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2 Abstract

The purpose of this project was to create a preliminary Monoclonal Antibody Production Facility that abides to the AICHE Student Design Competition Problem Statement. The facility has been designed to produce and store 1000 [kg/year] of monoclonal antibody product for up to one year. A process has been drafted to meet the yearly requirement through several unit operations: seed train reactors, production reactors, centrifuge and depth filtration, protein A chromatography, viral inactivation, anion exchange, and storage.

The production of MAb begins by cultivating CHO cells in eleven sequential seed reactors filled with media primed for optimal cell reproduction. CHO cell broths are moved from one seed reactor to the next seed reactor when the cell density reaches the experimental maximum of $7 \cdot 10^9$ [cells/L]. The final seed train reactor broth is evenly split into seven production reactors to optimize MAb production through fed-batch operation. Each production reactor operates at 33°C for 7.5 days with a fed flowrate of 25 [L/hr] and fed substrate concentration of 2.9 [g/L]. 1200 kg of yearly product is generated from the production reactors to account for losses in down-stream processes.

The solids from the cell broth are isolated through a centrifuge and depth filter harvesting operation. Harvesting will operate for 12 hours with the centrifuge having an equivalent area of 194 m² and having a depth filter rated for 12.9 µm. The remaining fluid is placed into a holding tank for protein A chromatography. The protein A functions by equilibrating, washing, eluting, and stripping for eight cycles to process all the solution from the holding tank. The protein A process operates for 50.8 hours and elutes 2800 L of usable solution per batch. The eluted material is then placed into an incubation vessel for two hours at a low pH to inactivate any potential viruses. Two filters are placed before a preparation tank to further separate any viruses. The preparation tank serves as a stage to titrate the solution to 7.5 pH and to increase the conductivity to 12 mS before flushing through the anion exchange column. All non-product materials will stick to the column walls as the MAbs will flow through and be placed into a storage tank. The final product will be stored at -20°C, therefore 5240 L of glycerol will be added to reduce the solution freezing temperature well below -20°C. Each batch produces 40,200 L drums of final product, yielding 1000 drums of product per year.

Each unit operation will require cleaning and preparation maintenance between batches. Proper SIP and CIP procedures have been created and cleaning times have been allocated for unit operations that require sanitation. Kill tanks have been allocated for spent solutions, where heat treatment is applied to raise the temperature to 80°C and cooled before being pumped into a municipal sewer system.

3 Introduction

A century ago Paul Ehrlich hypothesized that a “magic bullet” could be synthesized to directly target a disease¹. His hypothesis was proven later with the development of humanized Monoclonal Antibodies (MAbs). The MAbs have a human part which decreases the chance the antibody will be destroyed by the immune system, while the other part is mammalian and used to target the disease. MAbs have become a popular biopharmaceutical due to their ability to synergize with humans and high specificity to malignancies. MAbs are useful in treating rheumatoid arthritis, Crohn’s disease, lymphomas, and tumors.²

MAbs are the most successful class of biopharmaceutical with more than 50 MAbs being approved and sales expected to cross \$125 billion by 2020.³ In addition, over 300 MAbs are in clinical development today. With the ease and speed of producing MAbs and the ability to create a flexible plant for the production of multiple MAbs there is a large opportunity.

The project objective project is to design a large-scale manufacturing facility that has the flexibility to produce a variety of MAb products. The branch has already received FDA approval to produce one strain of MAb. The plant has potential to become a contract manufacturer in the production of various biopharmaceuticals. The plant design will be flexible to accommodate for titers of 1 to 2 g/L as well as 5 to 10 g/L and facilitate quick and sterile switches in manufacturing requirements.

The plant will consist of a media preparation area, seed train, production reactors, primary recovery, protein A chromatography, viral inactivation, purification polishing, storage of product, buffer preparation, and wastewater treatment. Each area of the plant will be discussed for purpose, design, economics, and safety more in depth throughout this report.

¹Adams, Gregory P and Louis M Weiner, “Monoclonal Antibody Therapy of Cancer,” *Nature Biotechnology*, September 2005, Vol 23, Number 9, pg 1147 -1157.

² Nicolaides, Nicholas C., et al. “Monoclonal Antibodies: a Morphing Landscape for Therapeutics.” *Drug Development Research*, vol. 67, no. 10, 2006, pp. 781–789.

³ Ecker SD, Jones D, Levine H. The therapeutic monoclonal antibody market. *Mabs*. 2015;7(1):9-14

4 Process Description

Upstream processing consisted of a seed train and production reactors. In the seed train, a small batch of cells was grown in successively larger batch reactors to produce 1000 [kg/yr] of product. The cells began in shake flask reactors of 0.01, 0.05, 0.1, and 0.15 L, then disposable rocking reactors of 1.5, 10 and 30, then a 200 L disposable agitated bag reactor, and finally permanent agitated batch reactors of 500, 1500 and 4000 L. The time in each reactor was optimized in MATLAB using ODEs and the Monod model for cell growth. The seed train used CD OptiCHOTM AGTTM media to facilitate growth. This media was a powdered formula prepared in the designated media preparation area. The broth in the final seed train reactor was evenly split into multiple production reactors.

