 78-93-3: immediate, delayed, fire.

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# 78-93-3 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:

XI F

Risk Phrases:

R 11 Highly flammable.
R 36 Irritating to eyes.
R 66 Repeated exposure may cause skin dryness or cracking.
R 67 Vapours may cause drowsiness and dizziness.

Safety Phrases:

S 16 Keep away from sources of ignition - No smoking.
S 9 Keep container in a well-ventilated place.

WGK (Water Danger/Protection)

CAS# 78-93-3: 1

Canada - DSL/NDSL

CAS# 78-93-3 is listed on Canada's DSL List.

Canada - WHMIS

This product has a WHMIS classification of B2, D2B.
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# 78-93-3 is listed on the Canadian Ingredient Disclosure List.

Section 16 - Additional Information

MSDS Creation Date: 7/21/1999
Revision #9 Date: 7/28/2008

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

APPENDIX F: CORRESPONDENCE WITH COMPANIES AND CONSULTANTS

PERVATECH
selective ceramic membranes
process design

Chemical and Biomolecular Engineering
University of Pennsylvania 11'
Att'n Mr. Tinatsei Prosper Ndoro
USA

Quotation 11.03.195-A
HybSi®

Enter, 5 April 2011

Subject: Module program for testing

Dear Mr. Tinatsei Prosper Ndoro,

Thank you very much for your request.
It's my pleasure to quote you as follows:

For correspondence or further information regarding this quotation, always mention the date, the Quotation number and Item description.

Position 1
4-tube module type PVM-043, including 4 ceramic HybSi pervaporation membranes.
Membrane surface area: 0,04 m^2.
Maximum process temperature for the membrane 150ºC.
Standard enclosure with O-ring EPDM-PC, max temperature 130ºC.
Kalrez O-rings are available upon request against extra costs.
Dimensions of the tubular ceramic membrane: 500 x 10 x 7 mm (Length x diameter outside x diameter inside), with the membrane coated on the inside of the ceramic tubes and the ends glazed.
Item price: € 5.250,00
Number of pieces: 1
Total position 1: € 5.250,00

Position 2
10-tube module type PVM-045, including 10 ceramic HybSi pervaporation membranes.
Membrane surface area: 0,1 m^2.
Maximum process temperature for the membrane 150ºC.
Standard enclosure with O-ring EPDM-PC, max temperature 130ºC.
Kalrez O-rings are available upon request against extra costs.
Dimensions of the tubular ceramic membrane: 500 x 10 x 7 mm (Length x diameter outside x diameter inside), with the membrane coated on the inside of the ceramic tubes and the ends glazed.
Item price: € 6.500,00
Number of pieces: 1
Total position 2: € 6.500,00

Pervatech BV			Telephone	: +31(0)547-383114
Rondweg 48	C of C	: 06088874	Fax	: +31(0)547-385153
7468MC ENTER	VAT	: NL807474411B01	E-mail	: info@pervatech.nl
The Netherlands	Bank	: ABN AMRO:53.00.24.802	web site	: www.pervatech.com

Position 3
54-tube module type PVM-039, including 54 ceramic HybSi pervaporation membranes.
Membrane surface area: 0,55 m^2.
Maximum process temperature for the membrane 150ºC.
Standard enclosure with O-ring EPDM-PC, max temperature 130ºC.
Kalrez O-rings are available upon request against extra costs.
Dimensions of the tubular ceramic membrane: 500 x 10 x 7 mm (Length x diameter outside x diameter inside), with the membrane coated on the inside of the ceramic tubes and the ends glazed.

Item price:	€ 9.500,00
Number of pieces: 1	
Total position 3:	€ 9.500,00

Costs for packaging and handling:	€ 150,00
Costs for transport:	€ 250,00
Insurance costs:	€ 217,00
Total costs:	**€ 21.867,00**

All prices are excluded VAT

Conditions

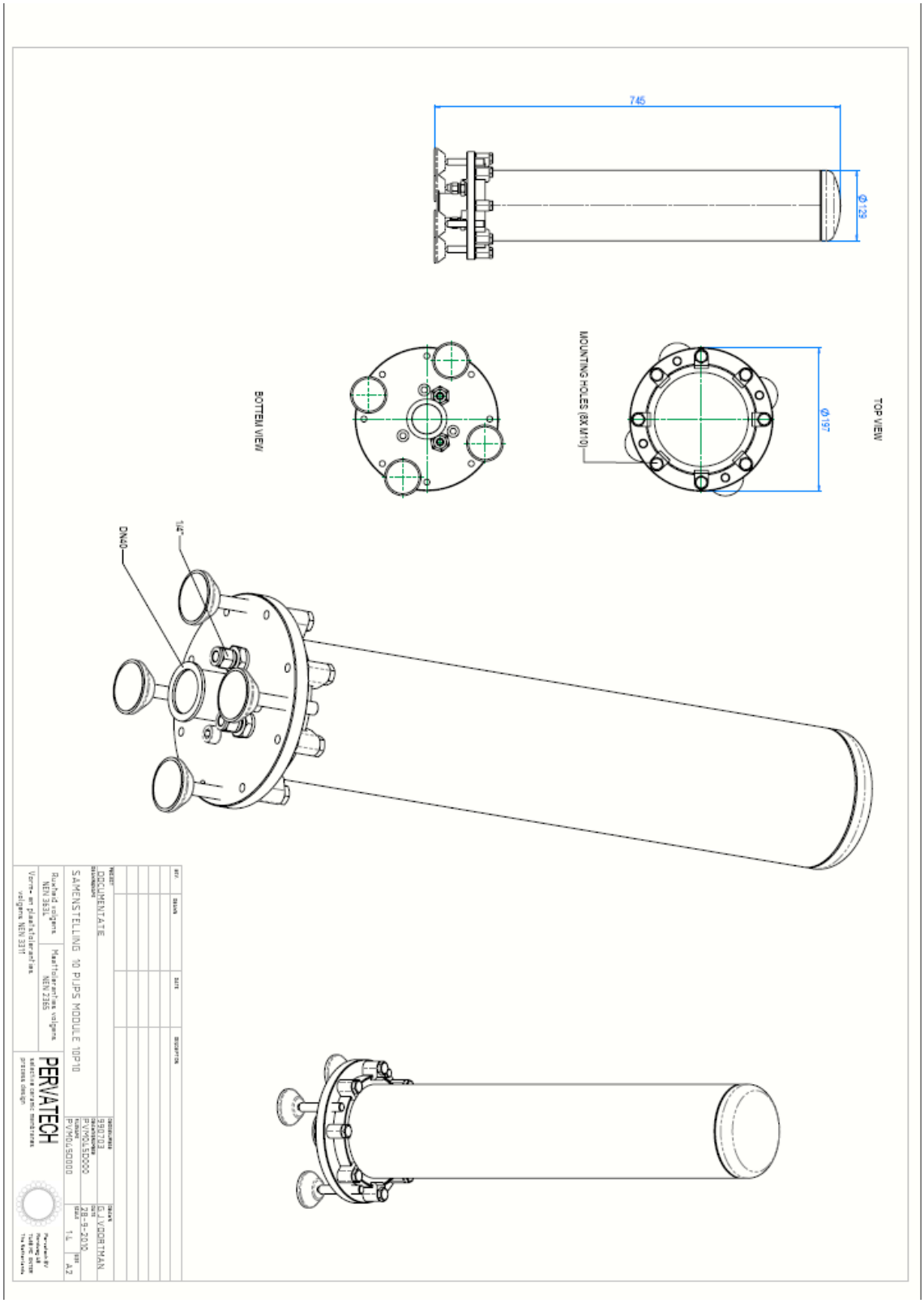
Delivery Time:	4 to 6 weeks, or to be agreed upon, depending upon stock and supply of raw materials for your order.
Delivery:	DDU (Delivery Duty Unpaid), shipment arranged for by Pervatech.
Validity of offer:	2 months
Valuta:	All quotations, invoices and payments are in Euro
Payment:	Shipment upon payment received.

Further our General Terms of Delivery are in force as enclosed in the email.
Looking forward to your most appreciated order.

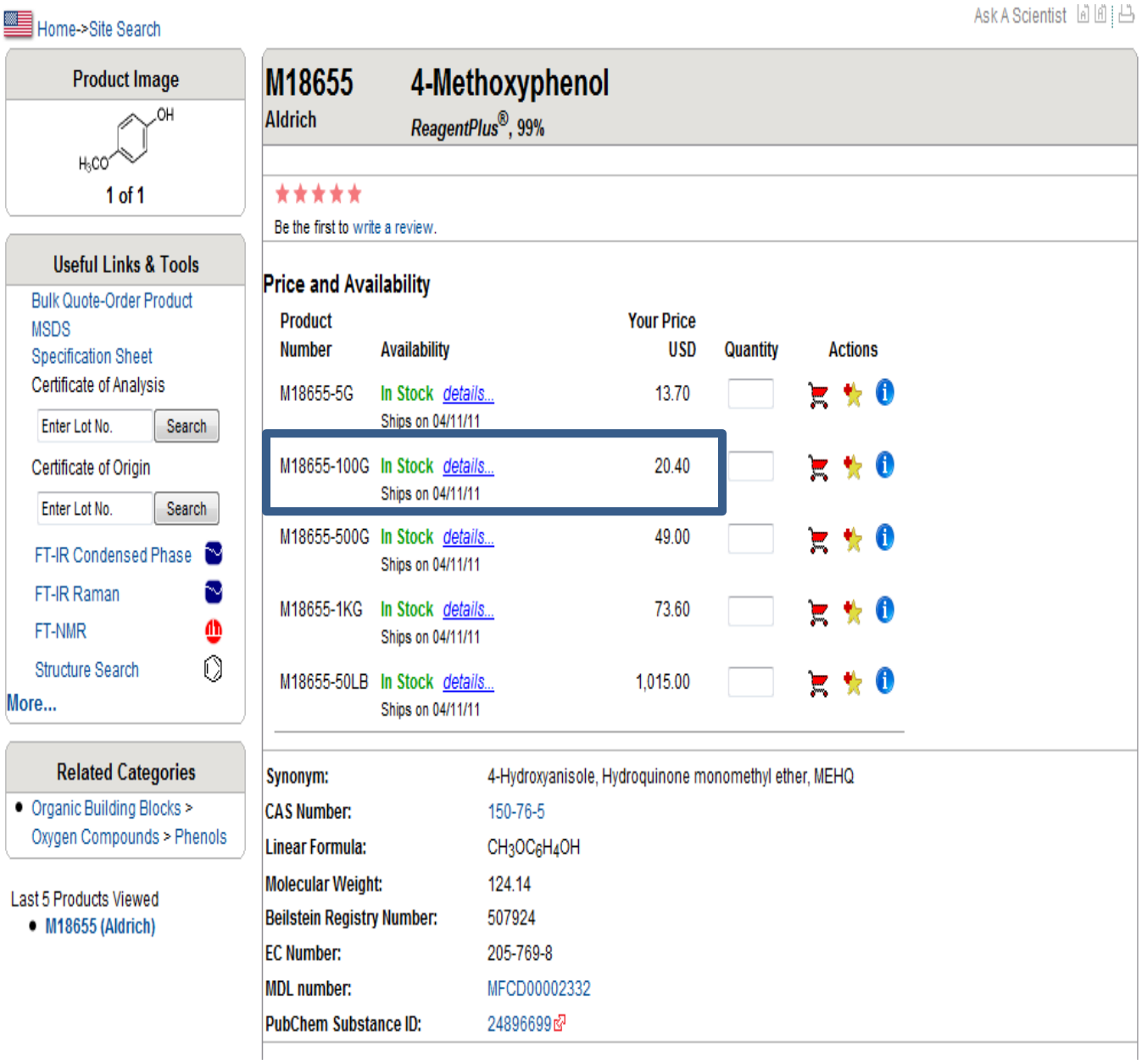
With kind regards

Frans Velterop
Pervatech BV
Managing director

Schematic of Pervaporation Module



Cost Estimate for 100 grams MEHQ from Sigma Aldrich



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Product Image

1 of 1

Useful Links & Tools

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MSDS
Specification Sheet
Certificate of Analysis
Enter Lot No. Search
Certificate of Origin
Enter Lot No. Search
FT-IR Condensed Phase
FT-IR Raman
FT-NMR
Structure Search
More...

Related Categories

- Organic Building Blocks > Oxygen Compounds > Phenols

Last 5 Products Viewed
- M18655 (Aldrich)

M18655 **4-Methoxyphenol**
Aldrich *ReagentPlus®*, 99%

★★★★★
Be the first to write a review.

Price and Availability

Product Number	Availability	Your Price USD	Quantity	Actions
M18655-5G	In Stock details... Ships on 04/11/11	13.70		
M18655-100G	In Stock details... Ships on 04/11/11	20.40		
M18655-500G	In Stock details... Ships on 04/11/11	49.00		
M18655-1KG	In Stock details... Ships on 04/11/11	73.60		
M18655-50LB	In Stock details... Ships on 04/11/11	1,015.00		

Synonym:	4-Hydroxyanisole, Hydroquinone monomethyl ether, MEHQ
CAS Number:	150-76-5
Linear Formula:	$CH_3OC_6H_4OH$
Molecular Weight:	124.14
Beilstein Registry Number:	507924
EC Number:	205-769-8
MDL number:	MFCD00002332
PubChem Substance ID:	24896699

Quotation for a Scheibel LLE column

---------- Forwarded message ----------
From: **Donald Glatz** <donald.glatz@modularprocess.com>
Date: Mon, Apr 11, 2011 at 2:27 PM
Subject: RE: Karr column for MAA purification
To: Ted Eckels <eckelse@seas.upenn.edu>

Edward:

Based upon your request for 8 theoretical stages, I would recommend a SCHEIBEL® Column. The size would be 14" diameter with fifty (50) agitated stages. The order-of-magnitude price for this column in 316SS construction is $350,000.

I hope this helps.

Best regards,

Don Glatz

Manager, Extraction Technology

SCHEIBEL® is a registered trademark of Koch-Glitsch, L.P.

****

Koch Modular Process Systems, LLC

45 Eisenhower Drive, Suite 350

Paramus, NJ 07652

Web: www.modularprocess.com

Tel: 201-267-8975

Fax: 201-368-8989

****

Correspondence with Project Advisor

---------- Forwarded message ----------
From: **Ted Eckels** <eckelse@seas.upenn.edu>

Date: Thu, Mar 24, 2011 at 9:54 PM
Subject: Column sizing

To: Stephen.M.Tieri@usa.dupont.com

Mr. Tieri,

We have been tweaking our MAA process. When we use structured packing, our tower diameters are dropping below 5 inches. For scalability, should we consider using random packing instead? Random packing results in a larger column diameter and might give better data.

We are using a column to purify some water to be recycled to the fermentors. This column will be very small (1-2 in) no matter what type of packing we use. We cannot use a flash vessel here because it would not provide necessary purity. Are there any alternatives?

We also forgot to ask you about what goes into the liquid-liquid extractor. We assume that we would fill it with some sort of random packing, but we are not sure how to size it, and we are not sure what type of random packing to use. Do you have any suggestions?

Thanks for your help.

Ted Eckels & Ben Galloway

--
Edward C. Eckels
School of Engineering and Applied Science
University of Pennsylvania, Class of 2011
Phone: 301.758.3289
eckelse@seas.upenn.edu

On Fri, Mar 25, 2011 at 2:25 PM, Stephen M Tieri <
Stephen.M.Tieri@usa.dupont.com> wrote:
Ted & Ben,

The main concern I have for any pilot facility, with respect to equipment selection & sizing, is that the equipment is representative of the type planned for use at the commercial scale. If the same type of equipment can
or will not be used, then the priority for selecting the pilot equipment is
what will generate the best / most directly scalable data for use in process design & equipment selection at the commercial scale. Overall, this really depends on the expected size of any commercial columns. Since
it is perfectly acceptable to use both structured or random packing for a commercial column, the choice is up to you. However, if you expected that
a commercial column would use trays instead of packing, I would suggest that it would be helpful (from a data quality standpoint) for the column to
have a diameter >6", ideally > 12". That said, I would consider column height, expected pressure drop, and potential cost differences at the commercial scale in selecting between column packing options for a pilot system. Unfortunately, my response does not directly answer your question,
but provides additional items to consider (and potentially work through). It might be helpful to make a quick estimate of column size at the commercial scale and estimate costs, and use the difference as the basis for your pilot packing (or trays?) selection.

It may be very difficult to both control and produce quality data (temperature profile) from a 1" column. With a column diameter in this size range, I would have significant concerns with heat losses from the vessel walls; as the mass of the vessel is significantly greater that the process material contained inside. I suggest using the 2" column, or possibly a 3 or 4" column operated in a batch mode. The other options depend on what you are trying to remove. If you provide me some info on what you are trying to remove in this water stream, I will try to identify
some other options. While I might expect you are trying to remove MAA

and/or MEK I will let you confirm first. What is the quantity of water that you are looking to recycle? I expect that quite a bit of water is involved, such that sending the water for disposal and using more fresh water is not a reasonable option.

There are many different types of liquid-liquid contacting methods, many of
which are illustrated in Perry's Chemical Engineers' Handbook (Chapter 15
in 8th edition). These can vary from simple decanters, to vessels with trays or packing, to agitated columns. There are also other options which
are not column designs. One fairly straight forward design is to use a static mixer, followed by a decanter vessel. Feed (pump) the liquids to an
in-line or static mixer (or a series depending on the necessary length), with the mixed flow feeding a vessel which will provide sufficient residence time for the materials & phases to separate. Overall, LLE equipment is very dependent on how quickly/cleanly the materials separate,
using data and observations gathered from lab experiments. If you provide
me with a bit more information, I may be able to provide some better suggestions.

Regards,

Steve

Ted Eckels
Re: Column sizing
04/01/2011 04:03
PM

Mr. Tieri,

We recently met with professor Fabiano and he suggested a few options to consider when taking into account the diameter of the columns. We cannot produce our product fast enough to warrant an increase in column size. However, we can increase the reflux ratio in the column to increase column size. Fabiano advised against this because you could store most of the MAA or MEK and then send it to an offsite vendor, like Koch-Glitsch. The vendor could then run the product in a large column and send us back data. The role of our plant would be to simply identify any kinks in the purification process, and any additional data about HETP and tray efficiency would be found later. Do you think that increasing the reflux ratio makes sense for producing reasonable data, or is just better to send the product off for further testing?

Our other question was about operation of the pilot plant. We originally assumed that the plant would be running 24/7, but we realized that this might not make sense on such a small scale. Would 8 hour shifts be appropriate for this process? If so, what are reasonable startup times and what should be done with residual liquids in the column or other parts of the purification process?

We are also going to call a company about sizing a Karr column for our process, which Fabiano recommended, as opposed to a packed column liquid-liquid extractor.

Thanks for you for your help

-Ted

Quoting Stephen M Tieri <Stephen.M.Tieri@USA.dupont.com>:

Ted,
I apologize for the delayed response, as I have only now opened my email for the weekend. I think that a Karr column is a reasonable choice for your pilot facility. One item to consider, since your process is pilot scale, is that it may be possible to use glass (clear glass like in the unit ops lab, not glass lined CS, depending on operating pressures) as the material of construction for the column and decanters. However, this could open the door to other operation issues, ex. if you want to see into the column to watch operation, heat losses could be an issue (since insulation will not be able to see through insulation). Although you could install removable insulation sections for process observation. If compatible with your materials, the potential to see into the process may be valuable in technology & process development.

Professor Fabiano's input opens an entirely alternate avenue to consider with respect to purification of your products and generation of your recycle streams. As I was not present for your discussion, I will do my best to provide guidance based on what was in your email.

There are many items to consider and reconcile when investigating contracting process development work and piloting. The first is cost; there will be investment savings in not installing the distillation columns and associated equipment and savings in the utilities to operate the columns. However, Koch-Glitsch will not do the work for free. Also, without the distillation equipment in your pilot plant, you will be constrained by the toller/contract manufacturer on both pricing and schedule. An additional thought is when someone else, or another group/organization/company, does the development work they gain the learnings not you or your company's technology group. While they can provide you with the process data (T,P, flows, etc.) collected during the distillation of your material, you will have limited input into modifying conditions to understand the impacts on your process. Even though you will be onsite for the toller/contract mfg. organization/equip manufacturer's distillation of you material, you will not get all of the small and seemingly inconsequential observations & learnings which turn out to be quite significant later, and arise from operating the equipment yourself.

It would have been helpful if you had included a pdf or sketch of your process to help refresh my memory of what your current process looks like. As I recall, your overall process recycles material from the distillation

columns back to fermentation. If I have confused your process with another, please forgive my memory. If the process is recycling material from the back/purification end, it will be difficult and more time consuming to wait for the next toller (or K-G) run of your material to then recycle that into the front end of your process and generate the next quantity of material for purification, etc.

Now that I've typed all of that, and remembering I was not present for your discussion, Professor Fabiano's insight & advice is directly on target. My guidance at this point is to do both. Yes both. Proceed with the smaller diameter columns in your pilot facility, since you have investigated the use of larger diameter columns and can describe why this not a great fit for your facility's scale. (Don't forget to add to report & presentation) Feel free to go with either the structured or random packing. Then, as a part of the pilot operation, overall process development, and detailed design of the commercial facility, produce a large quantity of intermediate material for distillation column optimization at K-G facilities. This would be completed during the early to mid front end loading project stages for the commercial facility, but once the pilot process has been proven operationally consistent and optimized.

Now, on to your operating time an schedule question. Whether or not to select an operating shift length of 8 or 12 hr (normal lengths), is somewhat separate from the choice of whether to run a 24/7 or 8 hr-5 day /week schedule. There are several frequently implemented operating schedules 24/7, 24/5 (24 hr op, but 5 days a week), 4 10's (4 10 hr days), 16/5 (16 hr op, but 5 days a week) and the standard 40 hr week (8 hr, 5 days/week). Also, consider that the time spent stopping and restarting your process will not be time when product is produced. You will likely not want to stop operating the process, after you have adjusted conditions and are anxious to see the impact, but may be forced to shut down because the end of the work- day is nearing and shutdown time is required. Modifying your schedule has the potential to significantly impact all of your equipment sizing, especially if you were to go to a 40 hr/week operating schedule, which would reduce your annual operating time. My suggestion and preference is to operate 24/7. However, given the scale you may be able to make some adjustments in # of operators/shift. What was your plan for staffing each shift (how many operators & what would they be used for?)? If using a 24/7 schedule, I would expect 4 operating shifts, regardless of 8 or 12 hr shift lengths.

Steve

---------- Forwarded message ----------
From: <lfabiano@seas.upenn.edu>
Date: Sun, Apr 3, 2011 at 12:26 PM
Subject: Re: Column sizing
To: Stephen M Tieri <Stephen.M.Tieri@usa.dupont.com>
Cc: eckelse@seas.upenn.edu

Hello Steve:

Thanks for your thoughtfull response.

To clarify what I told the team: The idea of going to a Koch_Glitsch was only to determine the HETP'S or tray efficiencies in a reasonably sized column. All other work can be performed in the pilot plant equipment whether it be a Karr column, a packed or staged extraction column and the distillation could be conducted in even an Oldershaw column. The Oldershaw in not very efficient and is not a commercial design to use practically but obtaining results might be good. Koch-Glitsch would not be involved, in my scenario, for anything more that mentioned above.

Len

[1] Alberts, Bruce *et al.* (2002). *Molecular Biology of the Cell*. New York: Garland Science. p. G:35
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[4] http://userpages.umbc.edu/~gferre1/bmoo.html
[5] Winkler M.: Fermentation process design *in* Principles of Industrial Enzyme Production and Utilization.
[6] http://www.iptonline.com/articles/public/IPT_16_2005_p84_88x.pdf
[7] Environmental microbiology
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[8] Manual on the causes and control of activated sludge bulking, foaming, and ...
By David Jenkins, Michael G. Richard, Glen T. Daig
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[17] http://www.springerlink.com/content/x8378j85vr552887/
[18] Yeast physiology and biotechnology
By Graeme M. Walker
[19] *http://www.ncbi.nlm.nih.gov/pubmed/16944129*
[20] Growing *E. coli* to high cell density—A historical perspective on method development
[21] http://www.bd.com/ds/technicalCenter/inserts/M17_Agar_&_Broth.pdf
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[25] http://www.springerlink.com/content/x8378j85vr552887/
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[28] Optimizationofthemevalonate-basedisoprenoidbiosyntheticpathway in Escherichiacoli for productionoftheanti-malarialdrugprecursor amorpha-4,11-diene
[29] http://www.sciencemag.org/content/314/5805/1565.full
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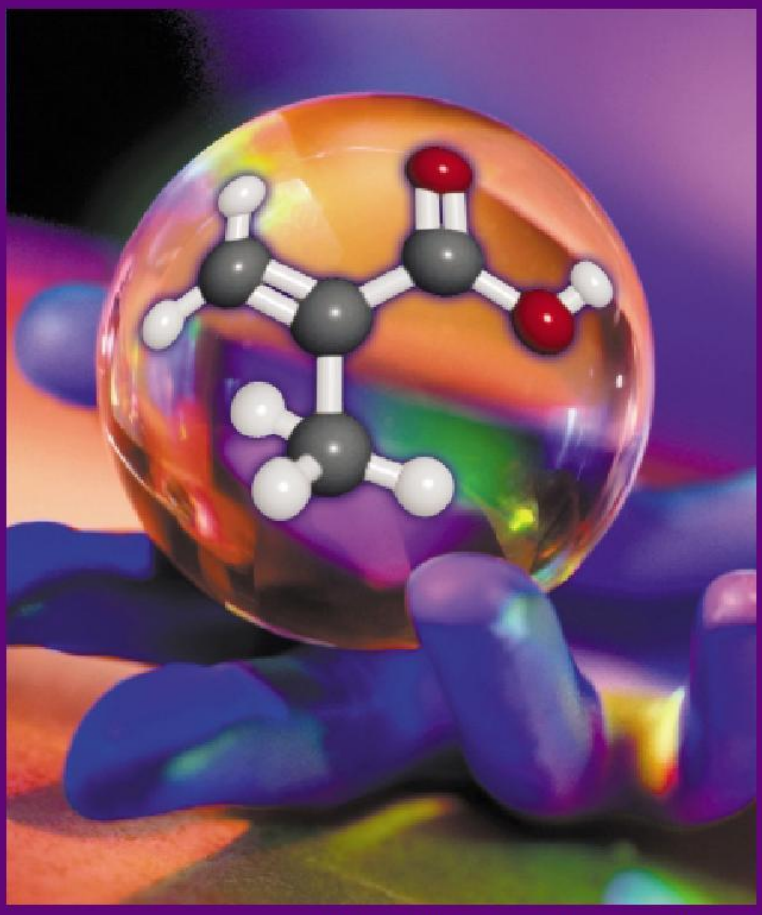
Methacrylic Acid
Safe Handling Manual



Methacrylate Producers Association, Inc. and
Methacrylates Sector Group of the European Chemical Industry Council

2007

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INTRODUCTION

This Manual provides general information on the safe handling of methacrylic acid and dealing with specific hazards in an appropriate manner. These hazards include health risks, environmental risks, and the potential of uncontrolled polymerization.

Properties and characteristics quoted in this Manual refer to methacrylic acid with a minimum content of 99 percent pure. They conform to specifications reported in the technical information bulletins issued by methacrylic acid manufacturers. Some of the physical data might be subject to minor changes due to variable concentrations of natural impurities.

Please read this entire Manual before handling methacrylic acid or before designing a storage system for methacrylic acid. All preventive measures described in this Manual must be followed to minimize the risks associated with this substance.

PREFACE

This Manual is a publication of the Methacrylate Producers Association, Inc. (MPA) and the Methacrylates Sector Group of the European Chemical Industry Council (CEFIC) and represents industry best practice. It provides general information to methacrylic acid users about the unique hazards associated with handling this chemical and measures to be followed to protect human health, equipment, and the environment. Methacrylic acid hazards include its corrosivity, combustibility, and its potential for unanticipated, uncontrolled, and rapid polymerization. Read and familiarize yourself with this entire Manual before using the information it contains. Also, thoroughly review your supplier's Material Safety Data Sheet for methacrylic acid before working with it. Additional information is available in the publication entitled "OECD SIAR Methacrylic Acid (CAS No. 79-41-4)", 2003. If you have any questions or need more detailed information, you should contact your methacrylic acid supplier.

This Manual was prepared by the following companies that are members of MPA and/or CEFIC: BASF SE (European Union) CYRO Industries (United States), Arkema Inc. (United States), Arkema France (France), Lucite International (United States, United Kingdom), Repsol Química, S.A. (Spain), Rohm and Haas Company (United States) and Evonik Röhm GmbH (Germany).

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TABLE OF CONTENTS

1. NAMES

Chemical Name	Methacrylic acid
Common Name	Methacrylic acid
Synonyms	2-Methacrylic acid
	α-Methyl acrylic acid
	2-Propenoic acid, 2-methyl
	2-Propenoic acid, α-methyl
CAS Registry Number	79-41-4
EINECS Number	201-204-4
Chemical Formula	$CH_2=C(CH_3)COOH$
UN Number	2531
IMDG-/ICAO-Class	8
ADA/RID Orange plate	89/2531
ADR/RID-Classification	8

2. PROPERTIES OF METHACRYLIC ACID

2.1 GRADES OF METHACRYLIC ACID

Glacial methacrylic acid is a refined grade of methacrylic acid and contains monomethyl ether of hydroquinone (MEHQ, CAS 150-76-5) as its inhibitor. Note that MEHQ is also known as para-methoxyphenol (PMP) and 4-Hydroxyanisole (HA). Grades are available with different levels and types of inhibitor. Specific information is available upon request from producers.

2.2 PROPERTIES AND CHARACTERISTICS OF GLACIAL METHACRYLIC ACID

The following values were taken from DIPPR (Design Institute for Physical Properties) where possible. DIPPR is a subsection of AIChE and specializes in compiling physical property data banks for various chemicals. The following is the most current information at the time of publication. Contact a producer for more up-to-date information or more detailed information about the properties of the grade of methacrylic acid.

Properties	**Values/Information**	**Notes**
Molecular Weight	86.09	G1
Physical State	Liquid above 15°C (59°F)	G1
Odor	Sharp, acrid, detectable at approx. 0.2 ppm	F
Color	Clear and colorless	
Solubility in Organic Solvents	Soluble in most solvents	
Light Sensitivity	Light promotes polymerization	
Hygroscopicity	Very hygroscopic	
Water Solubility	Totally miscible above 16°C (61°F)	F
Flammable Limits % by volume in air at 760 mm Hg minimum oxygen, calculated, %	1.6 (LEL), 8.7 (UEL) 7.2	G1 C12
Flash Point, Tag Closed Cup, DIN 51758 Abel-Pensky, EN 22719	67°C (153°F) 77°C (171°F)	G1 G2
Autoignition Temperature, approximate	435°C (815°F)	G1
Freezing Point, BS 523:1964	15°C (59°F)	G1
Vapor pressure, at 20°C (68°F), mbar (mmHg) at 40°C (104°F), mbar (mmHg) at 60°C (140°F), mbar (mmHg) at 100°C (212°F), mbar (mmHg) at 120°C (248°F), mbar (mmHg)	0.89 (0.67) 3.95 (2.96) 14.2 (10.6) 109.7 (82.3) 250.6 (188)	G1

Properties	Values/Information	Notes
Boiling Point, at 760 mm Hg (1013 mbar), DIN 51751 at 50 mm Hg (67 mbar), DIN 51751 at 10 mm Hg (13 mbar), DIN 51751	161°C (322°F) 90°C (194°F) 60°C (140°F)	G1
Equilibrium Concentration in air, calculated at 20°C (68°F), ppm (mg/m^3) at 25°C (77°F), ppm (mg/m^3) at 30°C (86°F), ppm (mg/m^3)	 878 (3369) 1300 (4988) 1905 (7309)	G1
Critical Volume, L/mol (ft^3/lb-mole)	0.28 (4.49)	G1
Critical Pressure, mbar (psia)	47900 (695)	G1
Critical Temperature, °C (K)	389 (662)	G1
Specific Gravity at 20°C (68°F), g/cm^3, DIN 53169	1.014	G1
Liquid Coefficient of Thermal Expansion, units of specific gravity/°C	0.0009786	G3
Specific Gravity of Vapor (air = 1)	2.97	C13
Liquid Viscosity at 20°C (68°F), cp (mpas)	1.54	G1
Surface Tension at 20°C (68°F), mN/m	65.9	G2
Heat of Combustion at 25°C (77°F), kJ/kg	23000	G1
Heat of Vaporization, kcal/mol (kJ/mol)	9.6 (40.2)	G1
Heat of Fusion, kcal/mol (kJ/mol)	1.926 (8.0625)	G4
Heat of Polymerization, kJ/kg	768	G5
Heat of Neutralization, kJ/kg	660	U
Specific Heat, at 20°C (68°F), kJ/kg, K at 100°C (212°F), kJ/kg, K	1.86 2.02	G1
Dissociation Constant at 25°C (77°F)	2.19 x 10 $^{-5}$	G6
Electrical Conductivity (range of estimates), mho/cm^2	4-10 x 10^{-5} (0%-0.8% H_20)	F
Refractive Index, n_D^{25} n_D^{20}	1.4288 1.4314	G1, 7 G8
National Fire Protection Assoc. Hazard Classification	3-2-2	G9
NPCA HMIS Rating	3-2-2	G10
Electrical Group Classification,IEC-78-8; NEC (US)	T2(300-450°C); Unclassified/Class I, Group D	G
Chemical Oxygen Demand (COD), weight oxygen/wt MAA	1.7	C14
Biological Oxygen Demand (BOD_5), wt oxygen/wt MAA	0.89	G11
pH of Methacrylic Acid in Water 100 ppm Methacrylic Acid 1000 ppm Methacrylic Acid 10,000 ppm Methacrylic Acid 100,000 ppm Methacrylic Acid	 3.8 3.3 2.7 2.3	 F F F F

Data Quality: G-Good, Reliable public source; F-Fair, Unverifiable or non-public source; U-Unknown source; C-Calculated by standard methods.