The production reactors produce the desired MAbs at 25 [pg protein/ cell-day]. Multiple production reactors were used to account for a worst case titer scenario of 1-2 [g/L]. As titers improve, 5 – 10 [g/L] may be possible and the excess reactors can be sold for a profit. The protein product was in a mixture of media, potential viruses, and cells at the end of production. The most common industrial separation practices consist of two stages: continuous disc stack centrifugation (DSC) followed by depth filtering. Centrifugation removed cells and cell debris from the mixture while depth filtration further separated the mixture.

Protein A chromatography separated the monoclonal antibodies from the post-depth filtered solution. This process worked in the same manner as ion exchange⁴ by passing a solution through a column filled with resin to bind the wanted product. The resin used in this operation, MAbSelect, had a high affinity (binding capacity) towards the desired product. A low pH buffer solution was passed through the column to remove the product and prepared the resin for the next cycle. The column was regenerated several times due to the high capital of MAbSelect resin.

Viruses formed during the seed train and production reaction phase. The FDA requires the process to achieve viral inactivation by two orthogonal processes. Inactivation can be achieved by multiple different methods, and the two methods used in this process are low pH and filtration. The pH was lowered for protein A chromatography, so a holding tank was used to ensure the proteins had been in the acidic environment for 0.5 – 2 hours. A filter was then used to remove the rest of the viruses.

The last unit operation in the process was polishing. Polishing uses anion exchange to remove the ions present and impurities such as DNA fragments or left-over viruses in the product stream. The process is referred to as flow through ion exchange because it does not require an elution step for product recovery. The anion exchange was done via a resin which is regenerated between cycles and batches. The product pH was brought to between 7 – 8.2 for optimal conditions for MAb flow through ion exchange¹⁰. Once the product had been run through the ion exchange column, the resin was regenerated using a NaCl solution. Polishing was the final step in purifying the product and resulted in a product stream containing the MAb with little other contaminants.

Monoclonal antibodies are stored in a cold environment because the compound starts to oxidize, and the proteins begin to proteolytic degrade at temperatures about 5°C⁵. Unfiltered microbes from the process can also consume MAbs when the product is stored at room temperature. The structure of antibody proteins is also susceptible to ice crystals; therefore, a Glycerol solution is typically added to reduce the freezing point to below -20 °C. Antibodies should also be stored in dark-colored vessels to prevent the breakdown from a UV source. The product can be stored under these condition up to one year.

⁵ Johnson, Mary. “Antibody Storage and Antibody Shelf Life.” Materials and Methods, 2 May 2019, www.labome.com/method/Antibody-Shelf-Life-How-to-Store-Antibodies.html.