Sources:

1 DIPPR
2 OECD/EU Risk Assessment
3 Chemical Properties Handbook-Coefficient of Thermal Expansion of Liquid: Organic Compounds.
4 Knovel Critical Tables – Physical Constants and Thermodynamics of Phase Transitions
5 Heats of Polymerization of Acrylic Acid and Derivatives," Evans and Tyrrall, Journal of Polymer Science, Vol 2, No 4, 1947
6 Lange's Handbook of Chemistry (15th edition) Table 8.8 pKa Values of Organic Materials in water at 25C
7 Riddick, J.A., Bunger, W.B., "Organic Solvents: Physical Properties and Methods of Purification, 3rd ed.," Wiley Interscience, New York (1970).
8 Weast, R.C., Astle, M.J., "Handbook of Data on Organic Compounds." CRC Press (1985).
9 Building and Fire Code Classification of Hazardous Materials.
10 National Paint & Coatings Association, Hazardous Materials Identification System.
11 Handbook of Environmental Data on Organic Chemicals
12 MOC = LEL x Stoichiometric O_2 moles (4.5 for Methacrylic acid)
13 $SG_{VAP} = MW_{MAA}/MW_{AIR}=86/29$
14 $COD=MW_{O2}xStoichoimetricO_2/MW_{MAA}=32x4.5/86$

3. TRAINING AND JOB SAFETY

Methacrylic acid is considered to be hazardous under the Occupational Safety and Health Administration's (OSHA's) Hazard Communication Standard (29 CFR 1910.1200) and the EU Classification Packaging and Labeling of Dangerous Substances Directive (67/548/EEC). Therefore, all employees must be provided with adequate health and safety information and training to handle methacrylic acid. The training requirements are listed in the Hazard Communication Standard and the EU Directive.

Before undertaking any training of the employees who are engaged in handling or processing methacrylic acid, the supervisor who will conduct the training must be thoroughly familiar with the storage, handling, and properties of methacrylic acid and with any applicable national, state or local governmental occupational safety and health regulations. Thoroughly review the supplier's MSDS and consult a safety specialist or your supplier before finalizing a safety review of the operations involving methacrylic acid.

4. INSTABILITY AND REACTIVITY HAZARDS

4.1 POLYMERIZATION

Methacrylic acid is highly reactive. Polymerization of methacrylic acid can be very violent, evolving considerable heat and pressure and ejecting hot polymer from the site of polymerization.

An explosion hazard exists if the material is in a closed or poorly vented container because pressure build-up can occur very rapidly. Commercially available methacrylic acid is inhibited with methyl ether of hydroquinone (MEHQ), which together with dissolved oxygen prolongs the usable shelf life, i.e., the time before spontaneous polymerization. Commercial grades of methacrylic acid generally have a shelf life of one year at ambient conditions and should be used within that time (see Section 8). Your supplier should be contacted for shelf life detailed information.

Methacrylic acid must never be handled or stored under an inert atmosphere. The presence of oxygen is required for the inhibitor to function effectively. A headspace containing sufficient oxygen should always be maintained above the monomer to ensure inhibitor effectiveness.

There are five main causes of unintended polymerization of methacrylic acid: overheating and photo-initiation, contamination, corrosion, inhibitor depletion, and inhibitor deactivation (via oxygen depletion).

4.1.1 Overheating and Photo-initiation

Commercially available methacrylic acid is inhibited with MEHQ that prolongs the usable shelf life, i.e., the time before spontaneous polymerization. However, this usable shelf life is reduced exponentially with increasing temperature. Therefore, overheating of methacrylic acid must be avoided. Throughout this manual, the recommended safe temperature range for storage of methacrylic acid is 18-40°C. The lower limit (18°C) is intended to provide a reasonable separation from the freezing point (15°C). The upper limit (40°C) is a temperature that testing and experience have shown to be acceptable for storing METHACRYLIC ACID, without degradation of safety or product quality, for periods of time typically occurring in a well-ventilated warehouse anywhere in the world, as long as shelf life is not exceeded. Higher temperatures can be safely tolerated for shorter times such as during transportation or inventory turnover. Never exceed 45°C during a thawing process. Consult your supplier of methacrylic acid to determine if a higher temperature is acceptable. Also, see Section 4.3 Effect of Freezing and Section 6, Thawing of Frozen Methacrylic Acid.

Methacrylic acid can polymerize as a result of photo-initiation. Where sight glasses or other transparent sections are needed for visual observation in methacrylic acid service, they should have covers to exclude light between observations. All containers such as IBC, drums, etc., used for storing methacrylic acid should be kept from the direct UV radiation and should be preferably opaque to direct UV radiation. Store only inside or in a shaded area.

4.1.2 Contamination

Good housekeeping must be exercised to avoid contamination of methacrylic acid monomer. Many compounds are known that promote its polymerization, such as peroxides and compounds which form peroxides and free radicals, including aldehydes, ethers, amines, azides, and nitric acid.

Care must be exercised to avoid contamination of monomer with polymerizing methacrylic acid. Such polymer "seeds" could be generated in localized or hot stagnant areas, such as dead-headed pumps, and heated transfer lines.

4.1.3 Corrosion

Corrosion in general can also pose a polymerization hazard. Metal ions can initiate polymerization. Metal ions result if there is corrosion in the storage or transportation system. Methacrylic acid will easily corrode iron, carbon steel and similar metals. Refer to 10.2.1 Materials of Construction for proper materials of construction. Corrosion can be caused by using an improper material of construction, accidental introduction of incompatible metals into the system such as leaving a tool in a storage tank, or the contamination of the methacrylic acid with materials such as mineral acids that are corrosive to normal methacrylic acid systems.

4.1.4 Inhibitor Depletion

Inhibitor is depleted with time. Elevated temperatures accelerate depletion. Observe the recommended storage time and temperatures to prevent depletion of the inhibitor as well as premature depletion of oxygen. A special depletion possibility exists during the freezing and thawing of methacrylic acid. See Section 4.3, Effect of Freezing.

4.1.5 Inhibitor Deactivation/Oxygen Depletion

Dissolved oxygen is necessary for MEHQ to function effectively, acting as the initial, and very efficient, radical scavenger. Therefore, methacrylic acid should never be handled under an oxygen-free atmosphere. A nitrogen/oxygen gas mixture containing 5-21% by volume of oxygen at one atmosphere should always be maintained above the monomer to ensure inhibitor effectiveness. Since methacrylic acid vapors in air do not form flammable mixtures at room temperature, air may be used for this purpose.

Oxygen is consumed slowly as part of the free radical scavenging mechanism. Observe the recommended storage time and temperatures to prevent premature depletion of oxygen as well as depletion of the inhibitor. The atmosphere above methacrylic acid in a closed system should be periodically replenished with air or a nitrogen/oxygen gas mixture containing 5-21% by volume of oxygen. Large volumes of liquid should be circulated with at least 5% oxygen present (See Section 9.2.4). Residues in transfer lines and other stagnant areas should be blown out with a nitrogen/oxygen gas mixture containing 5-21% by volume of oxygen, or should be designed to be self-draining.

4.2 POLYMERIZATION DETECTION

Methacrylic acid has the potential to polymerize very rapidly, generating a large amount of heat. A temperature rise that cannot be related to an external heat source should be considered an indication of a runaway polymerization. Observations and sampling of methacrylic acid may be able to indicate the onset of polymerization prior to an observable rise of temperature. The earliest indication of polymerization is the appearance of haze caused by methacrylic acid polymer, which is insoluble in methacrylic acid monomer. There is normally at least one hour after the first observance of haze and the onset of uncontrolled polymerization.

Occasionally on the plant scale, uncontrolled methacrylic acid polymerizations proceed slowly. Therefore, simple temperature rise may indicate an ongoing polymerization. In the case of a slow temperature rise, the presence of visible haze in the monomer should verify whether a polymerization is underway.

CAUTION: Even slow polymerization has the potential to accelerate into a runaway reaction. If the temperature rises above 40°C (104°F) and the rate of rise is greater than 2°C (3.6°F)/hour, and no source of external heat has been identified, this should be considered as the onset of polymerization. If the temperature rises at a rate greater than 5°C (9°F)/hour, the situation is considered critical.

4.3 EFFECT OF FREEZING

Freezing of methacrylic acid should be avoided. Thawing of frozen methacrylic acid can be extremely hazardous. Methacrylic acid freezes at 15°C (59°F). As freezing occurs, the first crystals formed contain pure monomer with very little inhibitor; the inhibitor is concentrated in the liquid phase. When freezing occurs in bulk containers, the first crystals, low in inhibitor, will form along the outer wall of the container. The inhibitor will be concentrated at the center. Improper thawing may lead to pools of monomer containing low levels of inhibitor. This condition is made worse by repeated freeze/thaw cycles if the methacrylic acid is not mixed well after each thawing. If possible, the acid should not be thawed by applying heat directly to the outside of the container because the potential of polymerization of the low-inhibited monomer along the walls is possible.

The temperature of the acid should be maintained at 18-40°C (64-104°F). The ideal storage temperature is 20-25°C (68-77°F). Other temperature targets between 18-40°C (64-104°F) may be needed on a seasonal or situational basis.

In the event freezing does occur, follow procedures in Section 7, Thawing of Frozen Methacrylic Acid.

4.4 HIGH TEMPERATURE DECOMPOSITION

Not all polymerization incidents involve rapid polymerization. Sometimes the reaction proceeds much more slowly. Still, in unvented containers or containers whose vent has plugged (note that small vents can plug easily because of methacrylic acid polymer), high temperatures and pressures can build up over time. If the temperature reaches 195-200°C (383-392°F), methacrylic acid will undergo degradation. It is well documented that methacrylic acid polymer will dehydrate to methacrylic anhydride and water at 200°C (392°F). Some evidence exists that methacrylic acid can undergo decarboxylation at 195°C (383°F) generating carbon dioxide. With either decomposition reaction, very high pressures can be generated in a short period of time in an unvented container. Rupture of the vessel is possible.

Note: In the event of an unintended polymerization in an unvented container, high pressures may persist long after the polymerization event is over because of the presence of decomposition gases.

5. RESPONSE TO UNCONTROLLED POLYMERIZATION

The techniques for responding to an uncontrolled polymerization of methacrylic acid are regularly reviewed for improvements. These will be updated as new information is developed. Use the information below to develop local Emergency Response Procedures. This section alone is not intended to serve as an Emergency Response Procedure by itself.

Approaching any container of methacrylic acid that is thought to be undergoing an uncontrolled polymerization is hazardous because of the possibility of the container's violent rupture. Do not approach a container of uncontrolled polymerizing methacrylic acid without prior emergency planning. Never approach a container of methacrylic acid after it has reached 55°C (131°F) or if the rate of temperature rise has exceeded 2°C (3.6°F)/hour. Consider this for all response choices.

The most effective response to an uncontrolled polymerization of methacrylic acid is the remote addition and mixing of shortstop inhibitor, phenothiazine (PTZ). The final concentration of PTZ in the methacrylic acid to be shortstopped should be in the range of 200 to 1,000 ppm. However, in the case of contamination, restabilization may not be possible at any concentration of PTZ, depending on the nature and concentration of the contaminant. While other choices, mentioned below, may be less expensive, they are also expected to be less effective unless done as extra activity to reduce consequences after shortstop inhibitor, PTZ addition, has already been done.

- Once it has been determined that an uncontrolled polymerization is occurring, (see Section 4.2, Polymerization Detection) establish emergency management control over the area including evacuation if necessary. Safe evacuation distances depend upon many factors including the rate of polymerization, the likelihood of vent pluggage, and equipment design (size, vent area, design pressure, etc.), etc. These should be planned for in advance and be a part of the local Emergency Response Procedures. **Consult your supplier for further advice in the development of your Emergency Response Procedures.**
- If possible, remove insulation from the vessel to allow cooling with fire water. Consider hazards in approaching the tank if the temperature is already above 55°C.
- Apply cooling water to coils, jackets, and to the exterior of the vessel to reduce the temperature of the methacrylic acid. The local emergency response team should be able to set up fire monitors to provide fire water for cooling. Externally applied water may also be effective in knocking down any vapors that may be released.
- If it is determined that it is safe to approach the vessel, the following can be tried to minimize the consequence of the polymerization.
    - Ensure adequate venting area by opening any closed top hatches, especially for tank trucks or rail cars. Caution: Do **not** attempt this if the temperature is already above 55°C or if a local pressure gauge indicates that the vessel is above atmospheric pressure or if the vessel is venting.
    - Add the shortstop inhibitor, PTZ, in a concentrated solution or slurry. Locations storing methacrylic acid should maintain a supply of PTZ for use when needed. MEHQ and HQ are not shortstop inhibitors. MEHQ or HQ can effectively extend the shelf life of methacrylic acid only if there is adequate oxygen in solution. Temperatures significantly above ambient may deplete the oxygen in solution and prevent MEHQ and HQ from working. Temperatures reached during a runaway polymerization will defeat the functionality of MEHQ or HQ. MEHQ or HQ will not have a significant impact if added to a runaway polymerization of methacrylic acid.

- Diluting the methacrylic acid with water may reduce the consequence of the polymerization if the temperature of addition is low enough, below 55°C (131°F). This should be done only if there is sufficient space left in the container to increase the volume by at least 30% with water addition and also allow mixing. Adding inadequate dilution water or inadequately mixing the dilution water with the reacting methacrylic acid or adding at a temperature that could allow the mixture to reach 100°C could increase consequences (increased venting because water is more volatile than methacrylic acid, increased probability of plugging the vent with a rise in the level of the liquid, and increased probability of breaching the vessel if reaction does not stop). Remember that shortstop inhibitor, PTZ, is not soluble in water. Addition of shortstop inhibitor, PTZ, and mixing should be done prior to any water addition.
- If impact to the environment and to personnel exposures is acceptable, consider discharging the contents of the container into a diked/bunded area.
- Consider moving a mobile container away from people and equipment; barricading of drums or totes/IBC is another option.

CAUTION: Do not attempt to mix the contents of the container unless either shortstop inhibitor or dilution water has been added to the methacrylic acid.

A vessel undergoing an uncontrolled polymerization may experience high enough temperature to cause venting. **Note: A polymerizing vessel that stops venting may have a plugged vent. The potential for a violent vessel rupture may exist for many hours**. Do not approach a vessel that has ceased venting until remote temperature sensing indicates that the vessel contents have returned to the ambient temperature.

6. THAWING OF FROZEN METHACRYLIC ACID

Methacrylic acid freezes at 15°C (59°F). Improper thawing of frozen methacrylic acid can be extremely hazardous. See Section 4.3, Effect of Freezing.

6.1 THAWING OF FROZEN DRUMS/IBC'S/TOTES

The thawing of frozen methacrylic acid in drums or other small containers can best be accomplished by placing the drums in a heated room at temperatures up to 40°C (104°F). This will allow the acid to melt slowly within a 48-hour period. Monitor the temperature of the room closely to prevent overheating. Drums should be rolled or rotated every 6-8 hours during thawing to mix the contents. IBC's/Totes should be placed on a shaker plate during thawing to mix the freshly thawed material and to speed up the thawing process. After thawing, methacrylic acid should be stored between 18-40°C (64-104°F), ideally 20-25^{o}C (68-77^{o}F).

- Never store adjacent to heat sources.
- Never withdraw material from a partially frozen or partially thawed container of methacrylic acid. Such material may have low inhibitor levels or it may contain most of the inhibitor required for the entire contents of the container.
- Never apply electric heating bands to thaw containers as these generate high surface temperatures.
- Never apply steam to the outside of the container.

6.2 THAWING FROZEN BULK CONTAINERS

CAUTION: Do not attempt to thaw a frozen bulk container of methacrylic acid unless you have received prior approval from your supplier. Specific training may be required.

If you can anticipate receipt of frozen containers of methacrylic acid, develop a thorough procedure for safe thawing in advance of the first receipt. Contact your supplier for advice on design and operations with regard to possible receipt of frozen methacrylic acid containers.

Bulk containers can be safely thawed through the use of tempered water systems. The temperature of the water should never exceed 45°C (113°F). Use only automatic temperature controlled, "Fail Safe" alarmed, tempered water systems. The temperature of both the circulating water and the thawed portion of the monomer should be closely monitored. If possible, the monomer should be well mixed to ensure that dissolved oxygen and the inhibitor is well distributed during thawing as well as to enhance heat transfer.

As soon as the methacrylic acid is thawed, maintain the temperature of the thawed methacrylic acid at 18-40°C (64-104°F) and empty the container into the methacrylic acid storage tank.

CAUTION: Never use live steam. Localized hot spots must be avoided.

Never use steam-water mixing nozzles or tees directly in heating coils, jackets, etc., for thawing. An increase in steam pressure or loss of water supply would create immediate high temperature conditions resulting in a polymerization.

Never remove any material from a partially frozen container. If possible contents should be thoroughly mixed during and after the thaw cycle to ensure uniform mixing of the inhibitor before any liquid is withdrawn.

If possible, mix by re-circulation, agitation, or by means of an eductor. Totally thawed methacrylic acid can be pumped in its entirety to a storage tank for completion of mixing.

If frozen methacrylic acid is discovered in a vessel after emptying, return warm methacrylic acid to the vessel to provide inhibitor and a heat transfer source for thawing.

Note: If you are surprised by a frozen container of methacrylic acid or cannot safely thaw the methacrylic acid in accordance with these guidelines, please contact your supplier immediately.

6.3 THAWING PLANT EQUIPMENT

A concise procedure for the safe thawing of a normal storage tank cannot be given. It depends upon the precise details of the equipment and the circumstances. Contact your supplier for advice.

Bulk containers can be safely thawed through the use of tempered water systems. The temperature of the water should not exceed 45°C (113°F). Use only automatic temperature controlled "Fail Safe" alarmed, tempered water systems. The temperature of both the circulating water and the thawed portion of the monomer should be closely monitored. The monomer should be well mixed to ensure that the inhibitor is well distributed and oxygen gets redissolved into the liquid.

As soon as the material is thawed, maintain the temperature of the thawed methacrylic acid at 18-40°C (64-104°F), and preferably between 20-25°C (68-77°F).

CAUTION: Never use live steam or electrical heating systems such as electrical tape systems or mantles. These may cause localized hot spots.

Never use steam-water mixing nozzles or tees directly in heating coils, jackets, etc., for thawing. An increase in steam pressure or loss of water supply would create immediate high temperature conditions.

Never remove any material from a partially frozen tank. Tank contents should be thoroughly mixed during and after the thaw cycle to ensure uniform mixing of the inhibitor before any liquid is withdrawn.

Mix by re-circulation, agitation, or by means of an eductor.

7. HEALTH CONCERNS

7.1 TOXICITY

The principal hazard of methacrylic acid is its corrosivity to tissue and mucous membranes. Direct skin contact causes severe burns if the acid is not immediately and thoroughly removed. Inflammatory symptoms and blister formation can appear as late as 24 hours after exposure. However, tissue destruction occurs within the first few minutes. Healing from such injuries is occasionally delayed. See Section 7.2.3, Contact with Skin for more information.

The cornea and mucous tissues of the eye region may be severely damaged by contact with methacrylic acid. If not flushed immediately and thoroughly with water, permanent damage may result. See Section 7.2.1, Contact with the Eyes for more information.

Although ingestion is not a typical route of exposure to chemicals in the industrial environment, if methacrylic acid is swallowed it severely damages the mucous membranes of the mouth, throat, esophagus, and stomach. See Section 7.2.4, Ingestion for more information.

Inhalation of high concentrations of methacrylic acid vapors or mists causes burns of the respiratory tract and the possibility of delayed formation of pulmonary edema. Inhalation of lower concentrations produces strong nasal irritation accompanied by lachrymation. No serious adverse health effects have been reported following single or repeated exposures to airborne concentrations of 10-20 ppm. See Section 7.2.2, Inhalation for more information.

Your supplier's current MSDS should be consulted for current toxicological information.

7.2 FIRST AID

In order to minimize adverse consequences of methacrylic acid incidents, all personnel assigned to work with methacrylic acid must be aware that prompt and appropriate response is essential. First aid must be administered immediately. One prerequisite for the proper management of incidents is the installation of a sufficient number of conveniently located emergency safety showers and a periodic testing program to ensure they are operative when needed.

All injured personnel should be referred to a physician who should be given a detailed account of the incident. Consideration should be given to supplying the physician or hospital emergency room, where medical help will be sought, with a copy of the supplier's MSDS. Medical management aspects of that document should be reviewed with the physician.

7.2.1 Contact with the Eyes

If even minute quantities of methacrylic acid enter the eyes, the eyes should be irrigated immediately and thoroughly with water for a minimum of 15 minutes. The eyelids should be held open and away from the eyeball during the irrigation to ensure contact of water with all the tissues on the surface of the eye and lids. The eye irrigation should be continued for a second period of 15 minutes if the odor of methacrylic acid persists. Obtain a Physician's assistance (preferably an eye specialist) or that of another trained emergency health professional as soon as possible and transport to a suitable clinic or hospital. No oils or oily ointments or neutralizers should be put in the eyes or on the eyelids unless prescribed by the physician.

7.2.2 Inhalation

Personnel affected by methacrylic acid vapors must be moved at once to an uncontaminated atmosphere. If an individual is not breathing, administer artificial respiration. Obtain a physician's assistance or that of another trained emergency health professional as soon as possible and transport to a suitable clinic or hospital. If breathing is difficult, trained personnel should administer oxygen.

7.2.3 Contact with Skin

The emergency safety shower should be used immediately to remove methacrylic acid. Once under the safety shower, immediately remove all clothing and shoes. Wash with large quantities of water. Continue washing for at least 15 minutes until odor has disappeared. Washing with soap may help remove residual methacrylic acid from the skin and reduce the severity of the injury. After showering, get immediate medical attention. No salves or ointments should be applied to chemical burns for at least 24 hours unless prescribed by a physician.

All contaminated clothing should be properly decontaminated before reuse. Where decontamination is not feasible, clothing should be disposed of properly. Contaminated shoes and other leather items cannot be decontaminated and should be discarded. Under no circumstances should contaminated clothing be taken home for laundering.

7.2.4 Ingestion

Although ingestion of chemicals is rare in the industrial setting, in the event of methacrylic acid ingestion the affected individual should be made to drink large quantities of water. Do not induce vomiting. Consult a physician.

7.3 INDUSTRIAL HYGIENE

Exposure to methacrylic acid by inhalation, ingestion, or skin or eye contact should be prevented by a combination of engineering controls and prudent work practices. Engineering controls such as closed systems and local exhaust ventilation should be the primary emphasis and must be in compliance with national, state and local governmental regulations. Personal protective equipment (PPE) must be used.

Occupational health standards-setting organizations have established workplace exposure limits expressed as parts of methacrylic acid per million parts of air (ppm). Examples of such organizations include: the Occupational Safety and Health Administration (OSHA) and the American Conference of Governmental Industrial Hygienists (ACGIH) in the U.S., the Health and Safety Executive (HSE) in the U.K., and the Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK) in Germany. In the past, these values have generally been 20 parts of methacrylic acid per million parts of air

(ppm). Therefore, the reader is advised to refer to up-to-date publications for the current values. These workplace exposure limits are time-weighted average values, which means that exposures in excess of 20 ppm are permitted providing that there are offsetting periods below 20 ppm such that the overall exposure averages 20 ppm or less for the 8-hour work day. In addition, some official standards include a "Skin" notation, indicating that methacrylic acid can be absorbed in harmful amounts through intact skin. Please refer to your relevant national or governmental authority for the current exposure standard information.

7.4 PERSONAL PROTECTIVE EQUIPMENT GUIDELINES

Personal protective equipment (PPE) is not an adequate substitute for engineering controls, safe work practices, and intelligent conduct on the part of employees working with methacrylic acid. It is, however, in some instances the only practicable means of protecting personnel, particularly in emergency situations.

Use the guidelines below as a starting point for developing your own Personal Protective Equipment procedures. The hazard of each task must be thoroughly assessed, appropriate personal protective equipment must be selected, and training in the correct use and care of the PPE must be provided.

Testing has shown that butyl rubber gloves and fluoroelastomer gloves provide superior resistance to permeation by methacrylic acid. Consult a methacrylic acid supplier for the most current information on glove materials.

7.4.1 All Personnel

All personnel who are in the general area where methacrylic acid is being handled should wear the appropriate personal protective equipment. This protective equipment should be worn even if an individual is not planning to come in contact with the methacrylic acid processing equipment.

7.4.2 Routine Work

Personnel engaged in routine work with a small risk of limited exposure, such as collecting a sample or operating processing equipment, should wear the following protective equipment: chemical-resistant gloves, hard hat, safety shoes, and chemical splash goggles. Depending on the situation, consider wearing chemical-resistant boots if walking surface contamination is anticipated and a face shield if methacrylic acid spray or mist is anticipated. In addition, air-purifying respiratory protective equipment should be worn if air monitoring has demonstrated that airborne concentrations of methacrylic acid are above the established exposure limit and below 200 ppm.

7.4.3 Non-Routine Work

Personnel engaged in non-routine work and/or work with moderate risk of exposure such as unloading tank trucks and rail cars, opening vessels, breaking lines, or cleaning minor spills and leaks, should wear the following personal protective equipment: acid suit, chemical-resistant boots and gloves, hard hat, and chemical splash goggles. Wear a face shield in the case of opening drums or lines that may be under pressure or an acid hood with an acid suit if there is a more serious splash potential. Air-purifying respiratory protective equipment may be worn if airborne concentrations of methacrylic acid are between 20 and 200 ppm. If greater than 200 ppm, a supplied-air respirator is required.

7.4.4 Emergencies

Any time there is a risk of exposure to airborne concentrations of methacrylic acid in excess of 200 ppm or to unknown airborne concentrations, full protective gear must be worn. In such events as major spills, vapor clouds or fire situations, wear full protective gear: a supplied air respirator in positive pressure mode, hooded acid suit, chemical-resistant boots, hard hat, and chemical splash goggles.

In the event of a release of methacrylic acid, the area should be evacuated immediately and should be entered only by properly trained personnel equipped with appropriate PPE. It is advisable to have several sets of PPE available at all times. This equipment should be stored outside of, but near, the area where the methacrylic acid is used.

8. FIRE AND EXPLOSION HAZARD

Methacrylic acid has a moderate flash point and low vapor pressure at typical handling temperatures. Ignition may occur, however, if excessive amounts of mist or aerosol have formed in the air. Ignition sources can be spark discharges from static electricity or any other source.

If a fire should occur, water can be used effectively since methacrylic acid and water are miscible in all proportions at temperatures above the freezing point of methacrylic acid or methacrylic acid/water mixtures: >15°C (59°F). Fire fighters should be informed about the water-miscible nature of methacrylic acid.

During transfer between containers, the containers must be electrically interconnected (bonded) and properly grounded/earthed. Splash filling into a tank should be avoided unless velocity is low enough to prevent static electricity build up. This can be achieved by using a dip tube. If mixing nozzles are used in storage tanks, the minimum storage volume should be chosen such that the liquid surface is 500 mm (20 inches) above the nozzle outlet at all times in order to avoid spraying.

In the event of major incidents involving large spills or fires in storage tanks or rail cars, an assessment of all pertinent facts is critical to the safe management of the situation. Factors to consider are the probability of a vapor cloud and its dispersion, explosion, corrosivity, and the effect of the fire and heat on surrounding objects or materials. Some situations in the past have been best managed by allowing a fire to burn out.

In case of fire, evacuate non-essential personnel to a safe location. Carbon dioxide or dry chemical extinguishers may be used on small fires. In addition to water, large fires may also be fought with "alcohol" or universal-type foam, water fog, carbon dioxide, or dry chemical methods. Consider permanent installation of fire monitors to enable the continuous application of fire fighting water to storage areas without personnel exposure. Containment of runoff of fire fighting materials should be planned as required by regional, country, state or local authorities. See Section 7.4.4, Emergencies, for PPE to be worn for all emergencies, including fire.

8.1 Special Hazards

Any drums or containers exposed to fire should be kept cool with water spray while personnel are fighting the fire. Fire fighting personnel should operate from a remote location if possible. In a fire, sealed containers may rupture explosively due to polymerization and autoignition of the vapors may occur.

The above information should be used to develop a Fire Emergency Response Plan.

9. STORAGE AND HANDLING

Please read this entire Manual before storing methacrylic acid or before designing a storage system for methacrylic acid. All preventive measures described in this Manual must be followed to minimize the possibility of an uncontrolled polymerization that may proceed with violence under certain conditions. See Section 4, Instability and Reactivity Hazards, for a description of the polymerization hazards.

9.1 DRUM/IBC/TOTE STORAGE

Methacrylic acid is commonly sold in either steel drums with a polyethylene liner or high molecular weight, high-density polyethylene drums. In some regions of the world, reusable stainless steel drums may be used. IBC/totes of various constructions (including Ultraviolet radiation protection and preferably a grounding option) may also be used and should be treated similarly to drums.

- The drums/IBC/Totes must be stored according to applicable national, state and local regulations. Lacking other guidance or limits, drums should be stored not more than 2 high and a path of 1.5 meters (5 feet) should be kept free around each block, to provide ventilation and both normal and emergency response access.
- Indoor storage is recommended. Warehouses must be well ventilated. The temperature of the warehouse should be between 18-40^{o}C (64-104^{o}F) and preferably <30°C (<86°F).
- If drums/IBC/Totes are stored externally, they should be protected from direct sunlight.
- If drums/IBC/Totes freeze, follow thawing procedures described in Section 7.1, Thawing Frozen Drums.

9.1.1 Drum/IBC/Tote Handling

It is preferable that drums/IBC/Tote be transported in a manner so as to ensure the temperature stays between 18-40°C (64-104°F). When a load of drums/IBC/Totes is received, open the doors carefully before entering. If a strong odor is present, indicating a leaking container, immediately call the supplier. See Section 11.2, Spill and Leak Control.

9.1.2 Drum/IBC/Tote Emptying

To empty a drum/tote/IBC in an area where flammable atmosphere may occur (e.g. Zone 2 rating) the following procedures must be followed:

- Before drums/IBC/Totes are opened, they should be supported and grounded/earthed.
- Drums/IBC/Totes and fittings should never be struck with tools or other hard objects that may cause sparking or puncture.

Note: Drum contents may be under pressure or vacuum.

When removing plugs (bungs) from a drum of methacrylic acid (or opening an IBC/Tote), the operator should wear PPE recommended in Section 8.4, Personal Protective Equipment Guidelines, and should use a bung or plug wrench. The operator should place the drum bung up, and loosen the bung. Note that the drum/IBC/Tote contents may be under pressure or vacuum. After the plug starts to loosen, it should be given not more than one full turn. If internal pressure exists, it should be allowed to escape to the atmosphere. Only then should the operator loosen the plug further and remove it.

The preferable safe method for emptying drums/IBC/Totes is by pump or by gravity. Note that electric pumps must comply with the area electrical classification. When emptying by gravity, use self-closing valves. Vent drum/IBC/Tote while emptying. Do not use pressure to empty drums.

Do not cut, drill, grind, or weld on or near drums/IBC/Totes. The heat from such work could ignite residual material in the drum/IBC/Tote. Residual vapors may explode on ignition.

Improper disposal or reuse of containers may be dangerous and/or illegal.

9.1.3 Drum/IBC/Tote Disposal

Empty drums/IBC/Totes are hazardous because of residual liquid and vapor. Dispose of drums/IBC/Totes in accordance with applicable regional, national, state, and local requirements. Before a drum/IBC/Tote is scrapped, it should be repeatedly washed with water to remove traces of methacrylic acid and then rendered unusable by crushing or piercing. Dispose of rinse water properly. See Section 11.1, Waste Disposal.

9.2 BULK STORAGE

Methacrylic acid must be stored under an atmosphere containing 5-21% oxygen. Methacrylic acid suppliers can provide information on special design features required to cope with specific hazards of bulk storage. Consider consequences/risk when planning an installation. Larger bulk quantities of the acid imply higher risk because of higher potential consequences. All efforts should be made for control and surveillance of the storage temperature within the prescribed limits of 18-40^{o}C (64-104^{o}F), and preferably <30^{o}C (<89^{o}F). Redundant control devices are strongly recommended.

Conduct a hazard review and/or risk analysis of the storage facility to ensure adequate safeguards are in place to reduce the risk of polymerization and exposure. Design the storage tank to maintain temperature within 18-40^{o}C (64-104^{o}F), and preferably <30^{o}C (<89^{o}F).

Bulk storage tanks may be positioned inside a heated building. This provides independence from weather conditions, thus reducing costs.

A typical installation for an outdoor tank is shown in the Appendix to this manual. The special features of the design are derived from long-term experience and have proven to be reliable.

For environmental reasons, tanks and pumps should always be positioned in a diked/bunded area. Structures made of concrete are attacked by methacrylic acid. Suitable coatings should be applied in order to prevent attack of the concrete. All applicable regional, national, state and local governmental regulations must be observed.

9.2.1 Materials of Construction

Preferred construction materials for tank, pump and pipe installations are stainless steels, e.g., EN 58 C or H; DIN 1.4571 or 1.4541; US 316L or 321. Aluminum alloys can be used for anhydrous methacrylic acid. Polyethylene, polypropylene, EPDM, or fluoropolymers are also suitable as materials of construction for methacrylic acid and may be useful for accessory equipment such as gaskets and valve parts. Carbon steel and other heavy metals must not be used due to the corrosive nature of methacrylic acid. Metals may also cause initiation of polymerization. All applicable regional, national, state and local governmental regulations must be observed.

9.2.2 Pressure Relief

There is no guaranteed or warranted method for relieving the pressure from a methacrylic acid runaway reaction and the consequent potential of violent rupture of the container. Therefore, low design pressure (API atmospheric) tanks are recommended. Although no detailed experience with runaway reactions in full size tanks is available, weak seam or frangible roof designs are believed to provide the best protection from a failure. Roof guide cables should be considered to control the trajectory of such a roof. In addition, oversized rupture disks or weight-loaded lids ("weighted manway cover") may be acceptable.

For the natural breathing of the tank, a conservation valve should always be installed. Various designs such as weight-loaded pressure pallet valves or breathing valves with flexible diaphragms are commercially available. Seal pots with glycol can be used instead of conservation vents if they are properly designed and maintained. They may also serve as an overflow device.

Uninhibited methacrylic acid vapor can condense or crystallize on cold surfaces, such as relief valve inlets or rupture disks. The uninhibited condensate may then polymerize. Pressure relief devices and their connected lines should be checked periodically for the presence of polymer and/or frozen methacrylic acid, to prevent interference with their proper operation. Relief valves and nozzles can be electrically traced and insulated to help control polymer formation. They can continue to be flushed continuously with dry air.

Environmental protection alternatives, such as containment of vapors with closed loop unloading or venting through a scrubber or incinerator, may also be considered.

9.2.3 Temperature Control

Because methacrylic acid freezes at 15^{o}C (59^{o}F), it is essential that reliable temperature control be installed on outdoor tanks in climates with temperatures expected below methacrylic acids' freezing point.

Insulate all tank surfaces, nozzles, manways and piping. All insulating structures should be covered with a protective shell to keep rainwater from penetrating into the insulation material. Humidity enhances heat loss and could therefore cause uncontrolled cooling of the stored material. Protruding surfaces should be entirely incorporated into the insulation mantle to prevent condensation or crystallization of acid vapors, which might result in polymerization.

Control of heat loss may not adequately protect methacrylic acid from freezing. Additional heating devices are needed to balance the energy loss and to keep the tank at the required temperature for storage. Either an internal heating coil or an external heat exhanger is suitable. The heat exchange surface must be generously designed for high-energy losses during unusually cold weather conditions.

The design of heating devices must include control instruments that are suitable to reliably prevent overheating the acid. One such system is a double-circuit warm water system with steam or hot water in the primary and a glycol-water mix in the secondary circuit. A heat exchanger is commonly used to achieve the transfer between the circuits. Provision must be made to automatically control and alarm the temperature in the secondary circuit at preset high and low points. Maximum permissible temperature in the secondary circuit is 45^{o} C (113^{o}F), minimum temperature should be set at 18^{o}C (64^{o}F). Under no circumstances should live steam come in contact with methacrylic acid. The primary circuit must shut off automatically if instruments fail and the preset high temperature is reached.

Transfer lines to and from the tank are particularly vulnerable to freezing in cold climates. All outdoor lines should be traced with warm water or self-limiting electrical heat tracing. The design of the heating system should be capable of meeting all potential heat losses. Only electrical heat tracing with self-limiting properties should be used and only then if it can be ensured that overheating of the material cannot occur.

The most reliable way to detect a runaway reaction is by continuously monitoring the temperature of the inventory. The temperature monitoring system must have redundancy and be capable of determining the absolute temperature of the bulk liquid as well as the rate of temperature rise. Recording the temperature is helpful, and the use of high temperature alarms is strongly recommended.

At least two independent thermosensors (thermocouples) should be installed near the bottom of the tank so that they are always covered by the monomer level. In larger tanks it is sometimes advantageous to have a second pair of sensors installed above the lower third of the total tank height. It is of the utmost importance that the thermosensors stop the primary heating circuit in the event that the preset high temperature point has been exceeded.

9.2.4 Pumps and Transfer Lines

It may be appropriate to locate pumps in a heated building or in heated cabinets. Care must be taken to ensure that pumps are never positioned close to a heat source such as radiators or steam pipes. In the event of polymerization, polymer adhering to pump rotors can exert centrifugal forces strong enough to shatter operating pumps.

Centrifugal chemical pumps or seal-less pumps, such as magnetic or canned motor pumps with external cooling, are appropriate for transfer services for methacrylic acid. Centrifugal pumps, either packed or with a gliding disk seal, may require more maintenance due to the poor lubrication properties of methacrylic acid. Canned motor pumps and magnetic coupled pumps have excellent performance properties with respect to leaks, but are sensitive to dry operation that usually leads to total loss of the pump. Therefore, a low/no flow switch should always be installed to protect pumps from dry operation or dead heading.

Care must be exercised to avoid deadheading of pumps since this might overheat the monomer. A temperature sensor or a flow control device combined with a motor switch should be installed on the pump discharge side and before the shutoff valve, preferably in the pump housing. For magnetic drive or canned pumps, a temp sensor must be installed within the pump body. Centrifugal pumps present the potential for dangerous splashes if methacrylic acid leaks through pump seals or glands. Therefore, pump seals/glands, flanged fittings, and valve stems should always be provided with splash collars.

Consideration should be given to unloading and re-circulating the material with the same pump. For this purpose the circulation loop should be routed into a dip pipe running through the tank roof reaching to the very bottom of the tank. An anti-syphon device such as a hole in the dip tube in the vapor space should be installed. The use of non-return (check) valves is not recommended in this application as they may plug. The circulation loop should be arranged so that methacrylic acid will always drain towards the storage tank in the event of an interruption in circulation or transfer.

The oxygen content of the inventory must be maintained to keep the inhibitor working. An air atmosphere is acceptable. In the event that less than 21% oxygen is desired in the vapor space, oxygen level must be reliably kept above 5%. Re-circulation of the contents on a regular basis, at least every 4-6 weeks will keep adequate oxygen dissolved in the liquid. Air is recommended for any purges that enter the tank such as level devices or pressure detection devices. In no event should inert gas such as nitrogen be allowed to completely purge the vapor space. [Reference Section 5.1.5].

Provision for draining lines is helpful in order to avoid stagnant material in case of an extended shutdown. The drainage valve(s) should be installed at the lowest point(s) of the pipe system. The drainage branch should be close coupled so as to not form a dead leg that can contain stagnant material.

To limit vapor emissions during monomer transfers, it is advisable to use a vapor return line to allow the exchange of acid vapor between the headspace of the storage tank and the shipping container. The vapor return line (back-venting pipe) in a closed loop unloading system should be designed with a slight inclination toward the storage tank so that condensed liquid can drain back into the tank. The vapor return line should be equipped with a shut off valve on the end connected to the transport vessel. Some locations may also require a flame arrestor on the end close to the tank. Insulation and tracing for such systems should be considered in cooler climates.

10. SHIPPING

10.1 GENERAL

When shipped in tank trucks or rail cars, methacrylic acid is transported in insulated containers, in compliance with ADR/RID/GGV/GGVE Class 8 Packing Group III specifications in Europe and as methacrylic acid, stabilized, 8, UN2531, PGII under US DOT regulations.

Tank trucks may or may not be heated. They should be fitted with at least one temperature gauge. Heat exchanger tank trucks are equipped with a special heating system to prevent the methacrylic acid from freezing. Temperatures greater than 40^{o}C can be hazardous and should be immediately investigated. The captive glycol-water system is heated by the tractor's radiator water by means of a separate trailer mounted exchanger. Proper design of such a heat exchanger will only compensate for heat loss, to avoid overheating. The temperature of the captive glycol-water mixture should not exceed 45^{o}C. Direct heating of the methacrylic acid with tractor radiator water is hazardous because it is too hot.

If the temperature has fallen below 20^{o}C (68^{o}F), or if as a result of some unforeseen event the truck cannot safely proceed to its destination in time, all efforts should be made to park the truck in a heated garage or carriers' heating station. The driver must inform the supplier as soon as possible.

Under no circumstances must a container of methacrylic acid be connected to a hot water supply or electrical heating without consent of the supplier.

CAUTION: Live steam must never be applied to containers to heat or thaw frozen methacrylic acid.

10.2 UNLOADING SITES

Tank truck unloading facilities should be level, constructed of concrete or other impervious material, and in a location where it can be easily and safely maneuvered. As methacrylic acid attacks concrete, an acid-resistant concrete coating is recommended. The drainage arrangements should be away from the truck and exposed structures, and suitable for the collection of spills for recovery or appropriate disposal. Where access to the top of the container is required, the site should be equipped with stairs and a platform or consider a fall arresting restraint cable system. An electrical grounding cable is required and must be attached to the transport vessel prior to unloading.

10.3 PROCEDURES FOR UNLOADING TANK TRUCKS

Prior to entering the unloading area, the tank truck should be visually inspected for leaks and other irregularities.

Prior to unloading, the following should be confirmed:

- The engine has been stopped.
- The truck brakes have been applied and the wheels are blocked.
- The tank is connected to the grounding/earthing cable.
- Personnel are wearing the correct PPE.
- Compare the trailer number with that on the bill of lading.
- The tank truck contents have been positively identified as methacrylic acid.
- All connections have been made and are correct.
- Check and record the temperature of the contents. Consult the supplier if the temperature is not within the range of 18-40°C (64-104°F).
- Verify that the receiving vessel will hold the entire contents of the tank truck or iso container.

Emergency showers and eye wash facilities must be available at unloading sites. During the unloading process, the area should be chained off and posted with signs warning others to stay away from the area. The driver must stay close to the vehicle during the unloading process, but not in the cab. The unloading should be continuously monitored until completed.

During cold weather the acid may be frozen, particularly in the valve area. A safe procedure for thawing the outlets is by winding cloth or other absorbent material around the line and then splashing it with warm water of 45°C (113°F). The procedure may have to be repeated several times until the plug has been completely thawed. Live steam must never be used to thaw unloading equipment.

Before transfer lines and vapor return lines are connected to the container, the contents of the tank truck must be positively identified as methacrylic acid. If a sample for testing or a retain sample is required, the operating personnel wearing the correct PPE should open the lid, first taking care to ensure that any accumulated overpressure is vented, and withdraw an appropriate amount of material.

Transfer of methacrylic acid should always be performed by pump. In several countries the use of compressed air for the transfer of flammable or combustible liquids is explicitly prohibited. In addition, the use of compressed air is extremely hazardous with respect to spills and splashes if the transfer hose should break or leak because the leak can continue until all the pressure drains from the leak. Under no circumstances may inert gases be used to transfer methacrylic acid because of the danger of spontaneous polymerization. In instances where compressed gas has been used to blow lines empty, a pressurized gas mixture of nitrogen with a maximum of 21% oxygen and a minimum of 5% oxygen has been used. A mixed gas system can be simple or complex, depending on the application and desired safeguards. A simple system employs regulators on the nitrogen and air sources. More sophisticated air-nitrogen mixing systems use oxygen sensors and flow controllers. The oxygen analyzer can feed into the flow controller and/or an emergency shutdown system. During system design, careful consideration must be given to the instrumentation failure modes.

If a spill or overflow should occur during an unloading operation, the pump should be turned off, valves closed, and the spill cleaned up before any other action is taken.

The unloading procedures should follow the following sequence:

1. Connect the vapor hose and open the valves to equalize pressure. A qualified person assigned for this duty should carefully check the transfer connections for proper alignment and to confirm that the storage tank is correct.
2. Remove the protection cap of the coupling. The protective caps on discharge pipes must be unscrewed with particular care because the pipes may be filled with acid if the bottom valve of the container is leaking.
3. Connect the liquid line and open the external valve.
4. Open the internal valve.
5. Start the pump. Once the flow has started, continue to monitor the vapor return line gauge to confirm the flow and to avoid pulling a vacuum that may implode the vessel.
6. When the vessel is empty, shut off the pump and close all liquid and vapor valves.
7. Drain and disconnect the hoses and replace the caps.
8. Leave the labels in place (according to the ADR/RID or IMDG or national guidelines).
9. Disconnect the earthing cables and remove the wheel chocks.
10. Verify that the vessel is empty. If the vessel cannot be emptied for whatever reason, contact immediately your supplier.

10.4 PROCEDURES FOR UNLOADING RAIL CARS

Written unloading procedures are required for safe operation and may be required by law.

In addition to the requirements for unloading tank trucks enumerated above in Section 10.3, in advance of unloading the rail car the following should be completed:

- Compare the car number with that on the invoice.
- Check and record the temperature of the contents if possible. Consult supplier if the temperature is not within the range of 18-40^{o}C (64-104^{o}F).
- Ensure that the train crew has accurately spotted the rail car at the unloading line.
- Secure the unloading track from rail access during unloading. At each accessible end of the car being unloaded, place derails approximately one car length away or else close and lock a gate or switch.
- Place a caution sign on the track or car warning persons approaching the car from the open end or ends of the siding. Leave the sign in place until after the car is unloaded and disconnected from the discharge connection.
- Verify that the receiving vessel will hold the entire contents of the rail car.

The unloading procedures should follow the following sequence:

1. Connect the vapor hose and open the valves to equalize pressure.
2. Remove the protection cap of the coupling.
3. Connect the liquid line and open the external valve.
4. Open the internal valve.
5. Start the pump. Once the flow has started, continue to monitor the vapor return line gauge to confirm the flow and to avoid pulling a vacuum that may implode the rail car.
6. When the rail car is empty, shut off the pump and close all liquid and vapor valves.
7. Drain and disconnect the hoses and replace the caps.
8. Leave the labels in place (according to the ADR/RID or IMDG or national guidelines).
9. Disconnect the earthing cables and remove the wheel chocks.
10. Verify that the vessel is empty. If the vessel cannot be emptied for whatever reason, contact immediately your supplier.

10.5 TRANSPORTATION INCIDENTS

In the event of a spill, fire, or suspected polymerization, immediately contact the appropriate local or national transportation emergency clearing organization. This would be, for instance, TUIS in Germany, CANTREC in Canada or CHEMTREC in the US.

If a shipment in a railcar, tank truck, drum, intermediate bulk container [IBC/ tote] becomes damaged so that delivery cannot be made safely, every effort should be made to move the container away from people and property. Police and fire departments are to be notified and the public is to be restricted from the area.

10.6 PERSONNEL PROTECTIVE EQUIPMENT WITH BULK CONTAINERS

A chemical resistant splash suit, gloves, boots, eye protection, and respiratory protection should be considered necessary when loading and unloading bulk containers. Safety glasses with chemical splash goggles and face shields are considered full eye protection. In the absence of a proper risk assessment, full protective PPE equipment should be required when sampling or making and breaking any connections.

10.7 THAWING SHIPPING CONTAINERS

Thawing of shipping containers can be extremely dangerous if proper procedures are not followed. See Section 6 for details. Methacrylic acid containers can be thawed safely with moderate heating and not steam. Bulk containers may be warmed in heated garages. Bulk containers with circulation systems can be thawed with tempered water through heating coils. Drums may be thawed by placing them in heated rooms at temperatures between 20^{o}C and 40^{o}C and then mixed by rolling the drum on the floor, on a drum roller or pallet wrap. Never use direct steam or electrical heating wrappers on drums or totes. Mixing is necessary to distribute the inhibitor and dissolved oxygen. Never remove acid from a partially thawed container.

11. ENVIRONMENTAL CONSIDERATIONS

National, state and local governmental regulations governing waste disposal require that producers and users of chemical products be fully aware of viable alternatives for the safe disposal of waste materials and to select and practice a disposal method or process that assures compliance with all applicable requirements. The treatment or disposal of methacrylic acid as a specific chemical can be determined by comparing the physical and chemical properties with regulatory standards.

Discharges into navigable waters, public or private sewers, or air, disposal in landfills, and by incineration, are all controlled by governmental (local, regional, national, and international) laws and regulations. Noncompliance is subject to criminal or civil penalties, or both.

11.1 WASTE DISPOSAL

Local, regional, national, and international regulations governing waste disposal make it essential for producers, suppliers, haulers, and users of monomers to be fully aware of viable options for the disposal of materials containing methacrylic acid. Materials to be disposed of include residues from production and cleaning operations as well as waste material from spills.

Minor spills of the methacrylic acid may be washed into a biological treatment plant after notifying local treatment facilities. Diluted methacrylic acid biodegrades readily in the environment. However, methacrylic acid may be toxic to the system if the bacteria have not been properly conditioned to it.

Accordingly, the initial feed rate should be low with a stepwise increase if a significant amount is to be fed into the treatment unit. The maximum concentration should not exceed 1000 mg per liter.

Solid material containing methacrylic acid, such as absorbents or polymeric material, can be disposed of by incineration. Disposal in landfills must be thoroughly reviewed with authorities and should be practiced only as a last choice.

For disposal of laboratory wastes or retained samples, great care must be exercised to keep methacrylic acid separate from incompatible materials, such as peroxides, which may initiate polymerization.

11.2 SPILL AND LEAK CONTROL

Emphasis should be placed on the prevention of leaks and spills through careful design and good operating procedures. Written spill and leak response procedures are recommended and may be required by law. Do not allow spills to enter drains, sewers or watercourses. Notify your appropriate regulatory body if spills or uncontrolled discharges enter watercourses.

Methacrylic acid is not likely to persist in surface waters over an extended period of time. All efforts must be made to prevent spills from running into public surface waters. In the event of accidental spillage of methacrylic acid into surface water or to a municipal sewer system, the responsible pollution control agencies must be promptly notified.

If there is a facility capable of treating methacrylic acid, small spills may be washed to the chemical waste treatment sewer with large amounts of water. Small spills of up to 5 liters can be suitably absorbed in commercially available spill cleanup kits.

Large spills should be contained, if possible, within a diked/bunded area. Stacking sand bags or similar material can be used temporarily. Avoid run-off into storm sewers routed to public waters. If possible, the spill should be recovered in appropriate containers for reuse or disposal.

11.3 AIR EMISSIONS

Air emissions are restricted and require permits in most locations. Emissions from storage, loading, and unloading facilities may be easily controlled by back venting transport containers to storage tanks through a vapor return line or vapor equalization line. Be careful to avoid contaminating other tank inventories when tanks are connected by a common header or exhaust system piping.

Scrubbing with sodium hydroxide or incineration are also acceptable treatments for methacrylic acid exhaust or vent gas. Spent or depleted scrubber solution must be disposed of properly by biological treatment or incineration.

In the EU, the storage and use of methacrylic acid may fall under the Integrated Pollution Prevention and Control Directive (96/61/EC). As of this writing, methacrylic acid in the U.S. is a SARA Section 312 reportable substance for inventory purposes. It falls under SARA Section 311 hazard categories: acute health, chronic health, reactive, and fire. Although not designated by name as hazardous by Superfund, methacrylic acid falls into its "Ignitable, Corrosive, Reactive" (ICR) category as a corrosive.

Please be aware that these regulations are constantly developing and other regulations may apply. Contact a methacrylic acid manufacturer for the most current MSDS for more complete and up to date information.

12. APPENDIX

Table 12-1: Key to Symbols in Figures 12-1, 12-2 and 12-3

Symbol	Definition
FAL	Flow alarm – low
FE	Flow element
FI	Flow indicator
FIC	Flow indicator/controller
JAL	Power alarm – low
JR	Power recorder
JSL	Power switch – low
JT	Power transmitter
LAH	Level alarm – high
LG	Level gauge
LI	Level indicator
LSHH	Level switch - high high (shuts down unloading pump)
PIC	Pressure indicator and control
PVRV	Pressure and vacuum relief valve
TAH	Temperature alarm – high
TAL	Temperature alarm – low
TC	Temperature control
TE	Temperature element
TI	Temperature indicator
TR	Temperature recorder
TSH	Temperature switch – high (shuts down pump)

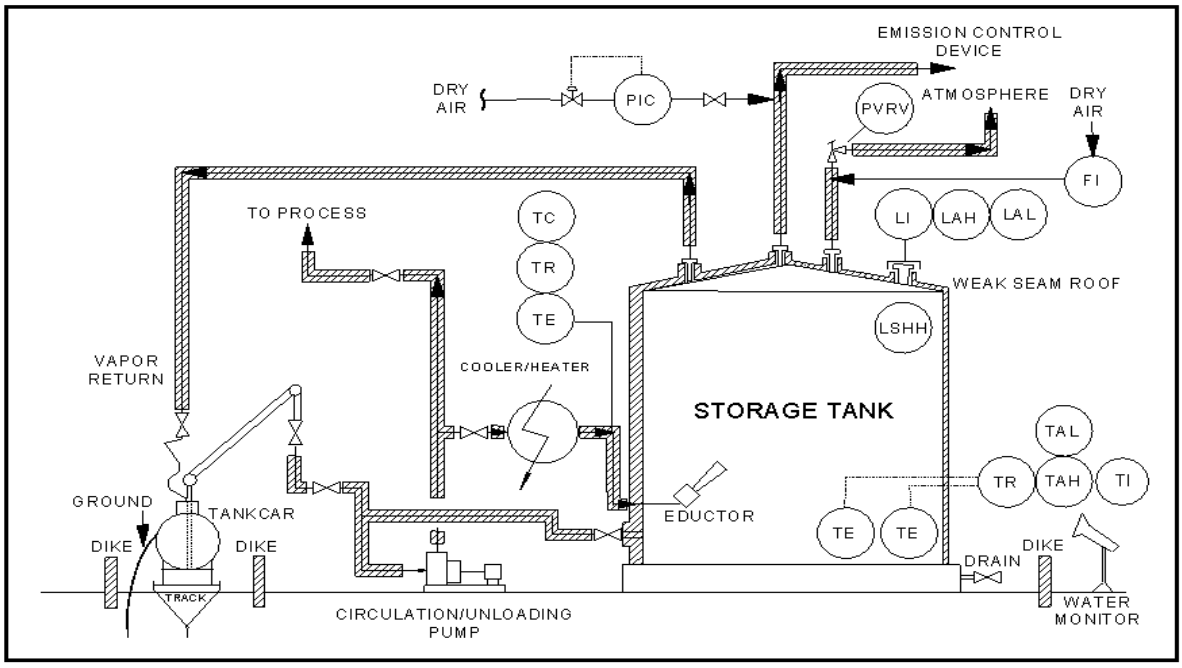

Figure 12-1: Example of a Methacrylic Acid Storage Facility
The example illustrates some of the safety features discussed in the booklet. Not all equipment or instrumentation required for operability is shown.

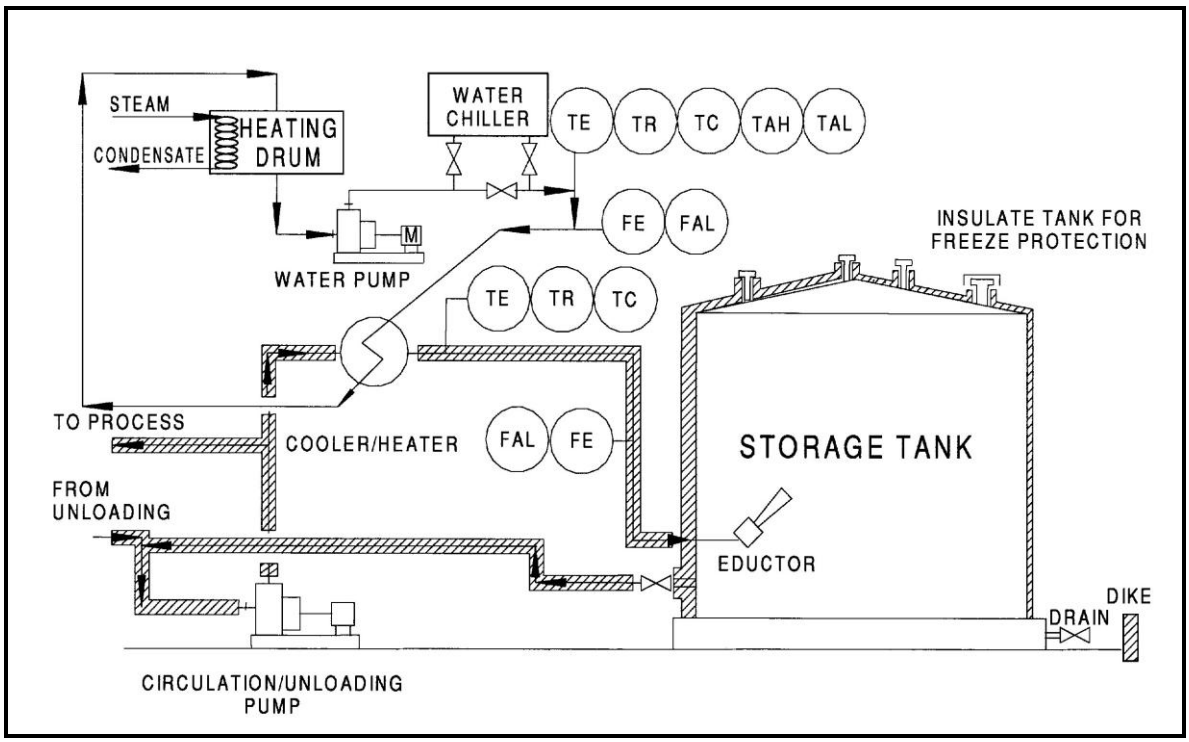

Figure 12-2: Example of a Methacrylic Acid Storage Tank Temperature Control System
This example illustrates some of the safety features discussed in this booklet. Not all equipment or instrumentation required for operability is shown.

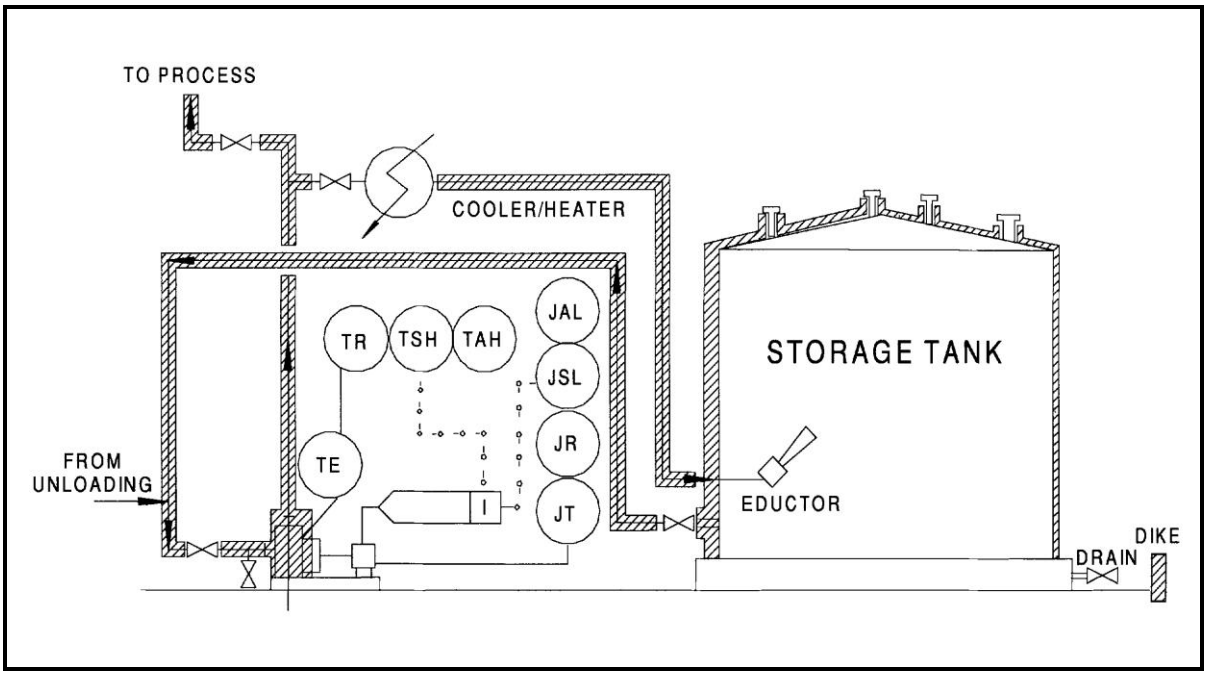

Figure 12-3: Example of a Methacrylic Acid Pump Loop
This example illustrates some of the safety features discussed in this booklet. Not all equipment or instrumentation required for operability is shown. See Table 13-1 for key to symbols.

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BioFlo 310 5.0 L COMPACT BENCHTOP FERMENTOR

Date: 04/08/11

Proposal Number: 040811-BF310-UPEN

Prepared By: George Faragalla

TABLE OF CONTENTS

1.0 General Description

The engineering embodied in the New Brunswick Scientific Co., Inc. fermentors and cell culture bioreactors represents the accumulation of many years' experience and achievement in the field of research & development, pilot and production scale fermentation equipment. The equipment has a history of successful performance in diverse biopharmaceutical applications, and will give years of trouble-free operation.

2.0 EQUIPMENT SPECIFICATIONS

The controller is designed to operate 24 hours per day, 7 days per week. The controller hardware is an embedded PC known as a single board PC that has the capability to control 1 to 4 reactors simultaneously.

1st Reactor Requirements: **Control System** consist of

1. **Utility Station** with built in **Controller**
2. Touch Screen **Display**
3. **Vessel** (2.5L, 5.0L, 7.5L, 14.0L)

Controller

4. Ability to control 1 to 4 reactors simultaneously
5. Maximum of 2 Displays (shows same images)
6. Two USB ports on Master System

* USB port to connect a box with 8 serial (RS232) Inputs and Outputs to connect and control up to 8 Mettler Toledo scales or other equipment with serial connections (8 serial ports can be shared between master and slave reactors).
* USB port to update the firmware/software service, export trend data and required for RS232 8-port (Serial) Controller when adding scales and other RS232 devices.

2nd, 3rd, and 4th Reactor Requirements:

7. Each reactor requires a **Utility Station**
8. No Controller required
9. No Display required
10. Vessel (Choose from any 4 sizes)

Utility Station

Each Utility Station manages one reactor and contains all the instrumentation to monitor and control all process variables, including:

* 3 built-in fixed speed pumps by Watson Marlow
* Temperature
* Agitation (motor)
* 3 Foam/Level
* pH
* pH2 or redox (Optional)

- DO
- DO 2 (Optional)
- Gas Flow with
  Standard:
  - One TMFC *0.4 to 20 SLPM (Standard Liters Per Minute)* with 4 solenoid valves

  Options:
  - Zero TMFC with 4 solenoid valves and manual Rotameter
  - Two, Three, or Four TMFC – Select gas flow range of *0.1 to 5, or 0.4 to 20 SLPM*

  - Fifth TMFC for Gas Overlay – Available with:
    - 4 solenoid valves with Rotameter
    - One TMFC with 4 solenoid valves with gas flow range of *0.1 to 5 SLPM*

- Analog Input and Outputs
  Standard:
  Total of 8 Analog Inputs
  - Four 4-20mA/0-5V switch able
    - One Input is dedicated for Gas Overlay (Option)
    - Three Inputs are available for user loop use
  - Four 0-5V Inputs
    - Are designated for the various TMFC options. When TMFC option is not installed the Inputs can be used for additional user loops.

  Total of 8 Analog Outputs
  - Four 4-20mA/0-5V switch able
    - One Output is dedicated for Gas Overlay Option.
    - Three Outputs are available for user loop use.
  - Four 0-5V Outputs
    - Are designated for the various TMFC options. When TMFC option is not installed the Outputs can be used for additional user loops.

Each TMFC occupies (1) 0-5V Analog input/output

Touch Screen Display

- The display is a 15-inch color touch screen mounted on the utility station, with flexibility to move in all four directions depending on user needs.
- User has the ability to monitor and control all process variables, trend graphs, alarms and more for up to four reactors with this one display by selecting Unit 1, Unit 2, Unit 3 or Unit 4.
- From the Display, users will have the ability to navigate to the following screens:

1. ***Summary Screen:*** This overview screen let users view all loop names, process values, setpoint values, output%, Control mode, Units, and Cascades. Add additional loops controlled via Analog and Serial I/O directly through this screen.

Additional Screens are associated where users can modify setpoints, control modes, configuration, and alarm screens of all controlled parameters except pumps.

2. ***Synoptic Screen:*** Provides a schematic overview of the entire control system. Users have the ability to monitor and adjust the set points and process variables of available loops. They also have the ability to view the open/closed status of control system valves.

3. ***Calibration Screen:*** From this screen users can calibrate all the probe parameter as specified in each configuration.

4. ***Cascade Screen****:* Enable/Disable cascades for any and all parameters as specified in each configuration, including dissolved oxygen and pH.

5. ***Trend Screen:*** This screen provides plotting for up to 8 parameters on a single screen and view real time trend graph for all of the 8 parameters.

   Advantages:
   - Enter Display High/Low for each parameter
   - Color code each parameter
   - Select None to delete a Trend Loop
   - Export data through USB flash drive
     Export data is available in Excel format
   - Choose sample time from minimum 5 seconds to maximum 60 seconds
     See below chart for total trend time:

Sample Time (SEC)	Total Trend Time (Hours)
5	12
15	36
30	72
60	144 (6 days)

   - In a single run, data is overridden after the maximum trend time. To save the trend data, export the data via USB FLASH DRIVE port.

6. ***Pump Screen:*** This screen controls all the pump parameters including 3 built-in fixed speed pumps on each Utility Station, and all additional pumps added through the available Analog Inputs and Outputs.
   - Users can select assignment to choose any of the 6 methods to operate the pump.
   - Users have the ability to calibrate the pumps on-screen and enter a value in ml/sec; thereafter view the total pump flow rates. Users can set cycle time of each pump in seconds.

7. ***Alarm Setup Screen:*** Define absolute or setpoint deviation alarm for any & all controlled parameters on a single screen. View history of all alarms during each run.

8. ***Setup Screen:*** This screen has 4 attached screens, including Controller Setup, Recipe Manager, System Settings, and Hardware Setup.

Controller Setup

- User can enter Unit Name,
- Select Fermentation or Cell Culture process
- Select Gas mixing options
- Select correct vessel size for accurate PI values
- Optional: 2nd DO & pH or 2nd DO & Redox – only available if this option is purchased
- Optional: Select Gas Overlay – only available if this option is purchased
- View Unit Type, Number of TMFC and TMFC Range (Set from the Factory)

Recipe Manager

- Save and load up to 10 recipes

System Settings

- Language: English
- Calibrate the touch screen display
- Set Time and date
- View current firmware and software versions
- Install software updates via USB flash drive
- Updates are available on NBS web site.

Hardware Setup

- View and set the fermentor hardware of 1 to 4 reactors
- Set Unit ID for software
- Set Scada parameters

  Protocol:

  ModBus

  AFS

9. ***User Selected Control Modes***
   - Auto
   - Manual
   - Off

3.0 STANDARD SYSTEM – CONTROLLED PARAMETERS (LOOPS):

Standard Parameters with 1 TMFC Includes:

1. Agitation
2. Temperature
3. pH
4. DO
5. Air
6. O2
7. 3rd Gas (depending on the gas formula)
8. 4th Gas (depending on the gas formula)
9. GasFlo (Set and view total gas flow rate)
10. Hi-Foam (Conductance)
11. Pump 1 (Pump Screen)
12. Pump 2 (Pump Screen)
13. Pump 3 (Pump Screen)
14. 7 Analog Inputs
15. 7 Analog Outputs

Option – Controlled Parameters (Loops):

16. pH2 or Redox
17. DO 2
18. Overlay with Rotameter or TMFC
19. RS232 8–port (Serial) Controller for scales and other RS232 device

* Each Utility station i capable of supporting up to **32** loops

4.0 Details

OTHER CONNECTIONS ON UTILITY STATIONS

Control Bus (RS485) Input and Outputs

1. Controller Input (Connects to the 2nd unit) - OPTION
2. Controller Output (Connects to the 1st unit) with 25 feet bus cable
3. External DO 2, pH2/Redox option installed in the field - OPTION

Standard Ethernet (One)

Future Expansion

Standard VGA (One)

Aux Monitor Video – (15 pin connector) for optional 2nd Touch Screen

Standard RS232 (One)

Aux Touch screen Display– (9 pin connector) for optional 2nd Touch Screen

Mechanical Subsystems

TEMPERATURE

SPECIFICATION	***Control (Range)***
Temperature	4°C to 80°C Rapid temperature shift at >1°C /minute for 2.5, 5 and 7.5L Vessel Rapid temperature shift at >0.8°C /minute for 14L Vessel

AGITATION

SPECIFICATION	***Control (Range)***
Agitation	50 to 1200 rpm 100 rpm +/- 1 500 rpm +/-2 1000 rpm +/- 5 1200 rpm +/- 10

PUMPS

3 Fixed speed pumps for control of nutrient, acid, base, foam or harvest. Unit is able to add additional fixed and variable speed pumps via available Analog Input/Outputs

SPECIFICATION	***Control***	***Speed rpm / Flow Rates***
Pump 1 **Watson Marlow 400F/B1 – one channel pump**	0 – 100% Output Ability to change flow rate % via controller	12 RPM / 0.12 to 7.1 ml/min depending on tubing size, cycle time & setpoint
Pump 2 **Watson Marlow 400F/B1 – one channel pump**	0 – 100% Output Ability to change flow rate % via controller	12 RPM / 0.12 to 7.1 ml/min depending on tubing size, cycle time & setpoint
Pump 3 **Watson Marlow 400F/B1 – one channel pump**	0 – 100% Output Ability to change flow rate % via controller	100 RPM / 1.00 to 59.0 ml/min depending on tubing size, cycle time & setpoint

pH

The output sends a signal to an acid, base pump and/or gas. An Ingold gel filled pH probe and housing is supplied. The pH control range is 2-14 pH.

SPECIFICATION	*Control (Range)*
pH	2 - 14

Option of 2nd pH is available: Allows users to switch to a second pH probe during a batch in case of a probe failure.

OR

Option of Redox is available: Allows users to measure oxygen reduction potential of the media.

DO

Measure the level of dissolved oxygen in the reactor with DO probe and maintain the DO level using agitation (RPM), gas(es) supplementation and/or nutrient pumps.

SPECIFICATION	*Control (Range)*
DO	0 – 200%

Option of 2nd DO is available: Allows users to switch to a second DO probe during a batch in case of a probe failure.

GAS (ES)

TMFC will control flow within +/- 0.1 slpm when gas(es) are turned off.

- Unit with one TMFC is built with 4 gas solenoid valves to automatically control the amount of air/gas(es) entering the vessel.
- Unit with zero TMFC is built with 4 gas solenoid valves and manually adds the total gas flow to the vessel via Rotameter.
- Unit with two, three and four TMFC are not built with 4 solenoids as each TMFC controls each gas.
- Gas Flow with

  Standard:
  - One TMFC *0.4 to 20 SLPM* with 4 solenoid valves

  Options:
  - Zero TMFC with 4 solenoid valves and manual Rotameter
  - Two, Three, or Four TMFC – Select gas flow range of *0.1 to 5* or *0.4 to 20 SLPM*
  - Fifth TMFC for Gas Overlay – Available with:
    - 4 solenoid valves with Rotameter
    - One TMFC with 4 solenoid valves with gas flow range of *0.1 to 5 SLPM*

In Fermentation Process – We recommend .4 to 20 SLPM for most cultures, and 0.1 to 5 SLPM for smaller size vessels or multiple TMFC.

BIOFLO 310 FERMENTATION VESSELS				
Total Volume	***2.5L***	***5.0L***	***7.5L***	***14.0L***
Working Volume	0.75L-1.75L	1.25L-3.75L	2.0L-5.5L	3.0L-10.5L
Headplate Ports	(1) 6.35 mm (9) PG 13.5	(3) 6.35 mm (10) PG 13.5 (1) 19mm	(3) 6.35 mm (12) PG 13.5 (1) 19mm	(3) 6.35 mm (12) PG 13.5 (1) 19mm

FOAM/LEVEL DETECTION

A level probe will be provided for level detection. The sensor will send an on/off output signal to the controller, and automatically works with the pumps to inform user of foam/level condition in the vessel.

5.0 Utility Requirements and Process Connections

Service/Utility	*Requirement*	*Connection*
Electrical	110/230 VAC, 50/60 Hz. Single Phase, 15 Amp (fluctuations not to exceed ±10%)	110/230 V Cable & Plug
Water Return (Outlet-Drain)	Open Drain	Quick Connect
Facility Water (Inlet)	3 GPM must be regulated to 10 PSIG	Quick Connect
Process Air	10 PSIG	Push on
Oxygen	10 PSIG	Push on
Exhaust	Open Exhaust	Quick Connect

BIOFLO 310 ENVIRONMENT REQUIREMENTS

* Ambient temperature range of 10°C to 35°C
* Relative humidity up to 80% non-condensing
* Require 4” space from back of BioFlo 310 Console to the wall for ventilation and cooling

BIOFLO 310 WEIGHT

Control Stations (Includes Display): 88 lbs.
Display Only: 15 lbs.

BIOFLO 310 DIMENSIONS:

BF 310 w/ Touch Screen: 18”W X 24”D X 34”H
BF 310 Utility Station: 11”W X 24”D X 28”H

6.0 Service

We at New Brunswick Scientific realize the importance of having minimum downtime in the daily operations of your fermentor. It is important for you to have immediate response to your service needs as they occur. It is our intention to maintain a partnership with you and to make sure you are operating as smoothly as possible at all times. New Brunswick Scientific has qualified professionals that are trained to deal with any problem that may affect your system, minimize downtime and restore your system to optimum operating levels of efficiency.

7.0 Spare Parts

New Brunswick Scientific Co., Inc. considers the ready availability of spare parts important to the economical and successful operation of this equipment. A complete spare parts list is an integral part of the instruction manual supplied with each unit. Most components are readily available for immediate shipment.

8.0 Start-Up and Operator Training

START-UP ASSISTANCE

A trained New Brunswick Scientific Service Engineer will provide up to (1) man-days of start-up assistance at the installation site. This assistance will include qualifying utility connections along with performing basic unit operation.

NOTE: All start-up assistance is to be performed within the validity of the warranty.

CUSTOMER TRAINING

Training programs can be conducted at New Brunswick Scientific's facility in Edison, New Jersey or On-Site at the operators facility. An additional daily charge plus all living and traveling expenses to be paid by the buyer.

9.0 Warranty

All equipment is warranted free from defects in material and workmanship for 12 months from the date of equipment start-up (not to exceed 60 days from delivery). The exceptions to this warranty are:

A. All glass parts which carry no warranty;
B. All electrodes, which are warranted for 15 months from the date of shipment from NBS, or 6 months from the date the customer accepts the equipment; whichever comes first.

Our obligation under this warranty is limited to repairing parts or providing replacement parts at no charge, which prove to be defective during the warranty period. A part shall be considered defective after inspection of NBS' technical staff. At NBS' option, we will repair or replace any defective part, which is returned to our plant in Edison, New Jersey, U.S.A., freight prepaid. The cost of shipping the repaired or replacement part will be borne by NBS.

Should the services of an NBS Engineer or Technician be required to the repair, travel time and expenses to and from the job site will be charged at the rates, which prevail at that time.

This warranty does not extend to equipment or parts that have been subjected to misuse, neglect, accident or improper installation or application; nor shall it extend to equipment or parts, which have been repaired or altered outside the NBS plant without prior approval by NBS.

10.0 PRICES & CONDITIONS

QTY	DESCRIPTION	LIST UNIT PRICE	DISCOUNTED PRICE
1	**BioFlo 310 Autoclavable Benchtop Fermentor Includes the following:** • Utility Station with built in controller • Four Gas mixing with • One thermal mass flow controller (0.4-20 SLPM) • Touch Screen Display • 5.0 Liter Total Volume Fermentor, • working volume Vessel Assembly. • Rushton type Impeller • Ingold pH and DO probe Kit	$38,690	$30,952
1	**M1230-3030** Water Prefilter Assembly	$770.00	$831.60
1	**M111702040** Air Prefilter Assembly (4 Manifold)	$500.00	$603.90
1	Biocommand Batch Control	$9,691	$5330,05
	DISCOUNT PRICE		**$37,552.05**

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NOTE:
Above quotation is valid for 60 days.
Limited availability on refurbished items.

F.O.B.: Destination
FREIGHT IS PREPAID AND ADDED TO THE INVOICE

11.0 SHIPMENT

Actual delivery date will be determined when order is received and purchase order has been generated.

12.0 PAYMENT TERMS

100% due 30 Days after delivery.

13.0 ORDERING INFORMATION

***MAIL PURCHASE ORDERS TO*:**	***TELEPHONE ORDERS TO*:**
Eppendorf North America	1-800-645-3050, 516-334-7500
102 Motor Parkway, Suite 410,	Fax Number: 1-516-334-7506
Hauppauge, NY 11788-5178	
In Canada:	In Canada:
Eppendorf Canada Ltd	800-263-8715, 905-826-5525
2810 Argentia Road, Unit #2	Fax Number: 905-826-5424
Mississauga, ONT L5N 8L2	

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PROPOSAL

BioFlo 610 65 Lit

Date: 04/08/11
Proposal Number: 040811-BF610-UPEN

Prepared By: George Faragalla

Eppendorf North America
102 Motor Parkway, Hauppauge, NY 11788
In the US: 800-645-3050 * In Canada 800-263-8715
www.eppendorf.com

Table of Contents

1.0 Engineering Philosophy

In developing their line of Sterilizable-In-Place fermentors, New Brunswick Scientifics' engineers decided to take a novel approach. The approach was to ask professionals in the biotech industry about the features that were needed in an industrial fermentor instead of following past design concepts. The result is a line of customizable equipment with the shortest lead-time in the industry, a competitive cost, and capable of expanding with the end user's process.

1.1 Key Features

Vessel
While other system manufacturers offer simple, generic vessels, with only a few penetrations for sterile media transfer or nutrient additions, New Brunswick Scientifics' standard vessels offer a large number of ports capable of being sterilized in place. Combining this fact with the overall design philosophy of the line, allows each vessel to be modified, after purchase, in order to accommodate additional functionality.

Support Skid
The piping system was designed to be as compact as possible so that even the largest system will occupy the smallest space. As with the vessel, the skid is designed with modularity in mind. The result is a piping frame capable of being modified to incorporate additional options after purchase

Controller
The control station's ergonomic and compact design allows for quick and easy access to all the necessary connections. A bright 15" adjustable color touch screen display provides the operator interface for controlling the system. Integration of auxiliary equipment or control software is easily managed through conveniently placed serial, USB, and analog inputs and outputs connections.

2.0 Standard System

2.1 Vessel

The BioFlo 610 comes standard with a sterilizable-in-place, type 316L stainless steel vessel that meets ASME and CE design requirements. Each vessel includes a motor, light, temperature probe, pressure gauge, sight glass, sparger, load cells, harvest valve, inlet filter, outlet exhaust filters and a rupture disk. Every surface in product contact is free from any pits, visible scratches, burrs, flash, folds, inclusions, crevices and nicks. All interior surfaces vessels are mechanically polished to a 20 microinch Ra better. All gaskets, diaphragms, O-rings, and other elastomeric components that are in product contact will be either Class VI steam-resistant (EPDM) or silicon. The vessels may be configured as either a cell culture unit or as a fermentation unit depending on the options chosen.

2.1.1 Vessel Data

CRITERIA	65.0L	125.0L

Total Capacity	65.0L	125.0L
Maximum Working Capacity	50.0L	100.0L
Minimum Working Capacity	16.0	32.0
Height to Diameter Ratio (nominal)	3:1	
Approximate Unit Dimensions **Width x Depth x Height**	48” x34” x92” 122cm x 86cm x 234cm	
Jacket Type	Open Type	
Top Head	Flat Plate	
Bottom Head	Flanged and Dished (F&D)	
Vessel Design Pressure	125.0 PSIG, full vacuum	
Jacket Design Pressure	50PSIG	
Vessel Design Temperature	149°C (300°F)	
Jacket Design Temperature	149°C (300°F)	
Vessel Interior Finish	≤20 Ra	
Vessel Exterior Finish	Brushed/Ribbon Polish	
Vessel Codes	CE,ASME	

2.1.2 Standard Vessel Ports and Penetrations

The table below represents the connections, locations and the typical uses of the connections and vessel penetrations that are included with each vessel. These will be the same for both the 65.0L and the 125.0L vessels.

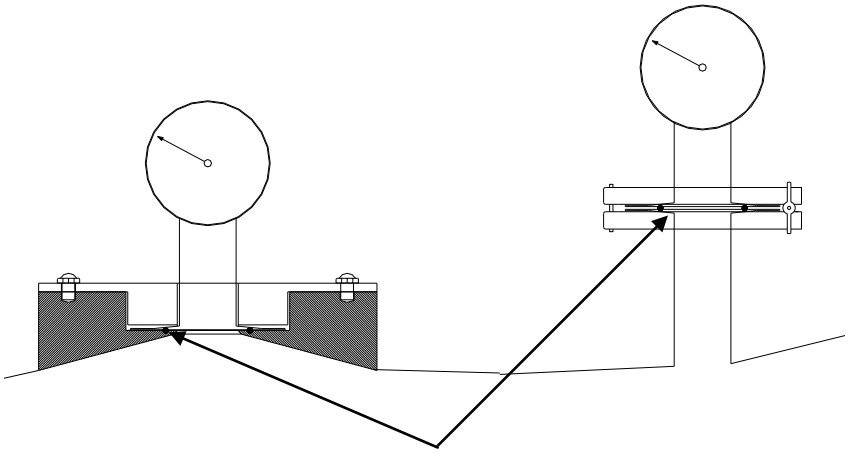
Location	Qty	Vessel Connection (Size)	Type	Typical Function
Head Plate	1	3”	FC	Exhaust Condenser w/ High Foam
	1	1 ½”	FC	Pressure Gauge / Spare
	1	1 ½”	FC	Spray Ball / Septum / Spare
	1	1 ½”	FC	Spray Ball / Septum / Spare
	1	1 ½”	FC	Septum / Spare
	1	PG 13.5	Threaded	Septum / Spare
	1	PG 13.5	Threaded	Hi-Foam/ Level High / Spare
	1	PG 13.5	Threaded	Foam/ Level Low / Spare
	1	2”	FC	Vessel Light
Bottom Head	1	2” (1” Radial Diaphragm Drain Valve)	FC	Drain Valve
	1	5.5”	Flange	Drive
Top Jacket	1	¾”	FNPT	Water Out/ Steam In
Bottom Jacket	1	¾”	FNPT	Water In/Steam Out
Bottom Side Wall	1	1.5” (Ingold 25 mm @ 15° Housing)	FC	pH
	1	1.5” (Ingold 25 mm @ 15° Housing)	FC	DO
	1	1.5” (Ingold 25 mm @ 15° Housing)	FC	Redox/ Spare pH/DO
	1	1.5” (Ingold 25 mm @ 15° Housing)	FC	Redox/ Spare pH/DO
	1	1 ½”	FC	RTD Thermowell
	1	1 ½”	FC	Sample Valve
	1	1 ½” (3/8” Sanitary Process Valve)	FC	Bottom Sparge
Upper Side Wall	4	1 ½” (3/8” Sanitary Process Valve)	FC	Addition Ports
	1	1 ½” (3/8” Sanitary Process Valve)	FC	Overlay
	1	1 ½”	FC	Pressure transmitter
	1	3”	FC	Sight Glass
	1	1 ½”	FC	Rupture Disk

FC - Flush Connection, FNPT – Female National Pipe Thread

2.1.3 Flush Connections

New Brunswick Scientific has improved upon the standard vessel design by changing many of the connections on the vessel to flush connections. With a flush connection, weld studs on the top, bottom, or sidewall of the vessel are avoided and a flush mounted, a tri-clamp type of connection can be made to allow an easier cleaning and sterilization. While still not practical for making all of the connections on the vessel, a flush connection provides an excellent solution for increasing the sterility and cleanability of the interior of the vessel.

As can be seen below the flush connection, on the left, is mounted flush with the interior of the vessel, while the tri-clamp actually extends away from the vessel. The net effect is the dead leg with a flush connection is virtually eliminated creating an easier to clean and more sterile vessel.



Jacket
The jacket is fabricated of type 316L stainless steel. The exterior of the jacket will be polished to match the exterior surface of the vessel and the mating welds will be completed with a ribbon finish.

Utility Skid
The piping for steam, water, air and other process gases is located on a skid-mounted piping module and offers the following features:

1. Automatic valves for controlling the steam supplies, heat exchanger, circulation pump and glycol while the unit is in sterilization and growth.

2. Stainless steel support and piping skid.

3. Welded or tri-clamp connections used in areas that are within the sterile envelope. **Note**: The sterile envelope includes all surfaces that are in product contact, consisting of the following:

- The process lines between the air inlet filter and the exhaust filter
- All vessel connections up to isolation valves
- All process steam lines up to isolation valves

- Inside of the rupture disk facing the interior of the vessel.

4. All valves, devices, and tubing within the sterile envelope are made of 316L stainless steel. Valves will be oriented to permit the best possible drainage, ease of operation and access for maintenance.

5. Fully drainable piping

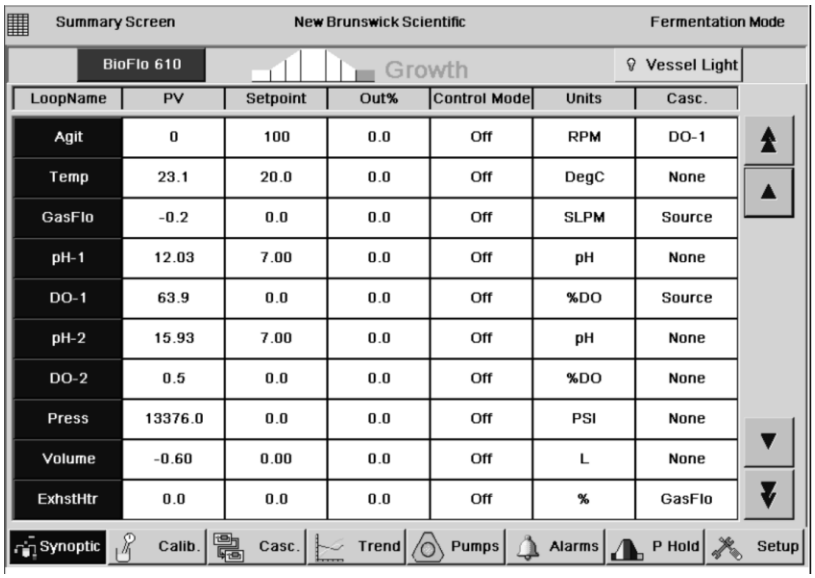
6. Designed with mobile compact footprint to minimize the amount of space the system takes up.

2.1.4 Controller

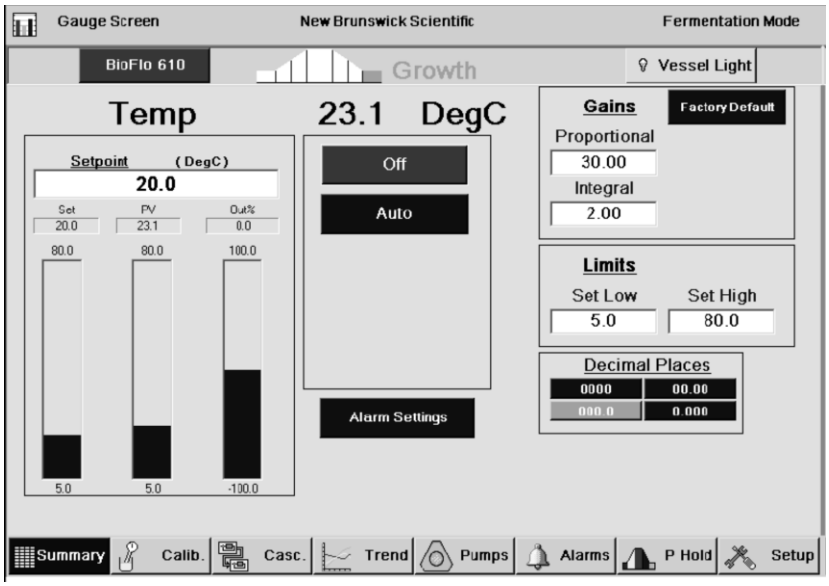
The control station consists of a splash proof cabinet and touch screen controller capable of operating at temperatures between 10°C and 30°C with humidity values up to 80% non-condensing relative humidity. Each controller will be equipped with a power switch, necessary I/O modules, necessary wiring, external connections for probes and transmitters, three pumps, gas mix solenoids, and a touch screen display. The control station is capable of operating 24 hours a day, 7 days a week without interruption and provides monitoring, controlling, and alarming for the equipment interfaced with and connected to the controller. The touch screen, also known as the OIT, allows for interaction with the control software via a digital display. From the Display, users will have the ability to navigate to the following screens:

NOTE: The following "screens shots" are for illustration purposes only. The actual screens may vary depending on the system configuration and software revision.

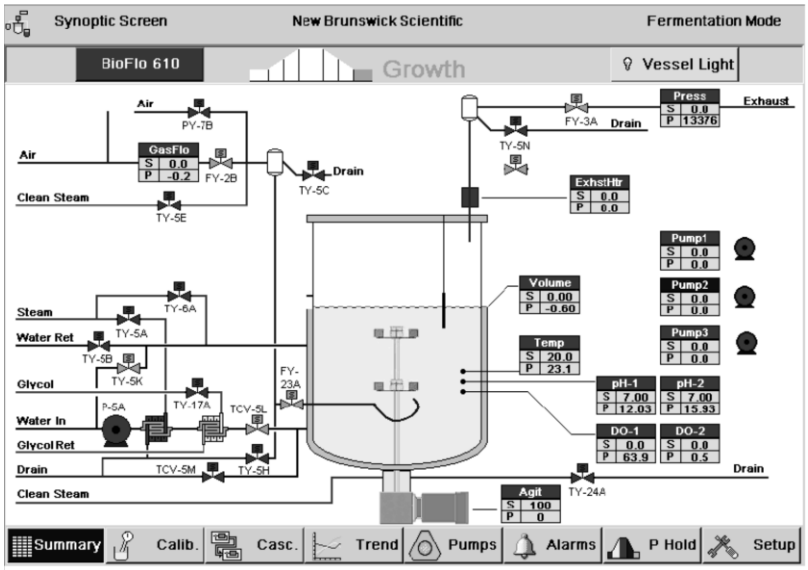
Summary Screen: This overview screen let users view all loop names, process values, setpoint values, output%, control mode, units, and cascades. Add additional loops controlled via Analog and Serial I/O directly through this screen. Additional Screens are associated where users can modify setpoints, control modes, configuration, and alarm screens of all controlled parameters except pumps.



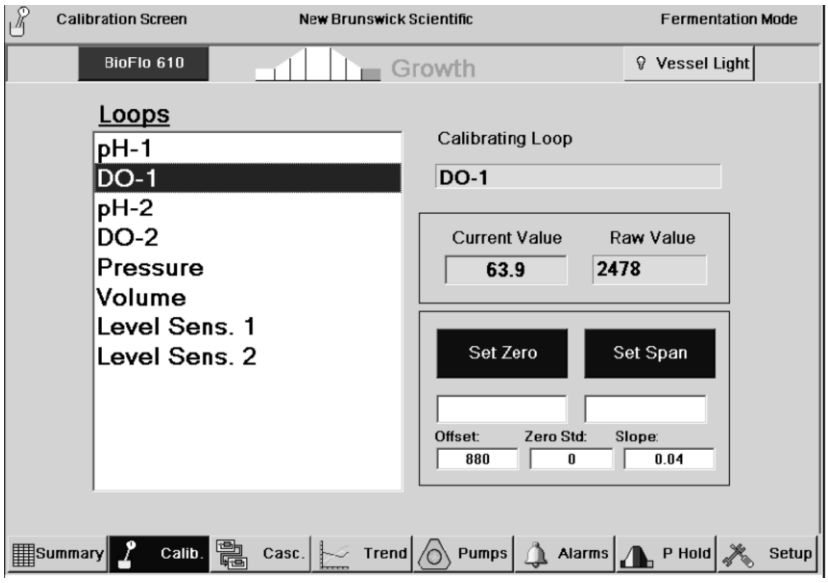
Gauge Screen: Turn the process loop to auto, manual and off. Set proportional and intregral values, enter high and low limits for process loop, link to alarm set up page.



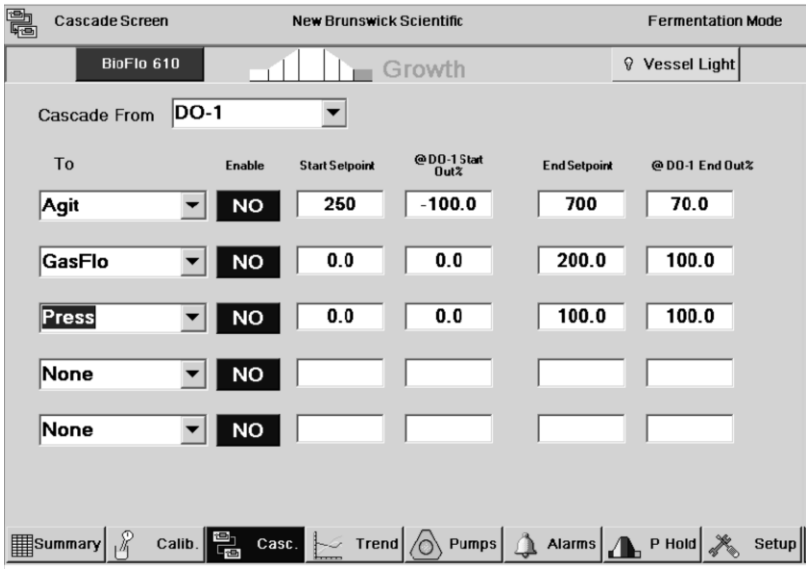
Synoptic Screen: Provides a schematic overview of the entire control system. Users have the ability to monitor and adjust the set points and process variables of available loops. They also have the ability to view the open/closed status of control system valves.



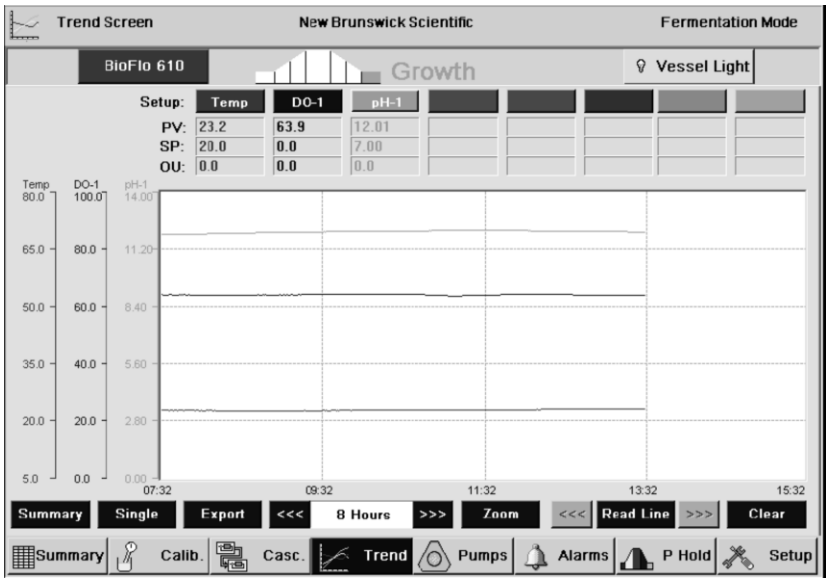
Calibration Screen: From this screen users can calibrate all the process parameters such as pH, DO, Redox, level, volume, and pressure.



Cascade Screen: Enable/Disable cascades for any and all parameters as specified in each configuration, including dissolved oxygen and pH.



Trend Screen: This screen provides plotting for up to 8 parameters on a single screen and view real time trend graph for all of the 8 parameters.

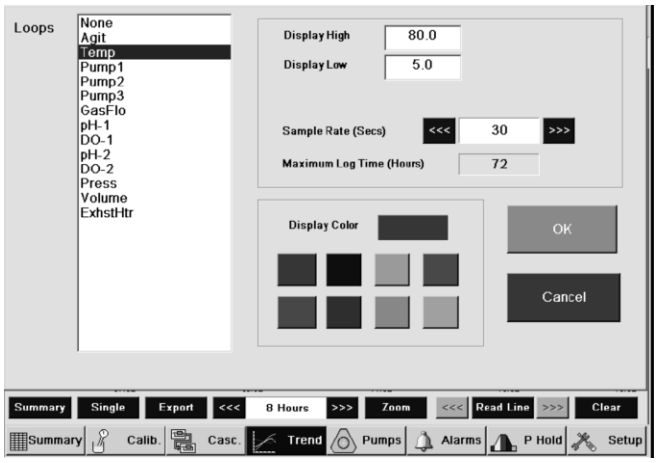


Features:

* Adjustable High/Low for each parameter
* Add or remove loops at any time
* Zoom feature for high detail
* Export data through USB port
    - Export data is available in Excel format
* Choose sample time from minimum 5 seconds to maximum 60 seconds
    - See below chart for total trend time:

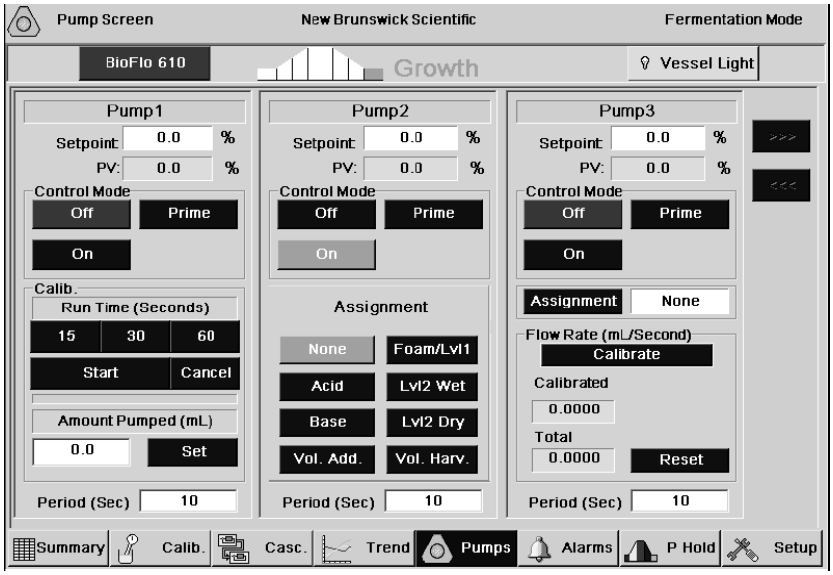
Sample Time (SEC)	Total Trend Time (Hours)
5	12
15	36
30	72
60	144 (6 days)

In a single run, data is overridden after the maximum trend time.

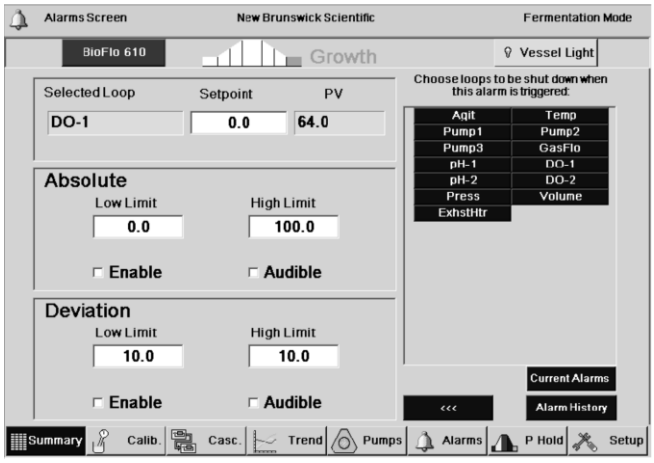


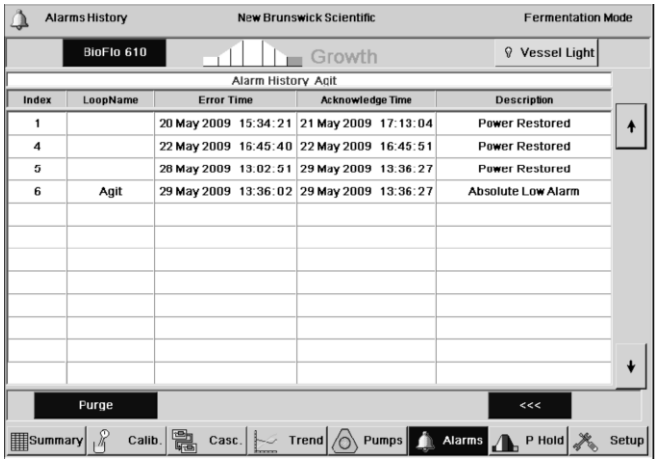
Pump Screen: This screen controls all the pump parameters including 3 built-in fixed speed pumps on each Control Station, and any additional variable speed pumps added through the available Analog Inputs and Outputs.

- Users can select assignment to choose any of the 6 methods to operate the pumps.
- Users have the ability to calibrate the pumps on-screen and enter a value in ml/sec; thereafter view the total pump flow rates. Users can set cycle time of each pump in seconds.

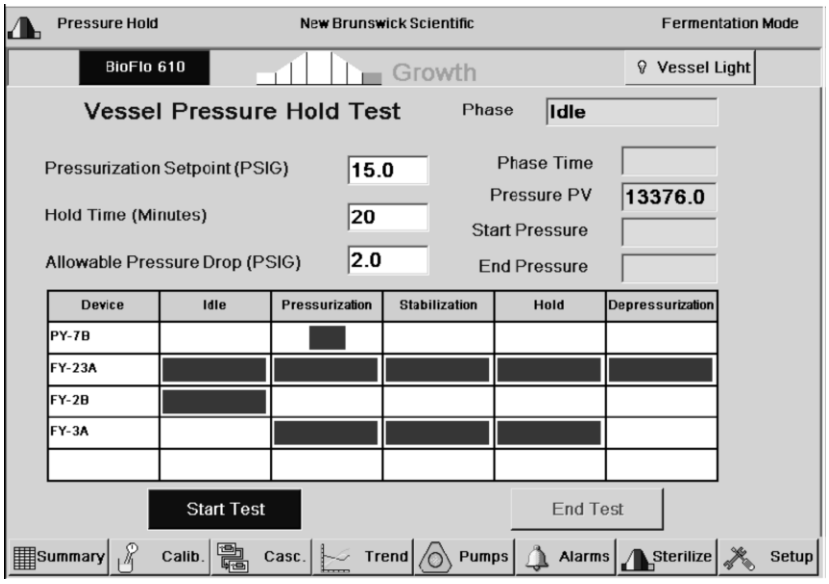


Alarm Setup Screen: Define absolute or setpoint deviation alarm for any & all controlled parameters on a single screen. View history of all alarms during each run.

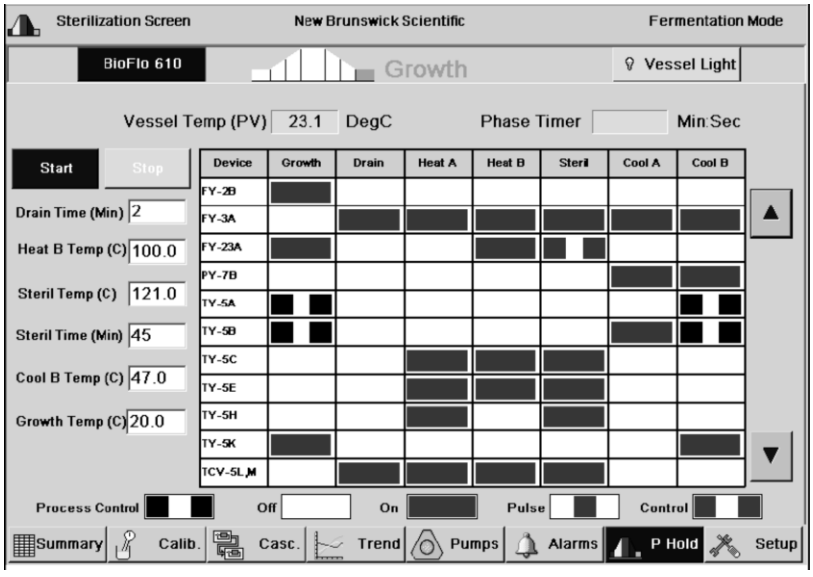




Pressure Hold Screen: This screen allows users to specify the test parameters and perform a pressure hold of the sterile envelope (2.1.3). It is used to check for leaks prior to initializing a sterilization cycle or after maintenance/service.



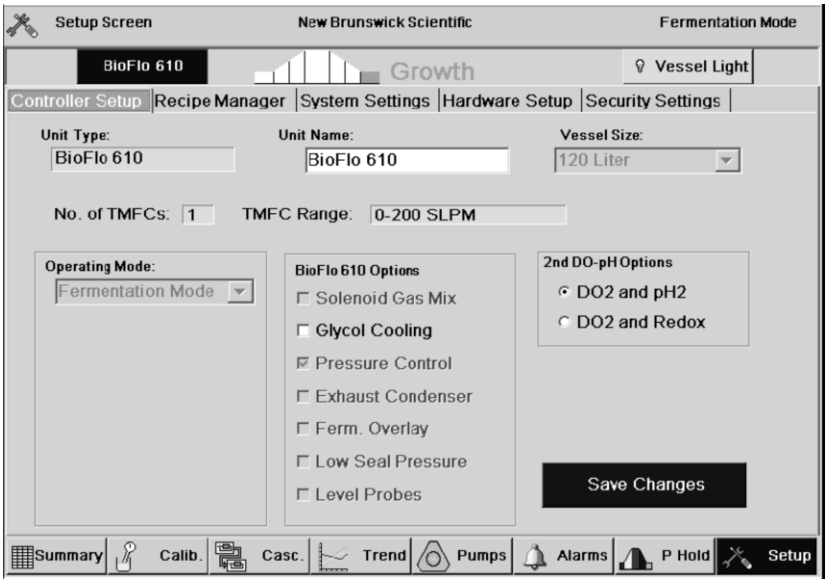
Sterilization Screen: This screen allows users to modify the sterilization temperature, hold time, drain time, and growth temperature. Users will have the ability to view sterilization valve sequences as well as a method for starting and aborting the sterilization sequence.



Setup Screen: This screen has 4 attached screens, including Controller Setup, Recipe Manager, System Settings, and Hardware Setup.

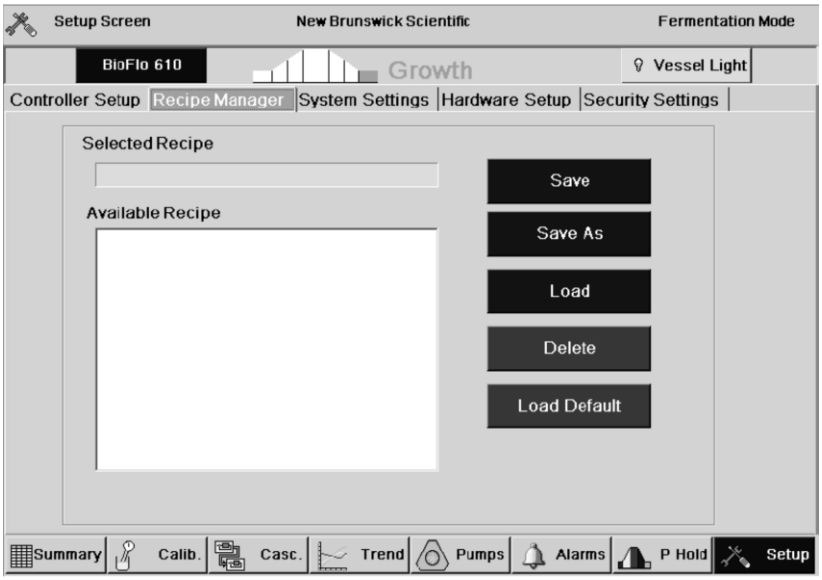
Controller Setup

- User can enter Unit Name,
- Select Gas mixing options
- Optional: 2nd DO & pH or 2nd DO & Redox – only available if this option is purchased
- View Unit Type, Number of TMFC and TMFC Range (Set from the Factory)



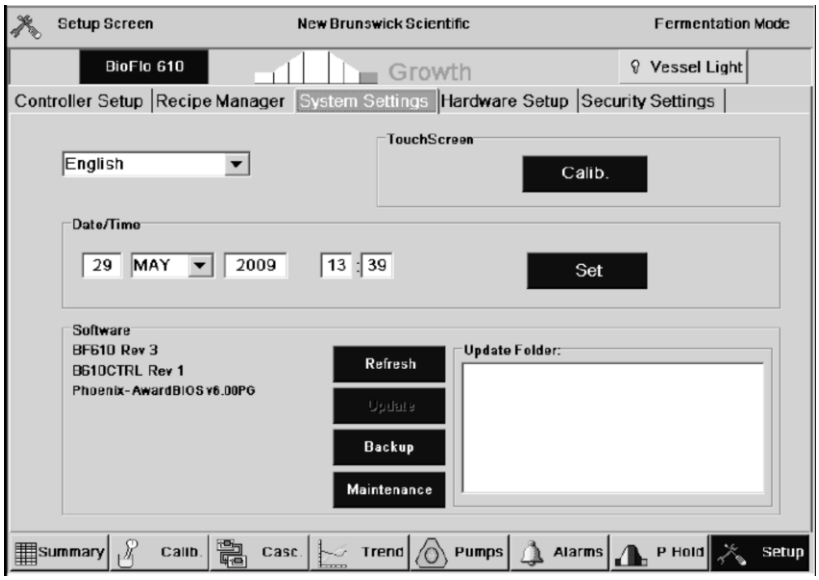
Recipe Manager

- Create, Save and load up to 10 recipes



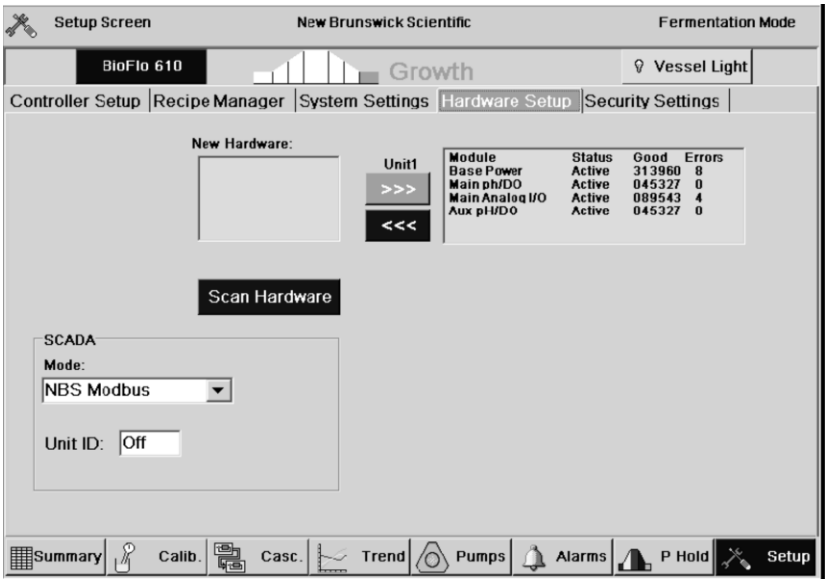
System Settings

- Language: English
- Calibrate the touch screen display
- Set Time and date
- View current firmware and software versions
- Install software updates via USB flash drive
    - Updates are available on NBS web site.



Hardware Setup

- View and set the system hardware
- Set Unit ID for software
- Set SCADA parameters



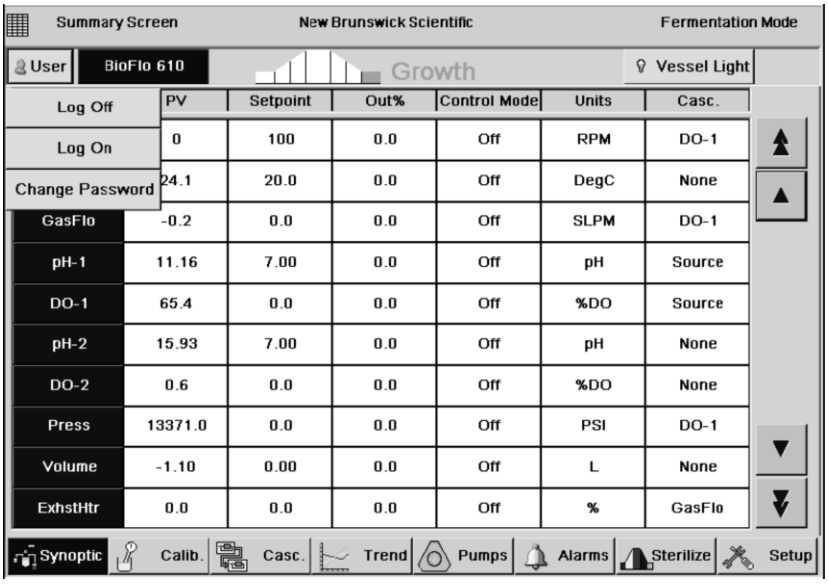
Additional Control Station features:

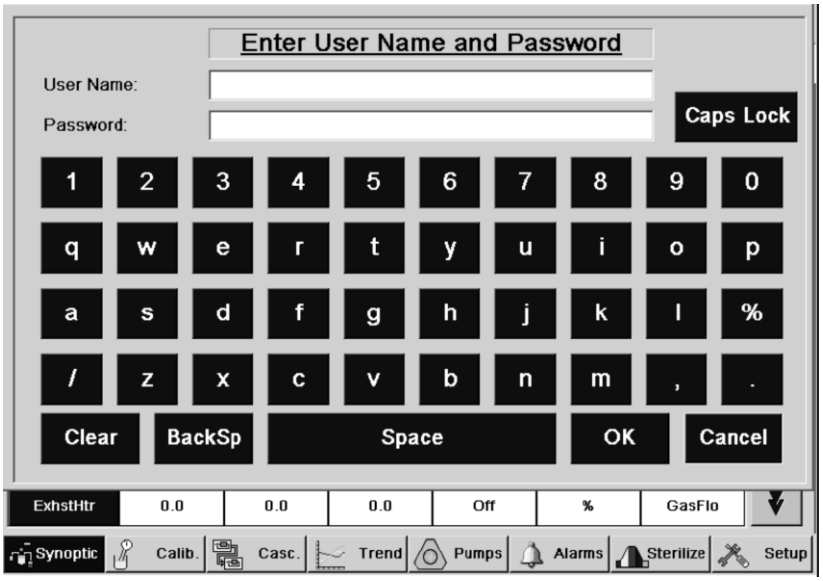
1. Two USB ports to update the firmware/software service, export trend data and required for RS232 (Serial) Controller when adding scales and other RS232 devices.
2. 3 built-in fixed speed peristaltic pumps by Masterflex
   a. (3) 100 RPM
3. 8 Analog Inputs and Outputs
   a. Four 4-20mA or 0-5V interchangeable Inputs
      Three Inputs are available for user loop use and one input is dedicated for Gas Overlay option.
   b. Four 0-5V Inputs
      Designated for the various TMFC options. When TMFC options are not installed the Inputs can be used for additional user loops.
   c. Four 4-20mA or 0-5V interchangeable Outputs and one output is dedicated for Gas Overlay option.
      Three Outputs are available for user loop use
   d. Four 0-5V Outputs
      Designated for the various TMFC options. When TMFC options are not installed the outputs can be used for additional user loops.

*Each TMFC occupies (1) 0-5V Analog input/output

2.1.5 Control System Security

The control system software has integrated security featuring multi-level access and individual username and passwords. Users will be assigned as Operators, Supervisors, or Administrators and each access level has pre-defined level specific privileges. Users with the appropriate access can either enable or disable this security feature depending on their preferences. When security is enabled persons without password access can only view the summary and trend screens.





2.2 Standard Mechanical Subsystems

2.2.1 Automatic Sterilization

A user defined sterilization program is used to automate the valve operation required to sterilize the vessel, and piping within the sterile envelope. This user can enter the vessel jacket drain length, heat B temperature, sterilization temperature, sterilization hold length, cool B and growth temperatures. Automatic sterilization program can be used to sterilize both full and empty vessels. The sterilization process is initiated from the sterilization screen on the OIT. Automating the sterilization process reduces operator to operator variations.

2.2.2 Temperature Control

The temperature control system consists of an RTD temperature sensor in a thermowell, located in the lower sidewall of the vessel, and two valves used to inject water or steam into the vessel jacket.

1. Temperature control range: 10°C above water supply temperature to 90°C ± 0.5°C*****.
2. The vessel temperature will be displayed on the OIT.
3. The temperature control loop is capable of heating the vessel at a rate of at least 1°C/minute.

****NBS recommends that when a system is to be used for a high temperature application (50°C or higher) that it be configured with an exhaust condenser and that the cooling water to the condenser be set at 8°C. In addition, gas flow for this anaerobic fermentation process should be limited to 0.2 vvm (vessel volumes per gas volume per minute). At the uppermost suggested gas flow of 0.2 vvm the evaporation rate will be approximately 2% of the vessel volume per day.***

2.2.3 Load Cells

The skid design of this system allows for the incorporation of load cells which measure the weight of the vessel and convert that information to a digital display of vessel volume. **Note**: Weight will be displayed on operator interface and can be setup as a control parameter for the pumps.

2.2.4 Pumps

3 Fixed speed pumps for control of nutrient, acid, base, foam or harvest. The unit can incorporate up to 3 additional fixed or variable speed pumps via available Analog Input/Outputs

SPECIFICATION	*Control*	*Speed rpm / Flow Rates*
Pump 1-3 **Masterflex** **Easy–Load** **One channel pump**	0 – 100% Output Ability to change flow rate % via controller	100 RPM / See table below for details on tubing size

Tubing Wall Thickness	*1/16 inch (1.6mm)*					
Inside Diameter inch (mm)	0.03 (0.8)	0.06 (1.6)	0.12 (3.1)	0.19 (4.8)	0.25 (6.4)	0.31 (7.9)
Hose Barb inch (mm)	0.06 (1.6)	0.06 (1.6)	0.12 (3.2)	0.19 (4.8)	0.25 (6.4)	0.38 (9.5)
Flow ml/revolution	0.06	0.21	0.8	1.7	2.8	3.8
100 RPM Flow ml/minute	6.00	21.6	80.0	170	280	380

2.2.5 Agitation System

SPECIFICATION		
BioFlo 610 65.0L	50-500 RPM	(Reference 1 and 2 below)
BioFlo 610 125.0L	50-500 RPM	(Reference 1 and 2 below)

1. "Measurement Accuracy" (difference between calibrated tachometer and set point) ±1% full scale

2. "Control" (deviation from set point)
   50-200 RPM ±2 RPM
   201-500 RPM ± 5 RPM

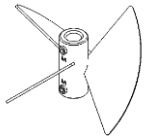
3. Digital display of RPM on the OIT.

4. Ball bearing shaft and housing with double mechanical seal (carbon to tungsten carbide) with sterile condensate lubrication.

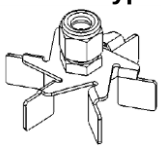
5. Four (4) type 316L stainless steel removable baffles are standard for fermentation vessels. Baffles will be optional for cell culture vessels. Baffles are electropolished to a 15Ra finish or better.

6. Impellers are adjustable in position along the shaft; alternate types of impellers can be substituted.

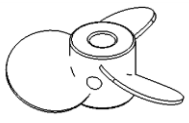
   Optional Impellers
   - **Pitched Blade Impeller** - Right and Left Handed



   - **Rushton type Impeller** - Standard with Fermentation System



   - **Marine Blade Impeller** - Right and Left Handed



2.2.6 Automatic Exhaust Pressure Control System

1. A sterilizable in place, heated filter will be installed on the vessel exhaust to insure that the filter keeps dry during the process run.
2. A pressure gauge will be provided for monitoring the pressure inside of the vessel.
3. Vessel pressure will be automatically controlled via an electronic pressure regulator (between1-15 PSIG)

2.2.7 Aeration System

Filtered, regulated, process air will be piped to the skid (by end user) and connected to a manifold outside the sterile barrier.

1. The gas supply line is sized to deliver an aeration rate of 1.5 vessel volumes per minute (VVM) of gas mixtures.
2. Available aeration configuration include:
   - Single gas system with rotameter
   - Two gas system (gas mixing via solenoids valves) with rotameter
   - Single gas system with Thermal Mass Flow Controller (TMFC)
   - Two gas system (gas mixing via solenoids valves) with TMFC
   - Two TMFC system
3. One sterilizable in place inlet filter with a stainless steel filter housing comes standard.
4. Air will enter the vessel though a removable ring sparger that is connected on the lower sidewall of the vessel.

3.0 System Options

The options presented below may be purchased at the onset of the project, or any time after the system has been installed at the end user's site. This is due to modular design concepts that were employed during the development of the product. The intent of the modular design was to allow each system to grow and adapt with the user's process needs. This feature allows the flexibly to purchase and install options that may have not been thought of when the equipment was ordered or were not needed due to the intended end use of the system.

3.1 Gas Inlet Line Options

3.1.1 Automatic Gas Flow Control

This feature uses 1 or 2 thermal mass flow controllers (TMFC) at various flow rates to automatically control the amount of air and other process gasses entering the vessel can be introduced into the media automatically through a DO control loop or through a user defined cascade system.

SPECIFICATION	*Range*	
Rotameter	1.5 – 75 SLPM* 3.0 -150 SLPM*	65.0L Fermentation 125.0L Fermentation
1 TMFC	1.5 – 75 SLPM 3.0 -150 SLPM	65.0L Fermentation 125.0L Fermentation
2 TMFC	1.5 – 75 SLPM, 0.6-32 SLPM 3.0 -150 SLPM, 1-64 SLPM	65.0L Fermentation 125.0L Fermentation

*Anticipated flow range

3.1.2 Fermentation Overlay Option

The fermentation overlay option allows an operator to redirect sparge gas into the head space of the vessel.

3.2 Exhaust Line Options

3.2.1 Exhaust condenser

A stainless steel exhaust condenser can be installed on top of the vessel to condense liquids contained in the process exhaust stream. Many cultures are grown at temperature above ambient and the condenser allows the system to retain as much volume as possible by counteracting evaporation.

3.3 Probe and Sensor Options

3.3.1 pH/DO SYSTEM

pH
The pH portion of this option outputs a control signal to an optional acid or base pump. An Ingold gel filled pH probe and housing is supplied. The pH control range is 2-14 pH.

SPECIFICATION	*Control*
pH	2 – 14 ± 0.1 units

DO
The DO portion of the option is used to control the amount of oxygen in the media. The level of dissolved oxygen in the system will be maintained using RPM, or airflow and O_2 supplementation if purchased.

SPECIFICATION (Range)	*Control*
D.O.	0 – 200% ± 0.1%

3.3.2 Spare pH/DO, Redox System

This option allows operators to run redundant probes during a batch in case of a probe failure. It also allows a user to integrate a Redox sensor if they choose to use one.

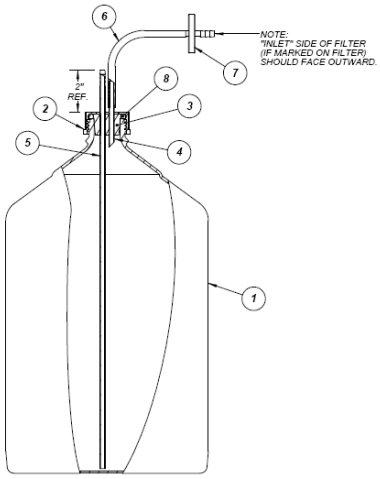
3.3.3 Foam/Level Detection

Up to two foam/level probes can be provided for detection of vessel volume or foam accumulation. The foam/level sensor will respond to conductivity measurements and will send an on/off output signal to an antifoam or other designated pump.

3.4 Addition and Sample Options

3.4.1 Addition Vessels

A variety of autoclavable vessels designed for vessel additions of acid, base, antifoam, nutrient or any other solution. These vessels can also be used to collect product or waste from a continuous process. For a complete list of addition vessels see the Addition Bottle/Containers/Tubing (Select the Required Options) in Section 7.



3.4.2 Re-Sterilizable Sample Valve

This option allows the user to minimize the possibility of contamination and take multiple samples from the same valve. The valve can be quickly and effectively sterilized after each sample so that it is ready for the next. The valves have sanitary tri-clamp fittings which allow for easy connections to sample apparatus.

3.4.3 Sterile Sampling Kit

Includes 3 assemblies of stainless steel Weir style diaphragm valve by port, sanitary fitting to hose barb conversion with clamps, gaskets, tubing and 500 ml glass bottles with 0.22 □m vent filter. This assembly when used with the resterilizable sampling valve on the 610 will allow users to collect sterile culture samples up to 500 ml. These assemblies are washable and reusable.

3.4.4 3/8" Resterilizable Addition Valve (maximum of 4)

This option provides operators with a way to make sterile additions for feedings, foam control, or pH control. These steam-through valves can be used multiple times during a run and there design allows for a sterile connection between addition bottle and the valve.

3.4.5 Spare Addition Isolation Valve (only)

Stainless steel Weir style diaphragm valve by port with 2 sizes of sanitary fitting to hose barb (1/4" and 1/8") conversion with clamps and gaskets. The spare addition isolation valve assembly provides the user with the flexibility to make multiple sterile additions to the vessel without having to remove the addition isolation valve that comes standard with the addition port, clean it, assemble it into a new tubing segment and autoclave it before they can make their next sterile addition to the vessel. The assemblies are washable and reusable

3.4.6 Single PG 13.5 or 1 ½" Tri-clamp 7-port QMI septum

A single or seven individual septum ports contained in a single housing for aseptic transfer of liquid from a sterile source to a sterile fermentor. Note: Septum is designed to resist positive pressure from vessel.

3.5 Miscellaneous Options

3.5.1 Glycol Heat Exchanger

This second heat exchanger allows the system to circulate glycol as a coolant to remove heat from the system. Unlike a single heat exchanger, which uses steam and water to heat the and cool an open loop system, this second heat exchanger allows for closed system and for glycol to be used as a cooling media instead of city water.

3.5.2 Transfer Line

Twenty feet of braided silicone hose with stainless steel sanitary fitting, gaskets and clamps on each side. This line attaches to the drain on one vessel and the addition port on the other vessel. Sterilization of transfer line is by steaming from the addition port steam supply on the receiving vessel to the low point condensate drain on the drain of the supply vessel.

3.5.3 Variable Speed Pumps

Additional variable speed peristaltic pumps can be included with the system. The operation of each pump is easily assigned and controlled from the operator interface and may be used for base, antifoam, nutrient, or perfusion.

3.5.4 Sprayballs

These sprayballs are specifically designed for each vessel to provide maximum cleaning fluid coverage within the vessel. Convenient tri-clamp connections allow for easy integration into an existing clean-in-place system.

3.5.5 8-port Serial controller (RS232)

This 8-port serial (RS232) communication controller connects to the control station via one of the USB ports and allows for the connection of up to 8 ancillary RS232 devices such as scales.

3.5.6 BioCommand SCADA Package(s)

Connect the controller to BioCommand for data logging or supervisory control. (System specification available upon request)

3.5.7 Validation Packages

Every system passes Installation Qualification and Factory Acceptance Testing before shipping. These Documents are available through several levels of validation packages designed to fit the needs of your process and facility.

Basic Validation
- Spare Parts List
- List of Materials and Lubricants in Product Contact
- Pressure Vessel Documentation
- Pressure Hold Test Certificate
- Media Hold Test Certificate
- Operational Qualification Documentation
- Installation Qualification Documentation

Basic Plus Validation
- ***includes Basic validation package**
- Blank copies of IQ/OQ Documentation
- Enhanced Spare Parts List
- Enhanced Pressure Vessel Documentation
- Calibration of Process Measurements
- Enhanced Material Certification of Parts in Product Contact

Enhanced Validation
- ***includes basic and basic plus validation packages**
- Weld Mapping of Process Piping W/Video Taping
- Passivation of Process Piping Certificate
- Temperature Mapping Test Procedure and Data
- Calibration Procedure for HP Agilent with Multiplexer and Type “T” Stainless Steel Thermocouples and Data
- Biological Challenge Test certificate

3.5.8 Utility regulators and filter kits

Utility Connection Kit – Converts sanitary (tri-clamp) fitting on the skid to MNPT (male threads) using compression type fittings to facilitate utility connection of the system when sanitary fitting is not required. This option is ideal for academic and non GMP applications.

Process steam regulator – (Stainless Steel regulator for clean steam applications)
Utility steam regulator and filter – (Bronze regulator for utility steam applications)
Water regulator and filter
Process and Instrument air regulator and filter

3.5.9 Steam Generators

NBS supplies electric steam generators to meet the BioFlo 610’s requirements. They are offered in either carbon steel or stainless steel. The LG15 generator in either carbon or stainless steel is appropriately sized to supply both utility and process steam. The stainless steel LG10 generator is sized to supply only process steam for applications requiring clean steam. Every generator constructed under A.M.S.E Boiler and Pressure Code Regulations and is ETL, CSA and UL approved and meets OSHA requirements. The user should check local codes in regards to a high pressure electric powered steam generator.

3.5.10 Chiller

NBS offers appropriately sized, air cooled chillers for the BioFlo 610. Chillers are used to facilitate rapid cool down post sterilization or for heat removal from exothermic processes. Used with our integrated secondary heat exchanger, the chiller provides a closed circulation loop that reduces the usage of facility water.

4.0 Utility Requirements

BioFlo 610 Utility Requirements	Size and Type	65.0L	125.0L
Electrical Requirements	L6-15 Plug	200-240VAC, 1ϕ , 50/60 Hz, 15 A	
Exhaust	¾" Male NPT	N/A	
Process Air (Gas 1)	3/8" Tri-Clamp	30 PSIG, 75 SLPM	30 PSIG, 150 SLPM
Oxygen (Gas 2)	3/8" Tube Push to Connect	30 PSIG, 32 SLPM	30 PSIG, 64 SLPM
Instrument Air	¼" Female NPT	80-100 PSIG, 2 scfm (56.5 SLPM)	
Process Steam	3/8" Tri-Clamp	35 PSIG, 10 lb/hr	
Utility Steam	¾" Tri-Clamp	35 PSIG, 50 lb/hr	35 PSIG, 100 lb/hr
Facility Water	¾" Tri-Clamp	30 PSIG, 4 GPM	
Water Return	¾" Tri-Clamp	Less than 15 PSIG back pressure	
Clean Condensate	¾" Female NPT	Gravity Drain	
Biowaste	¾" Tri-Clamp	Gravity Drain	
Glycol	¾" Female NPT	30 PSIG 4 GPM	30 PSIG 8 GPM

NPT – Female National Pipe Thread

5.0 Factory Inspection / Training

One (1) day Factory Inspection will be conducted at New Brunswick Scientific's facility in Edison, New Jersey. The end user will be responsible for all travel related expenses.

Factory Inspection allows a customer's appointed designee to represent them ensuring the system matches the purchase order and that any noted deviations have been addressed before shipment. NBS's standard factory one day inspection protocol includes a piping and instrument diagram review, system sterilization, and simulated growth operation. As an added bonus, the customer's appointed designee who participates in the Factory Inspection becomes familiar with system operation.

The one day of training must be scheduled within ten business (10) days of the written notification that the system is ready for testing. After 10 business days, it will be concluded that the customer has declined the option to perform the Factory Inspection. The customer may choose to decline the Factory Inspection at anytime by notifying NBS or their sales representative. The Factory Inspection is considered to be included with this quotation must be completed within 30 days from the initial written notification. Any exceptions to the above policy are subject to approval by NBS.

Additional training can be conducted at the customer site at a cost of $1,700 per day plus travel and related expenses.

6.0 Warranty

All equipment is warranted free from defects in material and workmanship for 12 months from the date of shipment from NBS, after equipment start-up (not to exceed 60 days from delivery), whichever comes first. The exceptions to this warranty are:

A. All glass parts which carry no warranty;
B. All electrodes, which are warranted for 15 months from the date of shipment from NBS, or 6 months from the date the customer accepts the equipment; whichever comes first.

Our obligation under this warranty is limited to repairing parts or providing replacement parts at no charge, which prove to be defective during the warranty period. A part shall be considered defective after inspection of NBS' technical staff. At NBS' option, we will repair or replace any

defective part which is returned to our plant in Edison, New Jersey, U.S.A., freight prepaid. The cost of shipping the repaired or replacement part will be borne by NBS.

This warranty does not extend to equipment or parts that have been subjected to misuse, neglect, accident or improper installation or application; nor shall it extend to equipment or parts which have been repaired or altered outside the NBS plant without prior approval by NBS.

7.0 System Configurations

Standard Unit – Refer to Section 2.0 for Specification	
BioFlo Fermentor w/ 65L Vessel Control System – NBS Reactor Process Controller with 3 built-in peristaltic pumps. Mobile Skid	$120,118.00
Impeller	
Bottom Drive	Included
Aeration	
Baffles	Included
Ring Sparger	Included
Vessel Additions and Sampling	
2 Addition Valves	$9,084.00
Resterilizable Sample Valve	$4,322.00
Gas Mixing and Control	
65L Two Gas - 1 TMFC (1.5-75.0 SLPM)	$15,720.00
Gas Overlay	
Without an Overlay valve	Included
Exhaust Condenser	
With Exhaust Condenser	$2,071.00
Vessel Pressure Control	
Automatic Pressure Control	Included
pH / DO / Redox Housing and Probes	
Ingold Polarographic 120 mm DO Probe/Cable/Housing	$2,961.00
Ingold Gel Filled 120 mm pH Probe/Cable/Housing	$1,594.00
Miscellaneous	
Level Probes (2)	$702.00
1.5" Tri-Clamp Septum 7 port	$606.00
TOTAL: $157,168.00	

Miscellaneous	
Low Seal Pressure Alarm	$2,687.00
Sterile Sampling Kit Assembly	$4,017.00
Utility Service	
Process Air Prefilter / Regulator Kit	$702.00
Utility Steam Prefilter / Regulator Kit	$4,221.00
Water Prefilter/Regulator Kit	$1,099.00
50'/16 meter RS-422 Serial Cable	$171.00
TOTAL PRICE	**$165,346**

8.0 Terms and Conditions

The following terms and conditions, "Terms and Conditions", apply to all bids, quotations, solicitations, and/or agreements between New Brunswick Scientific Co., Inc. herein referred to as "NBS" and the customer, its' representatives, and/or its' agents acting on its behalf herein referred to as "Purchaser or Customer". "Equipment" shall mean any equipment, machinery, parts, materials or services provided for in a Proposal and any contract resulting there from. "Proposal" shall mean the Terms and Conditions and the most recent proposal, bid or quotation of NBS provided to the Purchaser by NBS in connection with the transaction contemplated hereby.

8.1 Controlling Terms and Conditions

This Proposal alone constitutes an offer and all other offers or counteroffers whether conveyed by other documents or oral negotiations with respect to the subject matter hereof are hereby withdrawn, and of no further force and effect. This Proposal, including the Terms and Conditions, when duly executed by the parties, shall constitute the contract (the "Contract") resulting from our negotiations, regardless of any rejection thereof or statement to the contrary in any document soliciting the Proposal, or issued in response hereto unless such rejection statement, or response is expressly accepted in writing by an officer of NBS. Any conduct, which recognizes the existence of a contract shall constitute acceptance by both parties of the Terms and Conditions and rejection of all additional or different terms and conditions, proposed by Purchaser or incorporated by law. The applicability of this paragraph is an express condition of any contract being formed between the Purchaser and NBS.

8.2 Receipt of Purchase Order

Purchaser's Purchase Order shall be based on this Proposal. The terms of this Proposal shall control any terms and conditions contained in any purchase order issued in connection with the Proposal. The customer acknowledges and agrees that the Purchase Order is used for:

1. The Customer's convenience
2. Achieving compliance with the customer's internal procedures and requirements
3. The Contract that Customer accepted by execution of this Proposal is the document that governs the relationship of the parties

8.3 Shipments

Shipments will be F.O.B. Edison, NJ. USA, with insurance, freight and handling charges prepaid by NBS and billed to purchaser. Risk of loss shall pass to the purchaser upon delivery of equipment to carrier. Quoted shipment dates are approximate and are contingent upon timely

receipt pf purchaser's purchase order and supporting documentation. NBS reserves the right to request adjustments to the shipment schedule due to the nature of the work being performed.

Actual delivery dates will be determined when the order is received and purchase order has been generated.

8.4 Price

Prices quoted are valid for sixty (60) days from the date of this Proposal unless otherwise agreed to in writing by NBS. Prices do not include any applicable taxes or fees, such as sales or use tax, excise tax, property tax, customs fees (if applicable) or associated fees. All taxes and fees shall be the responsibility of Purchaser.

8.5 Payment Terms

30% at the time the order is placed;
30% 90 days after receipt of the purchase order;
40% against shipping documents;

8.6 Shipment Timeline

After order is placed and accepted, shipment will >><<<<weeks after receipt of order.

8.7 Change Orders

Any changes, modifications or deletions to the scope of the original purchase order must be made in writing by Purchaser and are subject to NBS' approval which approval shall not be unreasonably withheld. NBS will respond promptly to Purchaser on the impact, in terms of price and delivery that any change will cause. Prices quoted for such changes are valid for sixty (60) days.

8.8 Delays

The failure of NBS to perform any of the provisions of this Contract due to causes beyond the reasonable control of NBS, including but not limited to acts of God (including weather delays), disabling illness or death, acts of civil or military authority, labor trouble, inability to obtain necessary materials or components, war, riots, or fires shall not constitute a default under or a breach of the Contract and shall not subject NBS to any liability hereunder.

8.9 Liability

NBS shall under no circumstances be liable to Purchaser for special, incidental, exemplary, or consequential damages (hereafter referred to collectively as "consequential damages"), including, but not limited to loss of profits, anticipated revenue, interest, loss of use, loss by reason of plant shutdown, non-operation, cost of substitute equipment, facilities, services or utilities, costs incurred in removing defective of nonconforming Equipment and reinstallation of conforming Equipment delays in installation of the Equipment or completion of any project in which the Equipment is being installed or any other claims arising from any cause whatsoever whether or not such loss or damage is based in contract, warranty, tort (including negligence), strict liability indemnity or otherwise.

NBS' maximum aggregate liability for loss or damage arising under, resulting from or in connection with the supply or use of the Equipment provided under this Contract, or from the performance or breach of any obligation(s) imposed hereunder or otherwise, whether such liability arises from any one or more claims or actions for breach of contract, tort (including negligence), delayed completion, warranty indemnity strict liability or otherwise, unless otherwise limited by the terms

hereof shall be limited to the amount actually received by NBS hereunder Purchaser hereby releases NBS, its agent and employees from any further liability.

It is expressly agreed that this Contract sets forth the sole and exclusive remedies available to the Purchaser and that NBS' liabilities are limited as set forth herein. Purchaser acknowledges and agrees that NBS has not granted or assumed any other warranties, guarantees, duties, liability, or obligations, either express implied statutory at law or in equity. Purchaser further acknowledges and agrees that no breach of warranty or of contract or fault by NBS to fulfill any other conditions of the Contract shall in any manner alter limit or change the remedies available to Purchaser hereunder.

Governing Law and Form:

This Proposal shall be governed by and construed in accordance with the laws (but not the laws of conflict of laws) of and enforced solely in the courts of the State of New Jersey, USA and the jurisdiction of such courts over the parties is hereby expressly acknowledged. The parties expressly waive application and jurisdiction of the UN Convention on the international Sale of Goods.

8.10 Ordering Information

MAIL PURCHASE ORDERS TO:	***TELEPHONE ORDERS TO***:
New Brunswick Scientific Co., Inc.	Sales Dept.: 1-800-631-5417
Sales Department	
P.O. Box 125.005	Main Number: 1-732-287-1200
44 Talmadge Road	
Edison, NJ 08818-125.005	Fax Number: 1-800-489-1400

Mr. Ted Eckels
University of Pennsylvania
301-758-3289
220 South 33rd Street
Department Chemical and Biomolecular engineering
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PROPOSAL

BioFlo Pro 500 Liter Fermentation Systems with Allen Bradley PLC

Date: 04/08/11

Proposal Number: 040811-BFP-UPEN

Prepared By: George Faragalla

Table of Contents

1.0 General Description

The engineering embodied in the BioFlo Pro Fermentor series represents the accumulation of many years' experience and achievement in the manufacturing of fermentation systems. These fermentors are being used globally in applications ranging from polymer and biofuels research & development to pilot and commercial scale cGMP production of biologics. This commercial grade fermentor will deliver years of reliable operation.

The BioFlo Pro Fermentor series is fabricated in a modern plant under the control of a quality assurance system approved for the manufacture of ASME Section VIII Coded Pressure Vessels.

A staff of factory trained service engineers operating from key locations in the United States, and in most principal cities throughout the world, is available for technical assistance. Our service specialists are trained to diagnose problems and to adjust and service equipment promptly and efficiently in the field. NBS truly *differentiates itself from the competition* by having the following support capabilities:

- ***In-house Bioprocess Engineering Team***
- ***R & D Laboratories and Pilot Plant***
- ***Over 1.5 million dollars in segregated spare parts inventory***
- ***In-house Software Development Team***
- ***In-house Vessel Manufacturing Facility***
- ***Field Service Centers Worldwide***

2.0 Scope of Equipment Supply

NBS will design and fabricate the following fermentation system:

1. An ASME-coded / PED97/23-EC stainless steel vessel, process and utility piping skid and controller.

   Each system will come complete with:

   A. Fermentor vessel and Piping skid will come complete with:
      - Rushton-type impellers
      - Necessary fittings
      - Spare Ports
      - Required instrumentation agitation (motor and double mechanical seal)
      - Required instrumentation for temperature control (RTD)
      - Required instrumentation for automatic sterilization in place (SIP)

   B. Utility skid complete with:
      - Necessary piping
      - Required valves for automatic sterilization in place (SIP)
      - Devices necessary for achieving required vessel temperatures
      - Devices necessary for achieving required air flow (rotameter)
      - Devices necessary for achieving required manual vessel pressure control

      Note: The utility skid includes all of the connection points for integrating the system with the customer's utility piping.

   C. Operator manual.

3.0 Engineering Philosophy

In developing their line of Sterilizable-In-Place fermentors, New Brunswick Scientifics' engineers decided to take a novel approach. The approach was to ask professionals in the fermentation industry about the features that were needed in an industrial fermentor instead of following past design concepts. The result is a line of BioFlo Pro Fermentor systems that boosts one of the industries shortest lead-times, at a competitive price, and capable of expanding with the end user's process requirements.

3.1 Key Features

Vessel
While other system manufactures offer simple, generic vessels, with the least possible number of penetrations required for sterile media transfer or nutrient additions, New Brunswick Scientifics' standard vessels offer a large number of ports capable of being sterilized in place. Combining this fact with the overall modular design philosophy with standard options of the BioFlo Pro, allows each vessel to be modified, after purchase at the customer site, in order to accommodate additional functionality.

Piping Skid
The piping skid was designed to be compact decreasing the overall footprint of the system. As with the vessel, the skid is designed with modularity in mind. The result is a piping frame capable of being modified to incorporate additional standard options after purchase.

Controller

New Brunswick Scientific has broken away from the competition's rationale of developing proprietary controllers. Instead, NBS has decided to provide end users with the Allen Bradley CompactLogix programmable automation controller (PAC). The system was designed based upon the V-life cycle model as presented in the Good automation Manufacturing Practices guidelines.

4.0 Standard System

4.1 Vessel

The vessel is designed with a 3:1 height to diameter ratio to aid in the transfer of oxygen to the media. The vessel itself comes with a flanged and dished bottom head (F&D) to facilitate complete draining, and a spring assisted man-way head plate with a sanitary o-ring design. The vessel also comes standard with a jacket that allows water to be circulated around more than 60% of the vessel's surface. This feature enables the temperature of the vessel to change at 1^{o}C/minute allowing for rapid temperature increases, for induction, or decreases, for rapid cooling. The vessel comes complete with a with a 4" viewing window, a drain valve and was designed to be sterilized and cleaned in place, and meets ASME-codes as well as the PED97/23-EC directive.

4.1.1 75-Liter Vessel Assembly

CRITERIA		75-Liter
Total Capacity		75 liters
Maximum Working Capacity		60 liters
Minimum Working Capacity		32 liters
Unit Dimensions		80 inches L x 48 inches D x 96 inches H 203 cm L x 122 cm D x 244 cm H
Unit Weight	**Vessel**	275 lbs (125 kg)
	Electrical Cabinet/ Skid	500 lbs (227 kg)
	Piping Skid	300 lbs (136 kg)
Height-to-Diameter Ratio (nominal)		3:1
Jacket Type		Open Type
Top Head		Manway, ASME F&D
Bottom Head		ASME F&D dish
Vessel Design Pressure		40 PSIG (2.75 bar), full vacuum
Jacket Design Pressure		50 PSIG, (3.45 bar)
Vessel Design Temperature		149°C (300°F)
Jacket Design Temperature		149°C (300°F)
Vessel Interior Finish		20 Ra Mechanical Polish
Electropolished Interior Vessel		Optional
Vessel Exterior Finish		Brushed/Ribbon Polish around welds

4.1.2 150-Liter Vessel Assembly

CRITERIA	150-Liter
Total Capacity	150 liters

Maximum Working Capacity		120 liters
Minimum Working Capacity		45 liters
Unit Dimensions		80 inches L x 48 inches D x 96 inches H 203 cm L x 122 cm D x 244 cm H
Unit Weight	**Vessel**	400 lbs (182 kg)
	Electrical Cabinet/ Skid	500 lbs (227 kg)
	Piping Skid	300 lbs (136 kg)
Height to Diameter Ratio (nominal)		3:1
Jacket Type		Open Type
Top Head		Manway, ASME F&D
Bottom Head		ASME F&D dish
Vessel Design Pressure		40 PSIG (2.75 bar), full vacuum
Jacket Design Pressure		50 PSIG, (3.45 bar)
Vessel Design Temperature		149°C (300°F)
Jacket Design Temperature		149°C (300°F)
Vessel Interior Finish		20 Ra Mechanical Polish
Electropolished Interior Vessel		Optional
Vessel Exterior Finish		Brushed/Ribbon Polish around welds

4.1.3 300-Liter Vessel Assembly

CRITERIA		**300-Liter**
Total Capacity		300 liters
Maximum Working Capacity		240 liters
Minimum Working Capacity		68 liters
Unit Dimensions		80 inches L x 63 inches D x 123 inches H 203 cm L x 160 cm D x 312 cm H
Unit Weight	**Vessel**	500 lbs (227 kg)
	Electrical Cabinet/ Skid	500 lbs (227 kg)
	Piping Skid	350 lbs (159 kg)
Height to Diameter Ratio (nominal)		3:1
Jacket Type		Open Type
Top Head		Manway, ASME F&D
Bottom Head		ASME F&D dish
Vessel Design Pressure		40 PSIG (2.75 bar), full vacuum
Jacket Design Pressure		50 PSIG, (3.45 bar)
Vessel Design Temperature		149°C (300°F)
Jacket Design Temperature		149°C (300°F)
Vessel Interior Finish		20 Ra Mechanical Polish
Electropolished Interior Vessel		Optional
Vessel Exterior Finish		Brushed/Ribbon Polish around welds

4.1.4 500-Liter Vessel Assembly

CRITERIA		500-Liter
Total Capacity		500 liters
Maximum Working Capacity		400 liters
Minimum Working Capacity		103 liters
Unit Dimensions		80 inches L x 63 inches D x 123 inches H 203 cm L x 160 cm D x 312 cm H
Unit Weight	**Vessel**	600 lbs (272 kg)
	Electrical Cabinet/ Skid	500 lbs (227 kg)
	Piping Skid	350 lbs (159 kg)
Height to Diameter Ratio (nominal)		3:1
Jacket Type		Open Type
Top Head		Manway, ASME F&D
Bottom Head		ASME F&D dish
Vessel Design Pressure		40 PSIG (2.75 bar), full vacuum
Jacket Design Pressure		50 PSIG, (3.45 bar)
Vessel Design Temperature		149°C (300°F)
Jacket Design Temperature		149°C (300°F)
Vessel Interior Finish		20 Ra Mechanical Polish
Electropolished Interior Vessel		Optional
Vessel Exterior Finish		Brushed/Ribbon Polish around welds

4.1.5 1000-Liter Vessel Assembly

CRITERIA		1000-Liter
Total Capacity		1000 liters
Maximum Working Capacity		800 liters
Minimum Working Capacity		250 liters
Unit Dimensions		85 inches L x 80 inches D x 186 inches H 216 cm L x 203 cm D x 472.4 cm H
Unit Weight	**Vessel**	700 lbs (318 kg)
	Electrical Cabinet/ Skid	500 lbs (227 kg)
	Piping Skid	500 lbs (227 kg)
Height to Diameter Ratio (nominal)		3:1
Jacket Type		Open Type
Top Head		Manway, ASME F&D
Bottom Head		ASME F&D dish
Vessel Design Pressure		40 PSIG (2.75 bar), full vacuum
Jacket Design Pressure		50 PSIG, (3.45 bar)
Vessel Design Temperature		149°C (300°F)
Jacket Design Temperature		149°C (300°F)
Vessel Interior Finish		20 Ra Mechanical Polish
Electropolished Interior Vessel		Optional
Vessel Exterior Finish		Brushed/Ribbon Polish around welds

4.1.6 1500-Liter Vessel Assembly

CRITERIA	1500-Liter

Total Capacity		1500 liters
Maximum Working Capacity		1200 liters
Minimum Working Capacity		375 liters
Unit Dimensions		85 inches L x 85 inches D x 186 inches H 216 cm L x 216 cm D x 472.4 cm H
Unit Weight	**Vessel**	800 lbs (363 kg)
	Electrical Cabinet/ Skid	500 lbs (227 kg)
	Piping Skid	500 lbs (227 kg)
Height to Diameter Ratio (nominal)		3:1
Jacket Type		Open Type
Top Head		Manway, ASME F&D
Bottom Head		ASME F&D dish
Vessel Design Pressure		40 PSIG (2.75 bar), full vacuum
Jacket Design Pressure		50 PSIG, (3.45 bar)
Vessel Design Temperature		149°C (300°F)
Jacket Design Temperature		149°C (300°F)
Vessel Interior Finish		20 Ra Mechanical Polish
Electropolished Interior Vessel		Optional
Vessel Exterior Finish		Brushed/Ribbon Polish around welds

4.1.7 3000-Liter Vessel Assembly

CRITERIA		**3000-Liter**
Total Capacity		3000 liters
Maximum Working Capacity		2400 liters
Minimum Working Capacity		750 liters
Unit Dimensions		98 inches L x 102 inches D x 210 inches H 250 cm L x 260 cm D x 534 cm H
Unit Weight	**Vessel**	3600 lbs (1590 kg)
	Electrical Cabinet/ Skid	500 lbs (227 kg)
	Piping Skid	1000 lbs (454 kg)
Height to Diameter Ratio (nominal)		3:1
Jacket Type		Dimpled & insulated
Top Head		Manway, ASME F&D
Bottom Head		ASME F&D dish
Vessel Design Pressure		40 PSIG (2.76 bar), full vacuum
Jacket Design Pressure		100 PSIG, (6.89 bar)
Vessel Design Temperature		177°C (350°F)
Jacket Design Temperature		177°C (350°F)
Vessel Interior Finish		20 Ra Mechanical Polish
Electropolished Interior Vessel		Optional
Vessel Exterior Finish		Brushed/Ribbon Polish around welds

4.1.8 Materials and Finish

Vessel

Any metallic surface that may come in direct contact with the process liquid, including the vessel head, are fabricated of type 316L stainless steel. All internal vessel welds are ground

smooth, and are mechanically polished to match the surrounding finish. Each weld is free of ripples, pits, undercutting and other surface defects. Every surface in product contact is free from any pits, visible scratches, burrs, flash, folds, inclusions, crevices and nicks. All interior surfaces are polished to a 20 microinch Ra or better finish. The exterior of the vessel will be polished to a uniform finish with all welds ground and polished with a ribbon finish. All gaskets, diaphragms, O-rings, and other elastomeric components that are in product contact will be either “class VI” EPDM or silicon.

Jacket
The jacket is fabricated of type 316L stainless steel. The exterior of the jacket will be polished to match the exterior surface of the vessel and the mating welds will be completed with a ribbon finish.

4.1.9 Standard Vessel Ports and Penetrations

The table below represents the connections, and the typical uses of the connections that are included with this fermentor.

4.1.9.1 75-Liter Vessel Assembly

Location	Qty	Size	Type	Typical Function
Head Plate	1	2 inch	Tri-Clamp	Exhaust Condenser
	1	2 inch	Tri-Clamp	Sprayball/Charging Port
	1	2 inch	Tri-Clamp	Level/Foam/Sprayball
	1	2 inch	Tri-Clamp	Vessel Light
	1	1½ in	Tri-Clamp	Pressure Transducer
	1	1½ in	Tri-Clamp	Upper DP Transmitter
	1	1½ in	Tri-Clamp	Rupture Disk
Bottom Head	1	---	Flange	Motor Drive
	1	---	Flush Mounted	Drain Valve
	1	1½ in	NA Connect	Lower DP Transmitter
Upper Side Wall	1	½ inch	Tapered Tri-Clamp	Addition 1
	1	½ inch	Tapered Tri-Clamp	Addition 2
	1	½ inch	Tapered Tri-Clamp	Addition 3
	1	½ inch	Tapered Tri-Clamp	Addition 4
	1	½ inch	Tapered Tri-Clamp	Addition 5
	1	1½ inch	Tapered Tri-Clamp	Pressure Gauge
	1	¾ inch	NA Connect	Overlay
	1	4 inch	Tapered Tri-Clamp	View Port
Lower Side Wall	1	¾ inch	NA Connect	RTD Thermowell
	1	25 mm @ 15°	Ingold	pH
	1	25 mm @ 15°	Ingold	DO
	1	25 mm @ 15°	Ingold	Redox/Spare pH
	1	25 mm @ 15°	Ingold	Redox/Spare DO
	1	¾ inch	NA Connect	Thermometer/Spare RTD
	1	1½ inch	NA Connect	Sample
	1	1½ inch	NA Connect	Sparge
Jacket	1	¾ inch	FNPT	Water Out/Steam In
	1	¾ inch	FNPT	Water In/ Steam Out

4.1.9.2 150-Liter Vessel Assembly

Location	Qty	Size	Type	Typical Function
Head Plate	1	3 inch	Tri-Clamp	Exhaust Condenser
	1	2 inch	Tri-Clamp	Sprayball/Charging Port
	1	2 inch	Tri-Clamp	Level/Foam/Sprayball
	1	2 inch	Tri-Clamp	Vessel Light
	1	1½ inch	Tri-Clamp	Pressure Transducer
	1	1½ inch	Tri-Clamp	Upper DP Transmitter
	1	1½ inch	Tri-Clamp	Rupture Disk
Bottom Head	1	---	Flange	Motor Drive
	1	---	Flush Mounted	Drain Valve
	1	1½ inch	NA Connect	Lower DP Transmitter
Upper Side Wall	1	½ inch	Tapered Tri-Clamp	Addition 1
	1	½ inch	Tapered Tri-Clamp	Addition 2
	1	½ inch	Tapered Tri-Clamp	Addition 3
	1	½ inch	Tapered Tri-Clamp	Addition 4
	1	½ inch	Tapered Tri-Clamp	Addition 5
	1	1½ inch	Tapered Tri-Clamp	Pressure Gauge
	1	¾ inch	NA Connect	Overlay
	1	4 inch	Tapered Tri-Clamp	View Port
Lower Side Wall	1	¾ inch	NA Connect	RTD Thermowell
	1	25 mm @ 15°	Ingold	pH
	1	25 mm @ 15°	Ingold	DO
	1	25 mm @ 15°	Ingold	Redox/Spare pH
	1	25 mm @ 15°	Ingold	Redox/Spare DO
	1	¾ inch	NA Connect	Thermometer/Spare RTD
	1	1½ inch	NA Connect	Sample
	1	1½ inch	NA Connect	Sparge
Jacket	1	¾ inch	FNPT	Water Out/Steam In
	1	¾ inch	FNPT	Water In/ Steam Out

4.1.9.3 300-Liter Vessel Assembly

Location	Qty	Size	Type	Typical Function
Head Plate	1	3 inch	Tri-Clamp	Exhaust Condenser
	1	3 inch	Tri-Clamp	Sprayball/Charging Port
	1	3 inch	Tri-Clamp	Level/Foam/Sprayball
	1	2 inch	Tri-Clamp	Vessel Light
	1	1½ inch	Tri-Clamp	Pressure Transducer
	1	1½ inch	Tri-Clamp	Upper DP Transmitter
	1	1½ inch	Tri-Clamp	Rupture Disk
Bottom Head	1	---	Flange	Motor Drive
	1	---	Flush Mounted	Drain Valve
	1	1½ inch	NA Connect	Lower DP Transmitter
Upper Side Wall	1	½ inch	Tapered Tri-Clamp	Addition 1
	1	½ inch	Tapered Tri-Clamp	Addition 2
	1	½ inch	Tapered Tri-Clamp	Addition 3
	1	½ inch	Tapered Tri-Clamp	Addition 4
	1	½ inch	Tapered Tri-Clamp	Addition 5
	1	1½ inch	Tapered Tri-Clamp	Pressure Gauge
	1	¾ inch	NA Connect	Overlay
	1	4 inch	Tapered Tri-Clamp	View Port
Lower Side Wall	1	¾ inch	NA Connect	RTD Thermowell
	1	25 mm @ 15°	Ingold	pH
	1	25 mm @ 15°	Ingold	DO
	1	25 mm @ 15°	Ingold	Redox/Spare pH
	1	25 mm @ 15°	Ingold	Redox/Spare DO
	1	¾ inch	NA Connect	Thermometer/Spare RTD
	1	1½ inch	NA Connect	Sample
	1	1½ inch	NA Connect	Sparge
Jacket	1	¾ inch	FNPT	Water Out/Steam In
	1	¾ inch	FNPT	Water In/ Steam Out

4.1.9.4 500-Liter Vessel Assembly

Location	Qty	Size	Type	Typical Function
Head Plate	1	3 inch	Tri-Clamp	Exhaust Condenser
	1	3 inch	Tri-Clamp	Sprayball/Charging Port
	1	3 inch	Tri-Clamp	Level/Foam/Sprayball
	1	2 inch	Tri-Clamp	Vessel Light
	1	1½ inch	Tri-Clamp	Pressure Transducer
	1	1½ inch	Tri-Clamp	Upper DP Transmitter
	1	1½ inch	Tri-Clamp	Rupture Disk
Bottom Head	1	---	Flange	Motor Drive
	1	---	Flush Mounted	Drain Valve
	1	1½ inch	NA Connect	Lower DP Transmitter
Upper Side Wall	1	½ inch	Tapered Tri-Clamp	Addition 1
	1	½ inch	Tapered Tri-Clamp	Addition 2
	1	½ inch	Tapered Tri-Clamp	Addition 3
	1	½ inch	Tapered Tri-Clamp	Addition 4
	1	½ inch	Tapered Tri-Clamp	Addition 5
	1	1½ inch	Tapered Tri-Clamp	Pressure Gauge
	1	¾ inch	NA Connect	Overlay
	1	4 inch	Tapered Tri-Clamp	View Port
Lower Side Wall	1	¾ inch	NA Connect	RTD Thermowell
	1	25 mm @ 15°	Ingold	pH
	1	25 mm @ 15°	Ingold	DO
	1	25 mm @ 15°	Ingold	Redox/Spare pH
	1	25 mm @ 15°	Ingold	Redox/Spare DO
	1	¾ inch	NA Connect	Thermometer/Spare RTD
	1	1½ inch	NA Connect	Sample
	1	1½ inch	NA Connect	Sparge
Jacket	1	¾ inch	FNPT	Water Out/Steam In
	1	¾ inch	FNPT	Water In/ Steam Out

4.1.9.5 1000-Liter Vessel Assembly

Location	Qty	Size	Type	Typical Function
Head Plate	1	4 inch	Tri-Clamp	Exhaust Condenser
	1	4 inch	Tri-Clamp	Sprayball/Charging Port
	1	4 inch	Tri-Clamp	Level/Foam/Sprayball
	1	2 inch	Tri-Clamp	Vessel Light
	1	3 inch	Tri-Clamp	Spare
	1	2 inch	Tri-Clamp	Spare
	1	1½ inch	Tri-Clamp	Pressure Transducer
	1	1½ inch	Tri-Clamp	Spare
	1	1½ inch	Tri-Clamp	Rupture Disk
Bottom Head	1	---	Flange	Motor Drive
	1	---	Flush Mounted	Drain Valve
	1	3 inch	NA Connect	Lower DP Transmitter
Upper Side Wall	1	1 inch	Tapered Tri-Clamp	Addition 1
	1	1 inch	Tapered Tri-Clamp	Addition 2
	1	½ inch	Tapered Tri-Clamp	Addition 1
	1	½ inch	Tapered Tri-Clamp	Addition 2
	1	½ inch	Tapered Tri-Clamp	Addition 3
	1	1½ inch	Tapered Tri-Clamp	Pressure Gauge
	1	1½ inch	NA Connect	Overlay
	1	3 inch	Tapered Tri-Clamp	Upper DP Transmitter
	1	6 inch	Tri-Clamp	View Port
Lower Side Wall	1	¾ inch	NA Connect	RTD Thermowell
	1	25 mm @ 15°	Ingold	pH
	1	25 mm @ 15°	Ingold	DO
	1	25 mm @ 15°	Ingold	Redox/Spare pH
	1	25 mm @ 15°	Ingold	Redox/Spare DO
	1	¾ inch	NA Connect	Thermometer/Spare RTD
	1	1½ inch	NA Connect	Sample
	1	2 inch	NA Connect	Sparge
	1	2 inch	NA	Sight Glass
Jacket	1	1½ inch	FNPT	Water Out/Steam In
	1	1½ inch	FNPT	Water In/ Steam Out

4.1.9.6 1500-Liter Vessel Assembly

Location	Qty	Size	Type	Typical Function
Head Plate	1	4 inch	Tri-Clamp	Exhaust Condenser
	1	4 inch	Tri-Clamp	Sprayball/Charging Port
	1	4 inch	Tri-Clamp	Level/Foam/Sprayball
	1	2 inch	Tri-Clamp	Vessel Light
	1	3 inch	Tri-Clamp	Spare
	1	2 inch	Tri-Clamp	Spare
	1	1½ inch	Tri-Clamp	Pressure Transducer
	1	1½ inch	Tri-Clamp	Spare
	1	1½ inch	Tri-Clamp	Rupture Disk
Bottom Head	1	---	Flange	Motor Drive
	1	---	Flush Mounted	Drain Valve
	1	3 inch	NA Connect	Lower DP Transmitter
Upper Side Wall	1	1 inch	Tapered Tri-Clamp	Addition 1
	1	1 inch	Tapered Tri-Clamp	Addition 2
	1	½ inch	Tapered Tri-Clamp	Addition 1
	1	½ inch	Tapered Tri-Clamp	Addition 2
	1	½ inch	Tapered Tri-Clamp	Addition 3
	1	1½ inch	Tapered Tri-Clamp	Pressure Gauge
	1	1½ inch	NA Connect	Overlay
	1	3 inch	Tapered Tri-Clamp	Upper DP Transmitter
	1	6 inch	Tri-Clamp	View Port
Lower Side Wall	1	¾ inch	NA Connect	RTD Thermowell
	1	25 mm @ 15°	Ingold	pH
	1	25 mm @ 15°	Ingold	DO
	1	25 mm @ 15°	Ingold	Redox/Spare pH
	1	25 mm @ 15°	Ingold	Redox/Spare DO
	1	¾ inch	NA Connect	Thermometer/Spare RTD
	1	1½ inch	NA Connect	Sample
	1	2 inch	NA Connect	Sparge
	1	2 inch	NA	Sight Glass
Jacket	1	1½ inch	FNPT	Water Out/Steam In
	1	1½ inch	FNPT	Water In/ Steam Out

4.1.9.7 3000-Liter Vessel Assembly

Location	Qty	Size	Type	Typical Function
Head Plate	1	6 inch	Tri-Clamp	Exhaust Condenser
	1	4 inch	Tri-Clamp	Sprayball/Charging Port
	1	4 inch	Tri-Clamp	Sprayball
	1	2 inch	Tri-Clamp	Vessel Light
	1	3 inch	Tri-Clamp	Spare
	1	2 inch	Tri-Clamp	Spare
	1	1½ inch	Tri-Clamp	Pressure Transmitter
	1	1½ inch	Tri-Clamp	Spare
	1	2 inch	Tri-Clamp	Rupture Disk
	1	1½ inch	Tri-Clamp	Level
Bottom Head	1	---	Flange	Motor Drive
	1	---	Flush Mounted	Drain Valve
	1	2 inch	MNPT	Water In
	1	2 inch	MNPT	Water Out
	1	3 inch	NA Connect	Lower DP Transmitter
Upper Side Wall	1	1 inch	Tapered Tri-Clamp	Addition 1
	1	1 inch	Tapered Tri-Clamp	Addition 2
	1	½ inch	Tapered Tri-Clamp	Addition 1
	1	½ inch	Tapered Tri-Clamp	Addition 2
	1	½ inch	Tapered Tri-Clamp	Addition 3
	1	1½ inch	Tapered Tri-Clamp	Pressure Gauge
	1	1½ inch	NA Connect	Overlay
	1	3 inch	Tapered Tri-Clamp	Upper DP Transmitter
	1	6 inch	Tri-Clamp	View Port
Lower Side Wall	1	¾ inch	NA Connect	RTD Thermowell
	1	25 mm @ 15°	Ingold	pH
	1	25 mm @ 15°	Ingold	DO
	1	25 mm @ 15°	Ingold	Redox/Spare pH
	1	25 mm @ 15°	Ingold	Redox/Spare DO
	1	¾ inch	NA Connect	Thermometer/Spare RTD
	1	1½ inch	NA Connect	Sample
	1	2 inch	NA Connect	Sparge
	1	2 inch	NA	Sight Glass

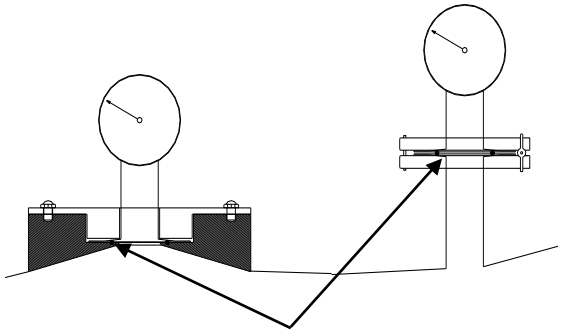
4.1.9.8 NA Connect

New Brunswick Scientific has decided to change some of the connections on the vessel from standard tri-clamps to a new type of connection known as NA-connect. With NA-connect, weld studs on the top, bottom, or sidewall of the vessel are avoided and a flush mounted, a tri-clamp type of connection can be made to facilitate cleaning and sterilization..

As can be seen below the NA-connect, on the left, is mounted flush with the interior of the vessel, while the tri-clamp actually extends away from the vessel. The net effect is the dead leg with NA-connect is virtually eliminated creating a more sterile connection with the vessel.

4.2 Piping Skid

4.2.1 Design Overview



The piping for steam, air and water is located on a skid-mounted piping module and offers the following features:

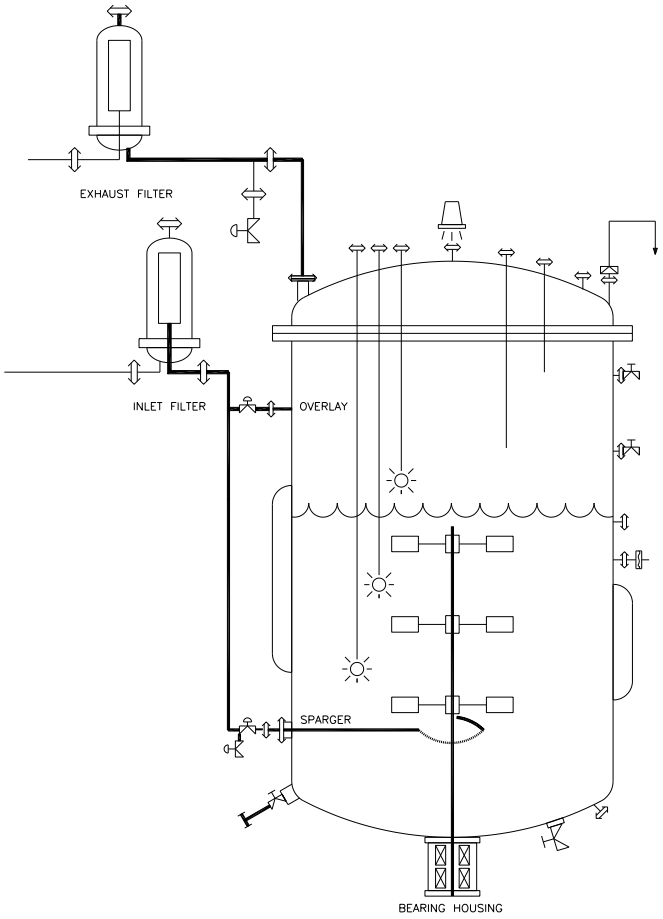
1. Automatic valves for controlling the fermentor while the unit is in sterilization and growth.

2. 304 Stainless steel skid, fully welded construction

3. Welded or tri-clamp connections used in areas that are within the sterile envelope. **Note**: The sterile envelope includes all surfaces that are in product contact, consisting of the following:

   - The process lines between the air inlet filter and the exhaust filter
   - All vessel connections
   - All process steam lines
   - Inside of the rupture disk facing the interior of the vessel.

4. All valves, devices, and tubing within the sterile envelope are made of 316L stainless steel. Valves will be oriented to permit the best possible drainage, ease of operation and access for maintenance.



STERILE ENVELOPE

5. Small footprint

4.3 Mechanical Subsystems

4.3.1 Temperature Control

The temperature control system consists of an RTD temperature sensor in a thermowell, located in the lower sidewall of the vessel, and two valves used to inject water or steam into the vessel jacket.

1. Temperature control range: 10°C above water supply temperature to 60°C ± 0.5°C.
2. The vessel temperature will be displayed on the operator interface terminal (OIT).
3. The temperature control loop is capable of heating vessel at a rate of 1°C/minute between 30°C and 60°C.

4.3.1.1 Automatic Sterilization

A user defined sterilization program is used to automate the valve operation required to sterilize the vessel, and piping within the sterile envelope as shown above in 4.2.1. This user can enter the vessel jacket drain length, heat B temperature, sterilization temperature, sterilization hold length, cool B and growth temperatures. Automatic sterilization program can be used to sterilize both full and empty vessels. The sterilization process is initiated from the sterilization screen on the OIT similar to the example shown below. Automating the sterilization process reduces operator to operator variations.

4.3.2 Agitation System

Vessel Size	*Power*	*Motor Drive*	*Speed Range*	*(#) Impeller Type*
75-Liter	1.5 hp	Bottom-Entering Vector Drive	50-500^ RPM	(3) Ruston * ‡
150-Liter	1.5 hp	Bottom-Entering Vector Drive	50-500^ RPM	(3) Ruston* ‡
300-Liter	3 hp	Bottom-Entering Vector Drive	45-450^ RPM	(3) Ruston* ‡
500-Liter	5 hp	Bottom-Entering Vector Drive	40-400^ RPM	(3) Ruston* ‡
1000-Liter	10 hp	Bottom-Entering Vector Drive	30-300^ RPM	(3) Ruston*
1500-Liter	20 hp	Bottom-Entering Vector Drive	25-275^ RPM	(3) Ruston*
3000-Liter	25 hp	Bottom-Entering Vector Drive	20-200^ RPM	(3) Ruston*

"^" Maximum agitation requires at least 0.1 vessel volumes per minute of airflow
"*" Impellers are Ruston "Like" impellers manufactured by New Brunswick Scientific.
"‡" Impellers are mounted to the shaft using a sanitary compression fitting designed to eliminate the necessary set screws.

1. Standard Allen Bradley Power Flex Variable Speed Frequency Drive (VFD)
2. Adjustable speed control with digital display of RPM on the OIT
3. Ball bearing shaft and housing with double mechanical seal (carbon to tungsten carbide) with sterile condensate lubrication.
4. Four (4) type 316 stainless steel removable baffles.
5. Impellers are adjustable in position along the shaft
6. Impeller shaft design eliminates the need for the typical fine reverse threads that can be easily be damaged by cross threading during maintenance.
7. Upper and lower impeller shafts can be disengaged and the upper can be removed from the vessel using the removable lifting lug for service without entering the vessel.
8. Pitched and marine blade impellers are also available.

4.3.3 Aeration System

Airflow for the vessel passes from an inlet port on the piping skid through:

- Pressure Gauge
- Process Air Filter
- Pressure Regulator
- Pressure Gauge
- Shut-off Valve (on two gas system)
- Rotameter or Thermal Mass Flow Controller (optional)
- Pneumatic Isolation Valve
- Steam-sterilizable cartridge-type absolute sterile filter(s) (0.2 micron pore size)
- Sparger on lower side wall.

Notes:

- All piping, valves and fittings in the air system are fabricated of Type 316L stainless steel. A diaphragm valve is used on the condensate line from the air inlet filter housing and on all process lines within the sterile envelope.
- All necessary valves, steam traps, and check valves are provided for sterilization of the

air inlet system in conjunction with the vessel.
- The stainless steel filter housings are furnished with condensate lines ending in steam traps, as well as ports that enable integrity testing of the filter cartridges
- The airline and filter is sized to deliver a maximum aeration rate of 1.5 vessel volumes per minute (1.5 VVM).

4.3.4 Exhaust System

Exhaust gas flows from the outlet port on the fermentor headplate through:
- A stainless steel exhaust condenser mounted on the fermentor headplate (if this option is selected) circulates cold water to condense and return much of the water vapor that is in the exhaust gas that is escaping the vessel.
- The exhaust filter heater is supplied with an electric band heater to reduce the likelihood that of the hydrophobic filter element becomes wetted out.
- All necessary valves and steam traps, are provided for the sterilization of the exhaust filter(s) in conjunction with the vessel sterilization.
- Cartridge-type, 0.45 micron depth filter is standard with system or choose the available 0.2 micron absolute filter.
- Manual pressure regulator
- Pressure gauge

Vessel pressure will be displayed on the OIT and automatically controlled (**automatic pressure control option**) by the system via a control valve, I/P, and pressure transducer. Note: A safety rupture disc is mounted on the bioreactor vessel in order to protect the vessel from accidental over-pressure.

***If automatic back pressure control is not installed, pressure will be manually controlled with a manual regulator. Automatic pressure control is standard with the 1000L, 1500L and 3000L fermentor systems.**

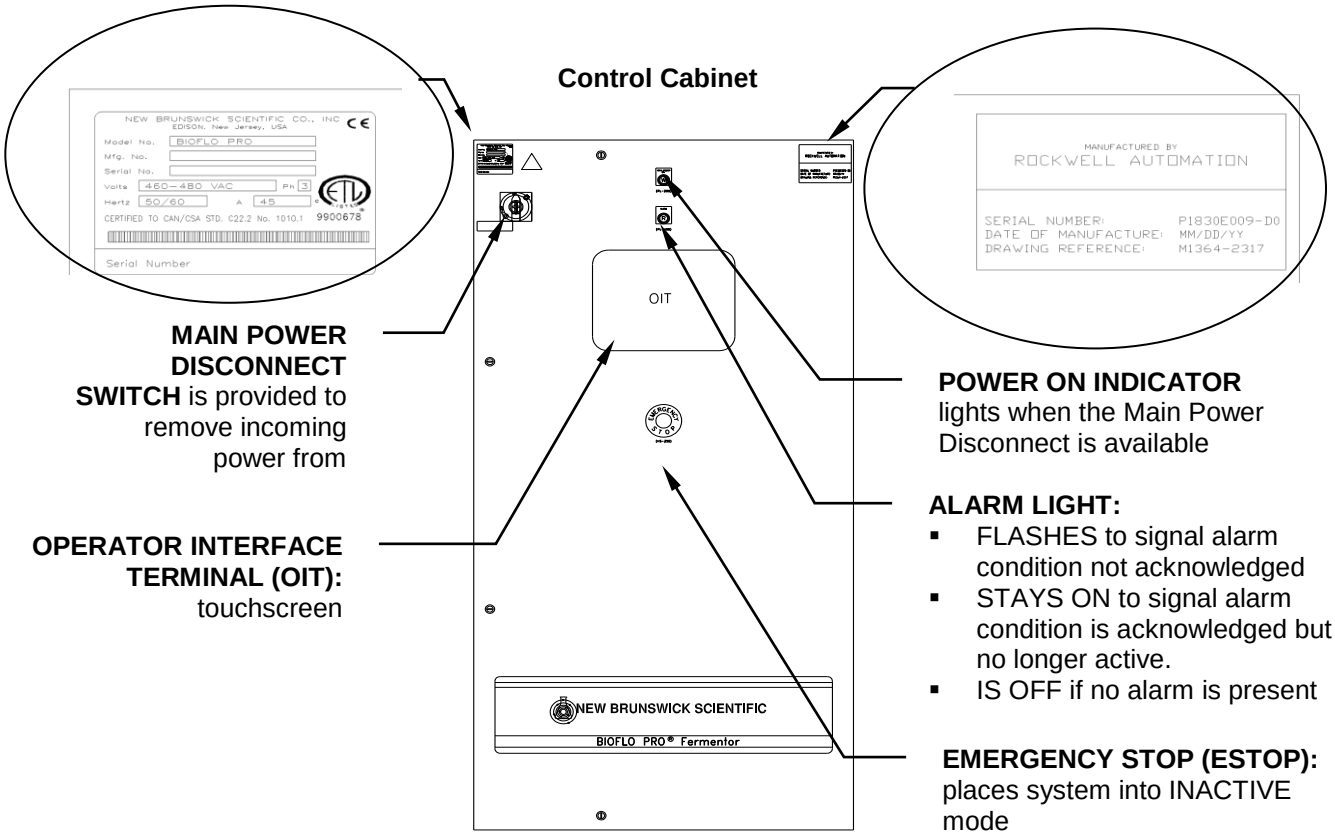
Notes:
- All piping, valves and fittings are fabricated of Type 316L stainless steel.
- A diaphragm valve will be installed on the condensate line from the exhaust filter housing to a steam trap.
- Exhaust filter(s) are sterilized in place during the vessel sterilization.

4.4 Controller

New Brunswick Scientific has differentiated itself from the competitors by offering an Allen Bradley CompactLogix programmable automation controller (PAC) as the standard method for controlling the processes associated with the operation of the bioreactor. Doing so has moved New Brunswick Scientific away from a proprietary package, to one that readily adapts to a wide range of data collection programs while providing a robust platform to control the entire process. Connectivity to the PAC is made possible by using either MODBUS Protocol

The code is compatible with ISA88 standard for batch processing and was developed based upon the current GaMP guidelines, good automated manufacturing practices, which allow end users to easily validate the operation of the system by following standard protocols available from New Brunswick Scientific.

The controller was developed to allow additional features and inputs to be installed and simply activated in the field thus permitting the system to grow with the end user's process.

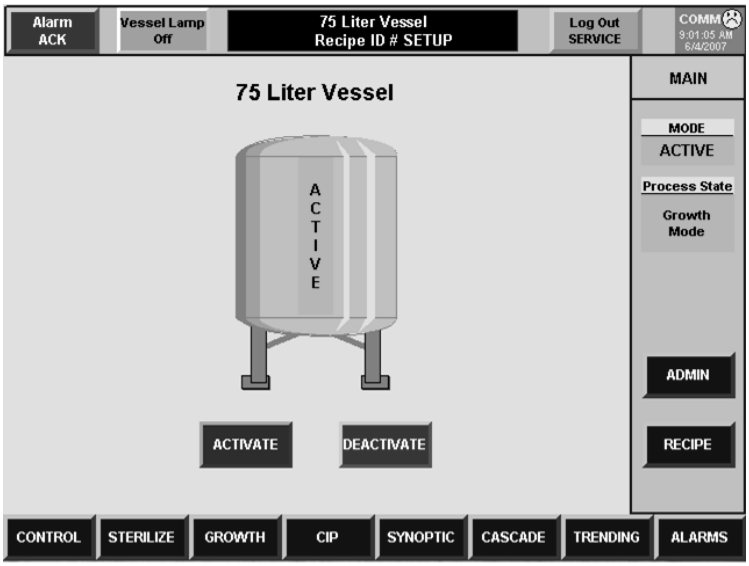

Control Cabinet
NEW BRUNSWICK SCIENTIFIC CO., INC
EDISON, New Jersey, USA
Model No. BIOFLO PRO
Mfg. No.
Serial No.
Volts 460-480 VAC Ph 3
Hertz 50/60 A 45
CERTIFIED TO CAN/CSA STD. C22.2 No. 1010.1
9900678
Serial Number
MANUFACTURED BY
ROCKWELL AUTOMATION
SERIAL NUMBER: P1830E009-D0
DATE OF MANUFACTURE: MM/DD/YY
DRAWING REFERENCE: M1364-2317
OIT
NEW BRUNSWICK SCIENTIFIC
BIOFLO PRO® Fermentor
MAIN POWER DISCONNECT SWITCH is provided to remove incoming power from
OPERATOR INTERFACE TERMINAL (OIT): touchscreen
POWER ON INDICATOR lights when the Main Power Disconnect is available
ALARM LIGHT:
FLASHES to signal alarm condition not acknowledged
STAYS ON to signal alarm condition is acknowledged but no longer active.
IS OFF if no alarm is present
EMERGENCY STOP (ESTOP): places system into INACTIVE mode
EMERGENCY STOP

4.4.1 Operator Interface

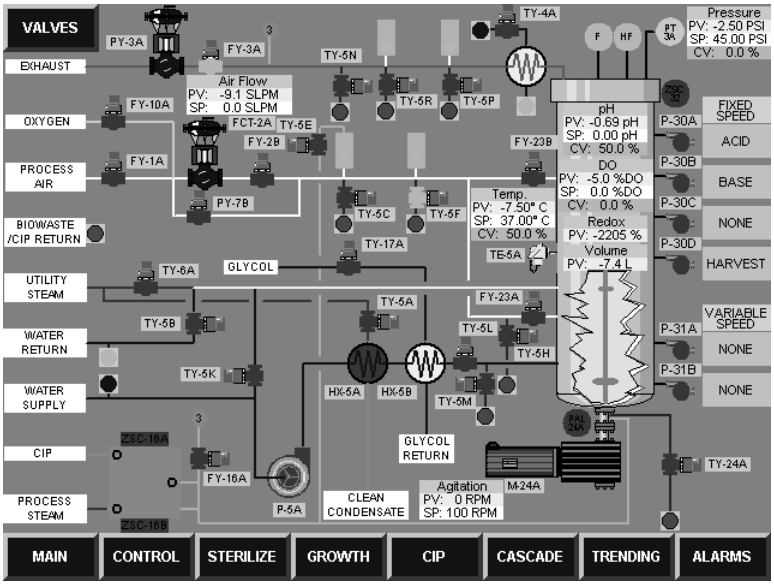
The operator interface is an Allen Bradley PanelView Plus that is mounted on the front of control cabinet. This PanelView Plus OIT offers Password Protection with operator, supervisor, administrator levels of access, and a Recipe Function to save, load or delete process recipes. From the interface operators can also have the ability to navigate to the following screens:

Note: The following example "screen shots" are for illustration purposes. The actual screens may vary depending on the vessel size, configured standard options and software version.

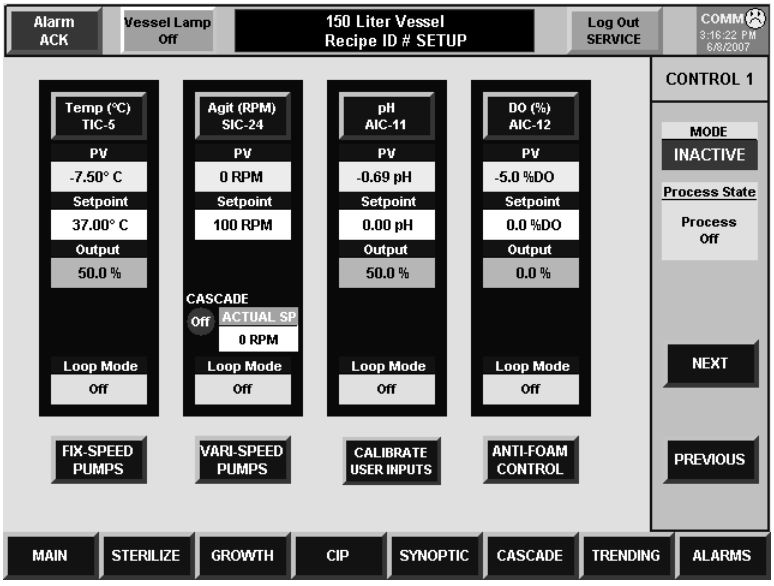
1. ***Main Screen:*** From this screen operators can load and edit recipes or deactivate the fermentor by turning off digital and analog outputs.



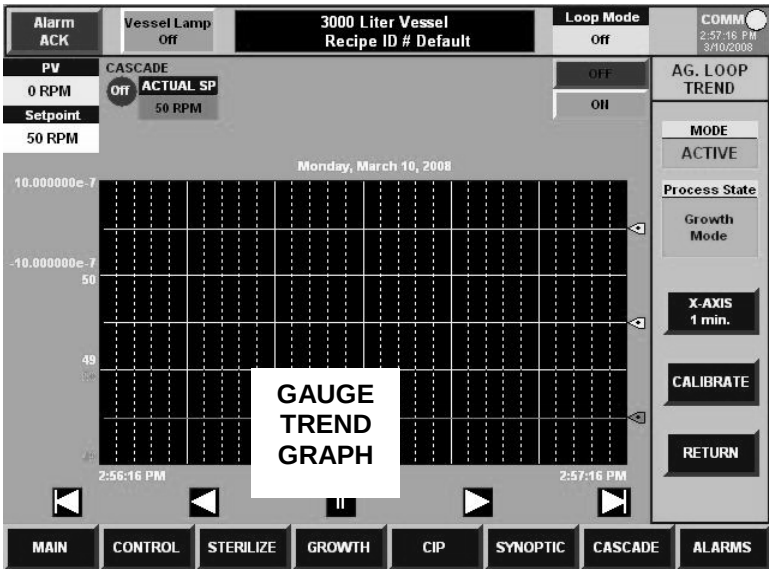
2. ***Synoptic Screen:*** Provides a system overview



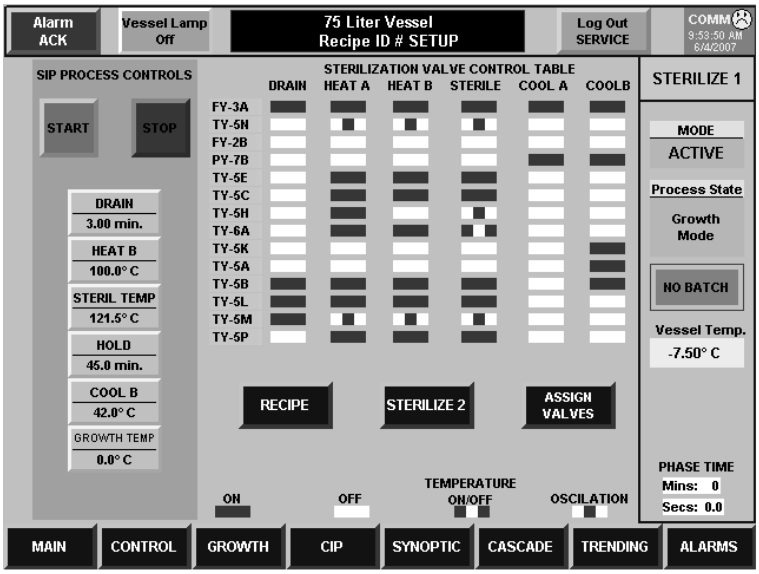
3. ***Control Screen:*** By navigating to this screen, operators can view process values of individual loops, modify setpoints and check the status of any cascaded loop.



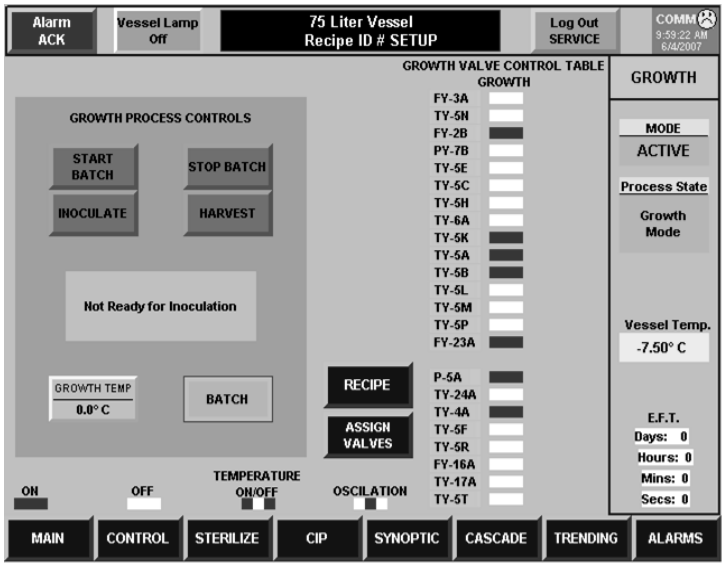
4. ***Gauge Screen:*** Each control loop enabled on the overview screen will have an additional screen associated with it where the tuning parameters of the loop can be modified and a real time trend graph for the loop can be viewed.



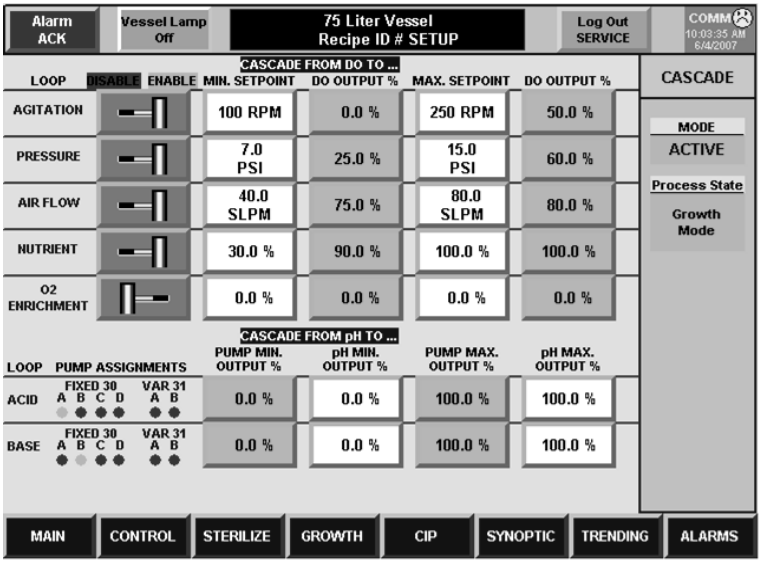
5. ***Sterilization Screen***: This screen allows users to modify the sterilization temperature, hold time, drain time, valve operating sequences, and growth temperature, as well as a method of starting or aborting an automatic sterilization sequence.



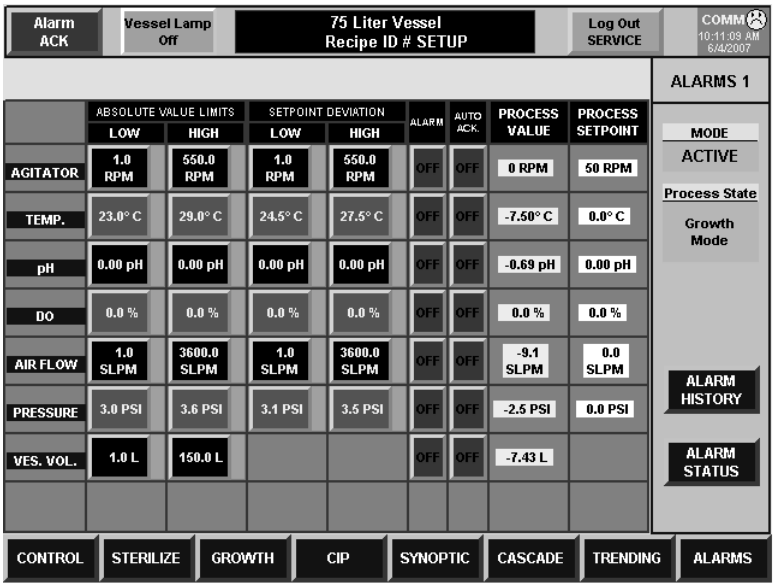
6. ***Growth Screen***: From this screen users can start a batch, inform the controller when the vessel has been inoculated, harvested, and is in CIP mode.

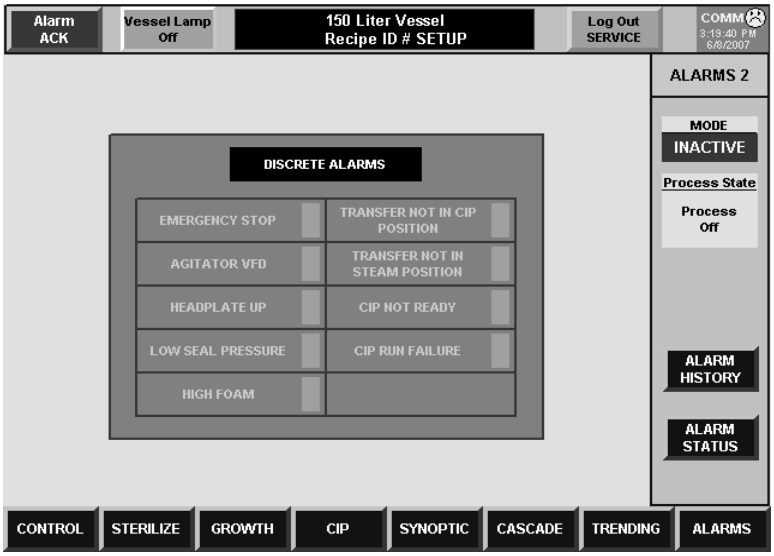


7. ***Cascade Control Screen****:* Enable/Disable cascades for control of dissolved oxygen or pH within the system.

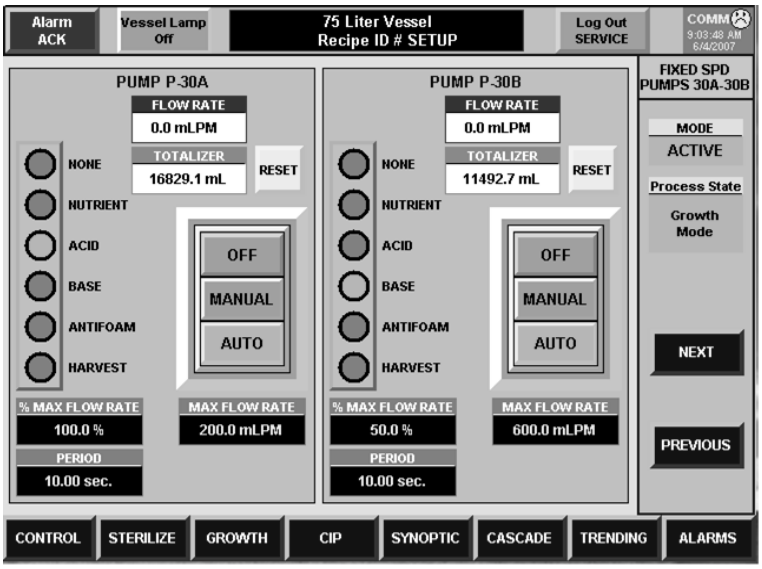


8. ***Alarm Setup Screen:*** Define absolute or setpoint deviation alarm for any system variable all on a single screen

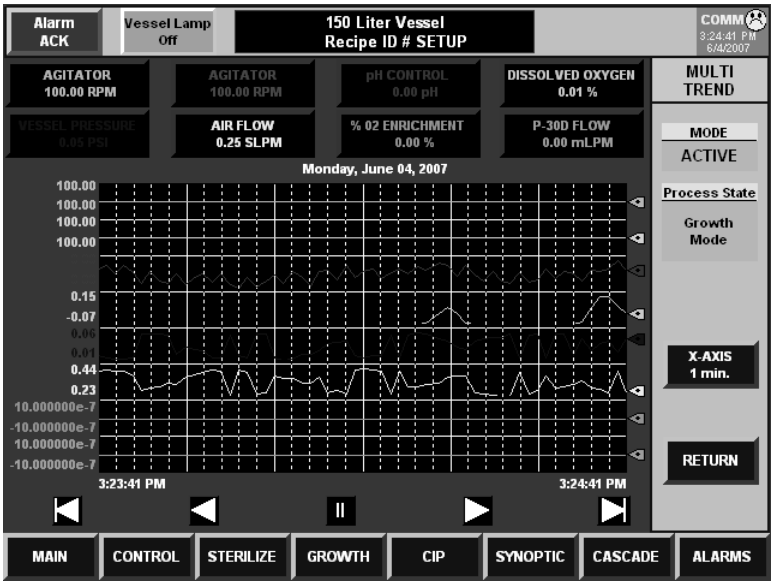

Alarm ACK
Vessel Lamp Off
75 Liter Vessel
Recipe ID # SETUP
Log Out SERVICE
COMM
10:11:09 AM
6/4/2007
ALARMS 1
ABSOLUTE VALUE LIMITS
SETPOINT DEVIATION
LOW
HIGH
LOW
HIGH
ALARM
AUTO ACK.
PROCESS VALUE
PROCESS SETPOINT
AGITATOR
1.0 RPM
550.0 RPM
1.0 RPM
550.0 RPM
OFF
OFF
0 RPM
50 RPM
TEMP.
23.0° C
29.0° C
24.5° C
27.5° C
OFF
OFF
-7.50° C
0.0° C
pH
0.00 pH
0.00 pH
0.00 pH
0.00 pH
OFF
OFF
-0.69 pH
0.00 pH
DO
0.0 %
0.0 %
0.0 %
0.0 %
OFF
OFF
0.0 %
0.0 %
AIR FLOW
1.0 SLPM
3600.0 SLPM
1.0 SLPM
3600.0 SLPM
OFF
OFF
-9.1 SLPM
0.0 SLPM
PRESSURE
3.0 PSI
3.6 PSI
3.1 PSI
3.5 PSI
OFF
OFF
-2.5 PSI
0.0 PSI
VES. VOL.
1.0 L
150.0 L
OFF
OFF
-7.43 L
MODE
ACTIVE
Process State
Growth Mode
ALARM HISTORY
ALARM STATUS
CONTROL
STERILIZE
GROWTH
CIP
SYNOPTIC
CASCADE
TRENDING
ALARMS


Alarm ACK
Vessel Lamp Off
150 Liter Vessel
Recipe ID # SETUP
Log Out SERVICE
COMM
3:19:40 PM
6/8/2007
ALARMS 2
DISCRETE ALARMS
EMERGENCY STOP
TRANSFER NOT IN CIP POSITION
AGITATOR VFD
TRANSFER NOT IN STEAM POSITION
HEADPLATE UP
CIP NOT READY
LOW SEAL PRESSURE
CIP RUN FAILURE
HIGH FOAM
MODE
INACTIVE
Process State
Process Off
ALARM HISTORY
ALARM STATUS
CONTROL
STERILIZE
GROWTH
CIP
SYNOPTIC
CASCADE
TRENDING
ALARMS

9. ***Pump Assignment Screen:*** When a pump option is selected, this screen provides users with a method of assigning the intended use of any pump as well as seeing the totaled pump flows.



10. ***Trend Screen:*** This screen provides a method to plot all of the available process variables on a single screen



5.0 System Options

The options presented below may be purchased at the onset of the project, or any time after the system has been installed at the end user's site. This is due to modular design concepts

that were employed during the development of the product. The intent of the modular design was to allow each system to grow with the user's process. This feature allows the flexibly to purchase and install options that may have not been thought of when the equipment was ordered or were not needed due to the intended end use of the system.

5.1 Air Inlet Line Options

5.1.1 Automatic Air Flow Control

This feature uses a thermal mass flow controller to automatically control the amount of air entering the vessel.

SPECIFICATION		*Control*
75-Liter	**Air**	2-90 SLPM ± 1.0% Full Scale
150-Liter	**Air**	4-180 SLPM ± 1.0% Full Scale
300-Liter	**Air**	7-360 SLPM ± 1.0% Full Scale
500-Liter	**Air**	12-600 SLPM ± 1.0% Full Scale
1000-Liter	**Air**	24-1200 SLPM ± 1.0% Full Scale
1500-Liter	**Air**	36-1800SLPM ± 1.0% Full Scale
3000-Liter	**Air**	72-3600 SLPM ± 1.0% Full Scale

5.1.2 Fermentation Overlay Option

This feature allows the system operator to divert the sparger gas entering the vessel to the headspace of the fermentor.

5.1.3 Dual inlet air filters

This provides an additional level of certainty by installing a second air inlet filter in series.

5.1.4 Dual inlet filters with integrity test ports

This option provides all of the valves and access ports to allow operators to perform a water intrusion test on the individual inlet filters without having to remove the filters from the system.

5.1.5 Oxygen Supplementation

Solenoid mixing valves controlled by the PLC are used to supplement the airflow with oxygen to match the demand of the culture based upon feedback from the dissolved oxygen loop.

5.2 Exhaust Line Options

5.2.1 Exhaust condenser

A stainless steel exhaust condenser can be installed on top of the vessel to condense liquids contained in the exiting process stream. The condenser is effective at reducing the rate of evaporation of the culture at growth temperatures that are less than 60˚C. When the condenser is ordered with the glycol option the condenser supply originates from chiller inlet process piping and it returns to the chiller outlet process piping and then back to the chiller to create a closed loop. The closed loop cooling reduces the consumption of single pass facility water through the condenser to system.

5.2.2 Integrity test ports for single exhaust filter

This option provides all of the valves and access ports to allow operators to perform a water intrusion test on the exhaust filter without removing the filter from the system.

5.2.3 Dual exhaust filters with integrity test ports

This option provides operators with a second exhaust filter which is installed in parallel with the first filter. This feature allows operators to introduce a sterile filter into the process in the event the initial filter becomes fouled. This option provides all of the valves and access ports to allow operators to perform a water intrusion test on an individual filter without having to remove the filter from the system.

5.2.4 Automatic Back Pressure Control

This feature provides a pressure transmitter, I/P, and control valve to automatically control the pressure inside of the vessel based upon a user entered setpoint.

SPECIFICATION	*Control*
Pressure	1 - 15 PSIG ± 1.0% Full Scale

5.3 Probe and Sensor Options

5.3.1 pH/DO SYSTEM

pH
The pH portion of this option outputs a control signal to an optional acid or base pump. A gel filled pH probe and housing is supplied. The pH control range is 2-12 pH.

SPECIFICATION	*Control*
pH	2-12pH ± 0.1pH

DO
The DO portion of the option is used to control the amount of oxygen in the media. The level of dissolved oxygen in the system will be maintained by enabling the cascades function for agitation, nutrient*, airflow*, O_2 supplementation*, vessel pressure* ("*" requires the system to be configured with additional standard options).

SPECIFICATION (Range)	*Control*
D.O.	0 – 100% ± 0.1% Full Scale

5.3.2 Spare pH/DO System

This option allows operators to switch to a second probe during a batch in case of a probe failure.

5.3.3 Retractable probe housing

This housing allows operators to change a probe during a batch by providing a method for isolating the probe from the vessel, sterilizing the new probe, and reintroducing the sterilized probe back into the vessel.

5.3.4 Foam Detection

A conductivity probe can be used to detect foam or liquid. The sensitivity of the probe is mechanically adjustable by the user. The probe will send an on/off output signal to either a fixed or variable speed pump that is assigned for antifoam.

5.3.5 Level Detection System

A conductivity probe can be provided for level detection. The sensitivity of the probe is mechanically adjustable by the user. The sensor will send an on/off output signal to the PLC which will inform operators of a high level condition.

5.3.6 High-High Foam Detection

A conductivity probe can be installed above the exhaust condenser for high-high foam detection. The sensitivity of the probe is mechanically adjustable by the user. The user can define/set if and how long the gas flow (automatic airflow option required) to the vessel will be interrupted in during a high-high foam condition.

5.3.7 Redox probe

End users are provided with a probe and a local display on the operator interface of the oxygen reduction potential of the media. **Note**: This option is for display only.

5.3.8 Vessel volume via differential pressure

By using pressure sensors at the top and bottom of the vessel, this option provides operators with an accurate and reliable way of obtaining the volume of liquid in the vessel. **Note**: Volume will be displayed on operator interface and will be used for display only.

5.3.9 Vessel weight via load cells

Using 3 independent load cells, a summing box, and flexible connections between the vessel and the piping skid, which allows the vessel to move freely, this option provides operators with the volume of liquid in the vessel with the assumption that 1 kg = 1L of water. Volume will be displayed on operator interface and will be used for display only.

5.3.10 Low Seal Pressure

By using a pressure switch on the seal condensate line, this option provides operators with an alarm indicating that the mechanical seal is being supplied at the required pressure.

5.4 Addition and Sampling Options

5.4.1 1/2" SIP/CIP Sample Valve

This resterilizable valve is located on the lower side wall of the vessel and can be used to collect samples of culture while maintaining the sterility of the culture inside the vessel. The standard valve configuration will allow the user to take aseptic samples from the vessel. Add the optional sterile sampling assembly kit to this sample valve if the process requires aerosol containment or a bioburden free sample.

5.4.2 Sterile Sampling Kit

Includes 3 assemblies of stainless steel Weir style diaphragm valve by port, sanitary fitting to hose barb conversion with clamps, gaskets, tubing and 500 ml glass bottles with 0.22 μm vent filter. This assembly when used with the resterilizable sampling valve on the 510 will allow users to collect sterile culture samples up to 500 ml. These assemblies are washable and reusable.

5.4.3 Addition Vessels

A variety of autoclavable vessels designed for vessel additions of acid, base, antifoam, nutrient or any other solution. These vessels can also be used to collect product or waste from a continuous process

5.4.4 1/2" SIP/CIP Addition Port

***maximum of 5 for 75-500L Systems**
***maximum of 3 for 1000-3000L Systems**
These resterilizable valve arrays (steam supply, vessel isolation, steam trap isolation valves) facilitate the connection of an autoclaved process diaphragm valve. After the connection has been competed, steam sterilized and cooled the assembly can be used

for bioburden free transfer / addition of liquids (antifoam, media, nutrient, acid, base) to the vessel.

5.4.5 1" SIP/CIP Addition Port

***maximum of 2 – only available on 1000-3000L Systems**
Same description as ½" valve above in 5.4.2.

5.4.6 Spare Addition Isolation Valve (1/2")

This includes stainless steel / EPDM (Class VI) sanitary diaphragm valve with multiple sanitary fitting to hose barb fittings with clamps and gaskets. The spare addition isolation valve assembly provides the user with the flexibility to make multiple sterile additions to the vessel without having to remove the addition isolation valve attached to the addition port, clean it, assemble it into a new tubing segment and autoclave it before they can make their next sterile addition to the vessel. The assemblies are washable and reusable.

5.4.7 Spare Addition Isolation Valve (1")

Same as 5.4.5 with 1" stainless steel valve.

5.4.8 7 port septum

The seven port septum contained in a single housing for aseptic transfer of liquid into the vessel. Septum requires a 1.5" sanitary port on the vessel headplate.

5.4.9 12 port septum

The twelve port septum contained in a single housing for aseptic transfer of liquid into the vessel. Septum requires a 2.0" sanitary port on the vessel headplate.

5.5 Miscellaneous Options

5.5.1 4 Fixed-Speed Pump Box

This includes four fixed speed peristaltic pumps with PWM (pulse with modulation) contained in a single box. The PWM modulates the pumps on time to emulate variable speed pumps. These pumps can be operated from the pump screen on the operator interface in a manual mode or in auto mode when they are assigned to a specific function (acid, base, antifoam, or nutrient). The flow of each pump is calibrated by the end user (ml/min) as required per the tubing material and internal diameter (ID). This information is used as the basis for the individual pump totalizers which can accurately calculate the volume in milliliters (ml) of each addition.

5.5.2 Variable Speed Pump (Maximum of 2)

Up to 2 variable speed peristaltic pumps can be included with the system. The operation of each pump is easily assignable from the operator interface and may be used for base, antifoam, nutrient, or perfusion. **Note**: the flow of each pump will be totaled by the controller and displayed on the OIT similar to the screen that is shown in 5.5.1.

5.5.3 CIP Interface

The option includes a transfer panel (with a magnetic switch) to change between process steam and CIP fluid so that the air inlet line, exhaust line, drain valve, sample valve, and addition valve(s) may be cleaned in place. Three spray balls are provided to insert into the vessel in order to clean the interior of the fermentor. **Note**: A CIP screen will be provided on the operator interface to assist operators with performing the CIP sequence.

5.5.4 Sprayballs Only

These sprayballs are specifically designed for each vessel to provide maximum cleaning fluid coverage within the vessel. Convenient tri-clamp connections allow for easy integration into an existing clean-in-place system.

5.5.5 Transfer Line

Twenty feet of braided silicone hose with stainless steel sanitary fitting, gaskets and clamps on each side. This line attaches to the drain on one vessel and the addition port on the other vessel. Sterilization of transfer line is by steaming from the addition port steam supply on the receiving vessel to the low point condensate drain on the drain of the supply vessel.

5.5.6 Glycol Heat Exchanger

This option places a second heat exchanger in the coolant circulation line. Unlike the first exchanger, which uses steam to heat the water in the temperature loop, the second allows glycol or chilled water to be used as a cooling media instead of city water.

5.5.7 Analog Inputs for Ancillary Devices

Up to 7 additional analog inputs are available for the connection of ancillary devices such as turbidity probes, gas analyzers, and glucose analyzers. The information can be viewed on the operator interface (millvolts) with user definable tags naming.

5.5.8 Utility regularor and filter kits

Utility Connection Kit – Converts sanitary (tri-clamp) fitting on the skid to MNPT (male threads) using compression type fittings to facilitate utility connection of the system when sanitary fitting is not required. This option is ideal for academic and non GMP applications.

Process steam regulator – (Stainless Steel regulator for clean steam applications)
Utility steam regulator and filter – (Bronze regulator for utility steam applications)
Water regulator and filter
Instrument air regulator and filter

5.5.9 BioCommand SCADA Package

Connect the controller to BioCommand for data logging or supervisory control. (System specification available upon request)

5.5.10 21 CFR Part 11 SCADA Package

For data logging and control in a 21 CFR Part 11 and Validated environment. (System specification available upon request)

6.0 Validation Packages

Every system passes Installation Qualification and Control System Inspection before shipping. These Documents are available through several levels of validation packages designed to fit the needs of your process and facility.

6.1 Basic Validation

Spare Parts List
List of Materials and Lubricants in Product Contact
Pressure Vessel Documentation
Pressure Hold Test Certificate
Media Hold Test Certificate

Control System Inspection Documentation
Installation Qualification Documentation

6.2 Basic Plus Validation

***includes Basic validation package**
As Built P&ID
Executable (blank) copies of IQ/OQ Documentation
Enhanced Spare Parts List
Enhanced Pressure Vessel Documentation
Calibration of Process Measurements
Enhanced Material Certification of Parts in Product Contact

6.3 Enhanced Validation

***includes basic and basic plus validation packages**
Weld Mapping of Process Piping W/Video Taping
Passivation of Process Piping Certificate
Temperature Mapping Test Procedure and Data
Calibration Procedure for HP Agilent with Multiplexer and Type “T” Stainless Steel Thermocouples and Data
Biological Challenge Test certificate

Utility Requirements

6.4 75-Liter Systems

Utility	Line size	Connection	Requirement
*Process Air	½ inch	Tri-clamp	3.19 SCFM (90 SLPM) 30-35 PSIG (4.1-5.5 bar)
Oxygen (optional)	½ inch	Tri-clamp	3.19 SCFM (90 SLPM) 35 PSIG (2.4 bar)
Exhaust	½ inch	Tri-clamp	0.5 PSIG maximum backpressure
*Instrument Air	½ inch	Tri-clamp	2 SCFM (56.5 SLPM) 80-100 PSIG (5.5-6.9 bar)
Process Steam	½ inch	Tri-clamp	10 lbs/hr (4.5 kg/hr) 35 PSIG (2.4 bar)
Utility Steam	¾ inch	Tri-clamp	50 lbs/hr (22.7 kg/hr) 35 PSIG (2.4 bar)
**Water Supply	¾ inch	Tri-clamp	4 GPM (0.91 m^3/hr) 30 PSIG (2.1 bar)
Water Return	¾ inch	Tri-clamp	20 PSIG maximum backpressure
Clean Condensate	1 inch	FNPT	Gravity drain
Biowaste	1 inch	Tri-clamp	Gravity drain
CIP Fluids (optional)	½ inch	Tri-clamp	14 GPM (3.18 m^3/hr)
Glycol (optional)	½ inch	FNPT	2 GPM (0.45 m^3/hr) 30 PSIG (2.1 bar)
Power	208-230 VAC, 50-60 Hz, Single Phase, 20 amps		

* Air should be dry (to -40ºC or F dewpoint) and contain less than 0.001 ppmw oil aerosol mist
**Water temperature must be at least 10ºC below Growth temperature

6.5 150-Liter Systems

Utility	Line size	Connection	Requirement
*Process Air	½ inch	Tri-clamp	6.38 SCFM (180 SLPM) 30-35 PSIG (4.1-5.5 bar)
Oxygen (optional)	½ inch	Tri-clamp	6.38 SCFM (180 SLPM) 35 PSIG (2.4 bar)
Exhaust	½ inch	Tri-clamp	0.5 PSIG maximum backpressure
*Instrument Air	½ inch	Tri-clamp	2 SCFM (56.5 SLPM) 80-100 PSIG (5.5-6.9 bar)
Process Steam	½ inch	Tri-clamp	25 lbs/hr (11.36 kg/hr) 35 PSIG (2.4 bar)
Utility Steam	¾ inch	Tri-clamp	125 lbs/hr (56.7 kg/hr) 35 PSIG (2.4 bar)
**Water Supply	¾ inch	Tri-clamp	6 GPM (1.37 m^3/hr) 30 PSIG (2.1 bar)
Water Return	¾ inch	Tri-clamp	20 PSIG maximum backpressure
Clean Condensate	1 inch	FNPT	Gravity drain
Biowaste	1 inch	Tri-clamp	Gravity drain
CIP Fluids (optional)	½ inch	Tri-clamp	14 GPM (3.18 m^3/hr)
Glycol (optional)	½ inch	FNPT	3 GPM (0.68 m^3/hr) 30 PSIG (2.1 bar)
Power	208-230 VAC, 60 Hz, Single Phase, 20 amps		

* Air should be dry (to -40ºC or F dewpoint) and contain less than 0.001 ppmw oil aerosol mist
**Water temperature must be at least 10ºC below Growth temperature

6.6 300-Liter Systems

Utility	Line size	Connection	Requirement
*Process Air	½ inch	Tri-clamp	12.75 SCFM (360 SLPM) 30-35 PSIG (4.1-5.5 bar)
Oxygen (optional)	½ inch	Tri-clamp	12.75 SCFM (360 SLPM) 35 PSIG (2.4 bar)
Exhaust	¾ inch	Tri-clamp	0.5 PSIG maximum backpressure
*Instrument Air	½ inch	Tri-clamp	2 SCFM (56.5 SLPM) 80-100 PSIG (5.5-6.9 bar)
Process Steam	½ inch	Tri-clamp	40 lbs/hr (18 kg/hr) 35 PSIG (2.4 bar)
Utility Steam	¾ inch	Tri-clamp	200 lbs/hr (90.8 kg/hr) 35 PSIG (2.4 bar)
**Water Supply	¾ inch	Tri-clamp	10 GPM (2.28 m^3/hr) 30 PSIG (2.1 bar)
Water Return	¾ inch	Tri-clamp	20 PSIG maximum backpressure
Clean Condensate	1 inch	FNPT	Gravity drain
Biowaste	1 inch	Tri-clamp	Gravity drain
CIP Fluids (optional)	½ inch	Tri-clamp	14 GPM (3.18 m^3/hr)
Glycol (optional)	½ inch	FNPT	6 GPM (1.37 m^3/hr) 30 PSIG (2.1 bar)
Power	208-230 VAC, 50-60 Hz, Three Phase, 30 amps		

* Air should be dry (to -40ºC or F dewpoint) and contain less than 0.001 ppmw oil aerosol mist
**Water temperature must be at least 10ºC below Growth temperature

6.7 500-Liter Systems

Utility	Line size	Connection	Requirement
*Process Air	½ inch	Tri-clamp	21.28 SCFM (600 SLPM) 30-35 PSIG (4.1-5.5 bar)
Oxygen (optional)	½ inch	Tri-clamp	21.28 SCFM (600 SLPM) 35 PSIG (2.4 bar)
Exhaust	¾ inch	Tri-clamp	0.5 PSIG maximum backpressure
*Instrument Air	½ inch	Tri-clamp	2 SCFM (56.5 SLPM) 80-100 PSIG (5.5-6.9 bar)
Process Steam	½ inch	Tri-clamp	45 lbs/hr (20.4 kg/hr) 35 PSIG (2.4 bar)
Utility Steam	¾ inch	Tri-clamp	225 lbs/hr (102 kg/hr) 35 PSIG (2.4 bar)
**Water Supply	¾ inch	Tri-clamp	12 GPM (2.73 m^3/hr) 30 PSIG (2.1 bar)
Water Return	¾ inch	Tri-clamp	20 PSIG maximum backpressure
Clean Condensate	1 inch	FNPT	Gravity drain
Biowaste	1 inch	Tri-clamp	Gravity drain
CIP Fluids (optional)	½ inch	Tri-clamp	16 GPM (3.63 m^3/hr)
Glycol (optional)	½ inch	FNPT	10 GPM (2.28 m^3/hr) 30 PSIG (2.1 bar)
Power	208-230 VAC, 50-60 Hz, Three Phase, 30 amps		

* Air should be dry (to -40ºC or F dewpoint) and contain less than 0.001 ppmw oil aerosol mist
**Water temperature must be at least 10ºC below Growth temperature

6.8 1000-Liter Systems

Utility	Line size	Connection	Requirement
*Process Air	1 inch	Tri-clamp	42.5 SCFM (1200 SLPM) 30-50 PSIG (4.1-5.5 bar)
Oxygen (optional)	1 inch	Tri-clamp	42.5 SCFM (1200 SLPM) 35 PSIG (2.4 bar)
Exhaust	1½ inch	Tri-clamp	0.5 PSIG maximum backpressure
*Instrument Air	¾ inch	Tri-clamp	27 SCFM (763 SLPM) 80-100 PSIG (5.5-6.9 bar)
Process Steam	1 inch	Tri-clamp	70 lbs/hr (31.75 kg/hr) 35 PSIG (2.4 bar)
Utility Steam	1½ inch	Tri-clamp	350 lbs/hr (158.76 kg/hr) 35 PSIG (2.4 bar)
**Water Supply	1½ inch	Tri-clamp	20 GPM (4.55 m^3/hr) 30 PSIG (2.1 bar)
Water Return	1½ inch	Tri-clamp	20 PSIG maximum backpressure
Clean Condensate	1½ inch	FNPT	Gravity drain
Biowaste	1½ inch	Tri-clamp	Gravity drain
CIP Fluids (optional)	1 inch	Tri-clamp	31 GPM (7.04 m^3/hr)
Glycol (optional)	1½ inch	FNPT	21 GPM (4.77 m^3/hr) 30 PSIG (2.1 bar)
Power	460-480 VAC, 50-60 Hz, Three Phase, 70 amps		

* Air should be dry (to -40ºC or F dewpoint) and contain less than 0.001 ppmw oil aerosol mist
**Water temperature must be at least 10ºC below Growth temperature

6.9 1500-Liter Systems

Utility	Line	Connection	Requirement
*Process Air	1 inch	Tri-clamp	63.8 SCFM (1800 SLPM) 30-50 PSIG (4.1-5.5 bar)
Oxygen (optional)	1 inch	Tri-clamp	63.8 SCFM (1800 SLPM) 35 PSIG (2.4 bar)
Exhaust	1½ inch	Tri-clamp	0.5 PSIG maximum backpressure
*Instrument Air	¾ inch	Tri-clamp	27 SCFM (763 SLPM) 80-100 PSIG (5.5-6.9 bar)
Process Steam	1 inch	Tri-clamp	80 lbs/hr (36.3 kg/hr) 35 PSIG (2.4 bar)
Utility Steam	1½ inch	Tri-clamp	400 lbs/hr (182 kg/hr) 35 PSIG (2.4 bar)
**Water Supply	1½ inch	Tri-clamp	25 GPM (5.46 m^3/hr) 30 PSIG (2.1 bar)
Water Return	1½ inch	Tri-clamp	20 PSIG maximum backpressure
Clean Condensate	1½ inch	FNPT	Gravity drain
Biowaste	1½ inch	Tri-clamp	Gravity drain
CIP Fluids (optional)	1 inch	Tri-clamp	34 GPM (7.72 m^3/hr) 25 PSIG (1.7 bar)
Glycol (optional)	1½ inch	FNPT	31 GPM (7.04 m^3/hr) 30 PSIG (2.1 bar)
Power	460-480 VAC, 50-60 Hz, Three Phase, 70 amps		

* Air should be dry (to -40ºC or F dewpoint) and contain less than 0.001 ppmw oil aerosol mist
**Water temperature must be at least 10ºC below Growth temperature

6.10 3000-Liter Systems

Utility	Line	Connection	Requirement
*Process Air	1½ inch	Tri-clamp	127.6 SCFM (3600 SLPM) 60-80 PSIG (4.1-5.5 bar)
Oxygen (optional)	1½ inch	Tri-clamp	127.6 SCFM (3600 SLPM) 35 PSIG (2.4 bar)
Exhaust	2 inch	Tri-clamp	0.5 PSIG maximum backpressure
*Instrument Air	¾ inch	Tri-clamp	29 SCFM (821 SLPM) 80-100 PSIG (5.5-6.9 bar)
Process Steam	1 inch	Tri-clamp	140 lbs/hr (63.5 kg/hr) 35 PSIG (2.4 bar)
Utility Steam	2 inch	Tri-clamp	1200 lbs/hr (544.3 kg/hr) 35 PSIG (2.4 bar)
**Water Supply	2 inch	Tri-clamp	40 GPM (9.1 m^3/hr) 30 PSIG (2.1 bar)
Water Return	2 inch	Tri-clamp	20 PSIG maximum backpressure
Clean Condensate	1½ inch	FNPT	Gravity drain
Biowaste	1½ inch	Tri-clamp	Gravity drain
CIP Fluids (optional)	1 inch	Tri-clamp	56 GPM (12.7 m^3/hr) 25 PSIG (1.7 bar)
Glycol (optional)	2 inch	FNPT	65 GPM (19.7 m^3/hr) 30 PSIG (2.1 bar)
Power	460-480 VAC, 50-60 Hz, Three Phase, 80 amps		

* Air should be dry (to -40ºC or F dewpoint) and contain less than 0.001 ppmw oil aerosol mist
**Water temperature must be at least 10ºC below Growth temperature

7.0 Service

We at New Brunswick Scientific realize the importance of having minimum downtime in the daily operations of your bioreactor. It is important for you to have immediate response to your service needs as they occur. It is our intention to maintain a partnership with you and to make sure you are operating as smoothly as possible at all times. New Brunswick Scientific has qualified professionals that are trained to deal with any problem that may affect your system, minimize downtime and restore your system to optimum operating levels of efficiency.

8.0 Spare Parts

New Brunswick Scientific Co., Inc. considers the ready availability of spare parts important to the economical and successful operation of this equipment. A complete spare parts list is an integral part of the instruction manual supplied with each unit. Most components are readily available for immediate shipment.

9.0 Start-Up and Operator Training

9.1 Start-Up Assistance

A trained New Brunswick Scientific Service Engineer will provide up to (1) man-days of start-up assistance at the installation site. This assistance will include qualifying utility connections along with performing basic unit operation.

NOTE: All start-up assistance is to be performed within the validity of the warranty.

9.2 Factory Inspection / Training

One (2) day Factory Inspection will be conducted at New Brunswick Scientific's facility in Edison, New Jersey. The end user will be responsible for all travel related expenses.

Factory Inspection allows a customer's appointed designee/expert to represent them

ensuring the system matches the purchase order and that any noted deviations have been addressed before shipment. NBS's standard factory one day inspection protocol includes a piping and instrument diagram review, system sterilization, and simulated growth operation. As an added bonus, the customer's appointed designee who participates in the Factory Inspection becomes familiar with system operation.

The one day of training must be scheduled within ten business (10) days of the written notification that the system is ready for testing. After 10 business days, it will be concluded that the customer has declined the option to perform the Factory Inspection. The customer may choose to decline the Factory Inspection at anytime by notifying NBS or their sales representative. The Factory Inspection is considered to be included with this quotation must be completed within 30 days from the initial written notification. Any exceptions to the above policy are subject to approval by NBS.

Additional training can be conducted at the customer site at a cost of $1,700 per day plus travel and related expenses.

10.0 Warranty

All equipment is warranted free from defects in material and workmanship for 12 months from the date of shipment from NBS, after equipment start-up (not to exceed 60 days from delivery), whichever comes first. The exceptions to this warranty are:

A. All glass parts which carry no warranty;
B. All electrodes, which are warranted for 15 months from the date of shipment from NBS, or 6 months from the date the customer accepts the equipment; whichever comes first.

Our obligation under this warranty is limited to repairing parts or providing replacement parts at no charge, which prove to be defective during the warranty period. A part shall be considered defective after inspection of NBS' technical staff. At NBS' option, we will repair or replace any defective part which is returned to our plant in Edison, New Jersey, U.S.A., freight prepaid. The cost of shipping the repaired or replacement part will be borne by NBS.

This warranty does not extend to equipment or parts that have been subjected to misuse, neglect, accident or improper installation or application; nor shall it extend to equipment or parts which have been repaired or altered outside the NBS plant without prior approval by NBS.

11.0 System Configuration

*DSU = part number determined by standard unit selection

ITEM	QTY	DESCRIPTION	PRICE
500-Liter SIP Fermentor consisting of the following			
1.	1	*500-Liter Vessel Assembly* ➢ 3:1 height to diameter ratio ➢ (3) Rushton impellers ➢ Spring assisted manway ➢ 4" sight glass ➢ Bottom drive with Double Mechanical Seal *Piping and Instrumentation* ➢ Valves for automatic sterilization ➢ Automatic temperature control ➢ Automation speed control ➢ Manual airflow control ➢ Manual back pressure control ➢ Inlet filter with test integrity ports ➢ Heated exhaust filter *Control Cabinet* ➢ 10" Touch Screen interface ➢ Industrial PLC ➢ Necessary I/O *Documentation* ➢ Operating Manual includes P & ID and 3-D Model ➢ User's Manual operating systems *Training* ➢ 2-days hands on training program at New Brunswick Scientifics' facility in Edison, New Jersey. *Set-up Assistance* ➢ 1-day Verification of Utilities and General Operating Procedure	**$228,100.00**
		500L Base System Price	**$228,100.00**
Air Inlet Line			
	Single Gas Mixing		**Included**
	Air is controlled via a thermal mass flow controller		**$5,005.00**
	Standard inlet filter with integrity testing		**Included**
	Without overlay valve		**Included**
Exhaust Line			
	Condenser is mounted on vessel to reduce dew point of exiting process stream		**$6,252.00**
	Single Exhaust Filter		**Included**
	Auto Pressure Control		**$6,161.00**

Sensors, Housings, and Transmitters		
	(1) Single 120 mm gel filled pH probe and cable	**$526.00**
	(1) Single 120 mm Ingold DO probe and cable	**$1,982.00**
	(2) SIP Housings (120 mm)	**$1,990.00**
	Dual pH/DO transmitter selected	**$3,098.00**
Addition Ports		
	(2) ½" Addition valves will be included with the system (contains steam crosses, steam traps, and interconnecting piping) (Maximum of 5 can be installed)	**$7,960.00**
	3" Tri-clamp Septum connection on the vessel head plate will be used for making additions	**$1,555.00**
Addition Options		
	(4) User definable 100 RPM pumps will be included with the system (flow rates between 1.26 and 16.8 liters/hour)	**$7,619.00**
Sample Port		
	A resterilizable sample valve will be included for sampling the content of the vessel	**$5,671.00**
Miscellaneous Options		
	Load Cell Option	**$18,880.00**
	500/300 Liter Glycol Option	**$2,688.00**
	Foam 300/500L Probe	**$1,759.00**
	High Foam 300/500L Probe	**$1,759.00**
	High High Foam 300/500L Probe	**$981.00**
	Sterile Sampling Kit Assembly (set of 3)	**$4,017.00**
	Transfer Line 20'	**$2,881.00**
	Total System Price	**$308,884.00**
Additional Options		
	Instrument Air Prefilter/Regulator Kit	**$497.00**
	Water Prefilter/Reg Kit (300/500L)	**$2,209.00**
	Main Steam Prefilter/Reg Kit (300/500L)	**$7,118.00**
	50'/16 meter RS-422 Serial Cable	**$171.00**
	BioCommand SCADA Package (Does not include software, just hardware for connection to BF Pro)	**$3,172.00**
	Total Price	$322,051.00

12.0 Terms and Conditions

The following terms and conditions,"Terms and Conditions", apply to all bids, quotations, solicitations, and/or agreements between New Brunswick Scientific Co., Inc. herein referred to as "NBS" and the customer, its' representatives, and/or its' agents acting on its behalf herein referred to as "Purchaser or Customer". "Equipment" shall mean any equipment, machinery, parts, materials or services provided for in a Proposal and any contract resulting therefrom. "Proposal" shall mean the Terms and Conditions and the most recent proposal,

bid or quotation of NBS provided to the Purchaser by NBS in connection with the transaction contemplated hereby.

12.1 Controlling Terms and Conditions

This Proposal alone constitutes an offer and all other offers or counteroffers whether conveyed by other documents or oral negotiations with respect to the subject matter hereof are hereby withdrawn, and of no further force and effect. This Proposal, including the Terms and Conditions, when duly executed by the parties, shall constitute the contract (the "Contract") resulting from our negotiations, regardless of any rejection thereof or statement to the contrary in any document soliciting the Proposal, or issued in response hereto unless such rejection statement, or response is expressly accepted in writing by an officer of NBS. Any conduct, which recognizes the existence of a contract shall constitute acceptance by both parties of the Terms and Conditions and rejection of all additional or different terms and conditions, proposed by Purchaser or incorporated by law. The applicability of this paragraph is an express condition of any contract being formed between the Purchaser and NBS.

12.2 Price

Prices quoted are valid for 60 Days unless otherwise agreed to in writing by NBS. Prices do not include any applicable taxes or fees, such as sales or use tax, excise tax, property tax, customs fees (if applicable) or associated fees. All taxes and fees shall be the responsibility of Purchaser.

12.3 Receipt of Purchase Order

Purchaser's Purchase Order shall be based on this Proposal. The terms of this Proposal shall control any terms and conditions contained in any purchase order issued in connection with the Proposal. The customer acknowledges and agrees that the Purchase Order is used for:

1. The Customer's convenience
2. Achieving compliance with the customer's internal procedures and requirements
3. The Contract that Customer accepted by execution of this Proposal is the document that governs the relationship of the parties.

12.4 Payment Terms

30% at the time the order is placed;
30% 90 days after receipt of the purchase order;
40% against shipping documents;

12.5 Shipments

Shipments will be F.O.B. Edison, NJ. USA, with insurance, freight and handling charges prepaid by NBS and billed to Purchaser. Risk of Loss shall pass to Purchaser upon delivery of Equipment to carrier. Quoted shipment dates are approximate and are contingent upon timely receipt of Purchaser's purchase order and supporting documentation also on Purchaser responding on a timely basis with the necessary approvals of engineering drawings etc. as described herein. NBS reserves the right to request minor adjustments to the shipment schedule due to the nature of the work being performed.

Shipment Timeline:
After order is placed and accepted, shipment will be 12 -16 weeks after receipt of order.

12.6 Change Orders

Any changes, modifications or deletions to the scope of the original purchase order must be made in writing by Purchaser and are subject to NBS' approval which approval shall not be unreasonably withheld. NBS will respond promptly to Purchaser on the impact, in terms of price and delivery that any change will cause. Prices quoted for such changes are valid for sixty (60) days.

12.7 Delays

The failure of NBS to perform any of the provisions of this Contract due to causes beyond the reasonable control of NBS, including but not limited to acts of God (including weather delays), disabling illness or death, acts of civil or military authority, labor trouble, inability to obtain necessary materials or components, war, riots, or fires shall not constitute a default under or a breach of the Contract and shall not subject NBS to any liability hereunder.

12.8 Liability

NBS shall under no circumstances be liable to Purchaser for special, incidental, exemplary, or consequential damages (hereafter referred to collectively as "consequential damages"), including, but not limited to loss of profits, anticipated revenue, interest, loss of use, loss by reason of plant shutdown, non-operation, cost of substitute equipment, facilities, services or utilities, costs incurred in removing defective of nonconforming Equipment and reinstallation of conforming Equipment delays in installation of the Equipment or completion of any project in which the Equipment is being installed or any other claims arising from any cause whatsoever whether or not such loss or damage is based in contract, warranty, tort (including negligence), strict liability indemnity or otherwise.

NBS' maximum aggregate liability for loss or damage arising under, resulting from or in connection with the supply or use of the Equipment provided under this Contract, or from the performance or breach of any obligation(s) imposed hereunder or otherwise, whether such liability arises from any one or more claims or actions for breach of contract, tort (including negligence), delayed completion, warranty indemnity strict liability or otherwise, unless otherwise limited by the terms hereof shall be limited to the amount actually received by NBS hereunder Purchaser hereby releases NBS, its agent and employees from any further liability.

It is expressly agreed that this Contract sets forth the sole and exclusive remedies available to the Purchaser and that NBS' liabilities are limited as set forth herein. Purchaser acknowledges and agrees that NBS has not granted or assumed any other warranties, guarantees, duties, liability, or obligations, either express implied statutory at law or in equity. Purchaser further acknowledges and agrees that no breach of warranty or of contract or fault by NBS to fulfill any other conditions of the Contract shall in any manner alter limit or change the remedies available to Purchaser hereunder.

Governing Law and Form:
This Proposal shall be governed by and construed in accordance with the laws (but not the laws of conflict of laws) of and enforced solely in the courts of the State of New Jersey, USA and the jurisdiction of such courts over the parties is hereby expressly acknowledged. The parties expressly waive application and jurisdiction of the UN Convention on the international Sale of Goods.

13.0 Ordering Information

***MAIL PURCHASE ORDERS TO*:**	***TELEPHONE ORDERS TO*:**
Eppendorf North America	1-800-645-3050, 516-334-7500
102 Motor Parkway, Suite 410,	Fax Number: 1-516-334-7506
Hauppauge, NY 11788-5178	
In Canada:	In Canada:
Eppendorf Canada Ltd	800-263-8715, 905-826-5525
2810 Argentia Road, Unit #2	Fax Number: 905-826-5424
Mississauga, ONT L5N 8L2	