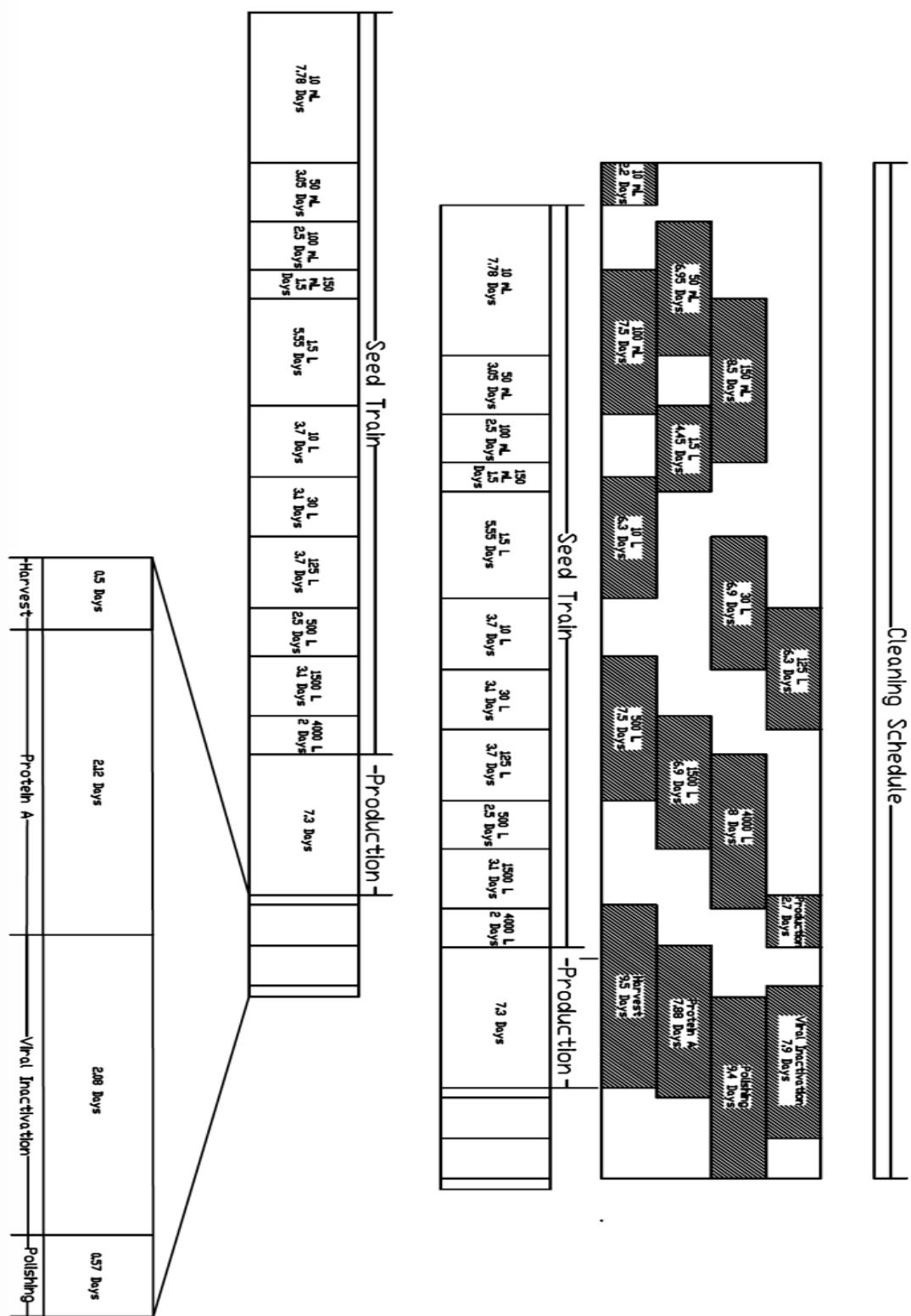


Figure 1: A timeline showing two batches staggered by 10 days. Also, shows the unit operation durations and cleaning schedule.

5 Unit Descriptions

This section describes the process in depth with accompanying low- and high-level PFDs. More detailed PFDs of individual unit operations can be found in the subsequent sections. A low-level PFD of the entire process is shown below including media preparation, seed train, buffer preparation, production, centrifugation, protein A chromatography, viral inactivation, polishing, storage, boilers, CIP/SIP system, WFI tank and waste disposal:

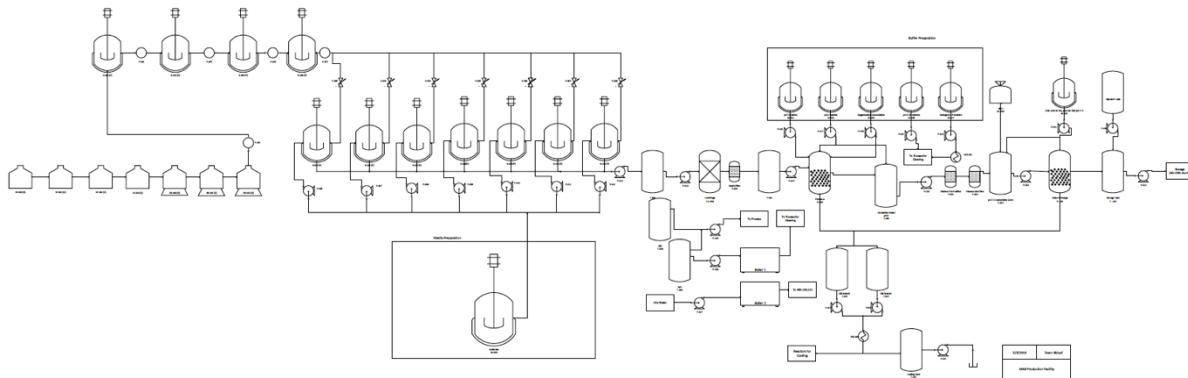


Figure 2: The above figure shows a low level PFD of the MAB production facility.

5.1 Media Preparation

5.1.1 Assumptions

A Chemically Defined Medium (CDM) will be used to adhere to the animal free facility standard. The media is assumed to be powdered components. The media contains substrate for cell growth and protein production.

5.1.2 Design

The media preparation area will be in a room near the seed train system for convenient transport of media. An off the shelf media called CD OptiCHO™ AGT™ will be used since data for CHO cell growth using this media was attainable. The room will need to contain stainless steel mixing equipment, analytical balances, measuring equipment, and sanitation instruments. The facility should be able to produce 5 mL to 40450 L of medium to accommodate seed train incremental batch volume increases and the production phase. An autoclave will be available to sterilize media and media production equipment.

5.2 Seed Train

5.2.1 Assumptions

It was assumed the Monod model was an accurate representation of CHO cell growth. The Monod model has proved useful as model across other biological systems and scales.⁶ The Monod model constants, K_s and Y , were assumed to be similar to values found in the literature

⁶ Craven S, Shirsat N, Whelan J, Glennon B. Process model comparison and transferability across bioreactor scales and modes of operation for a mammalian cell bioprocess. *Biotechnol Prog*. 2013;29:186–196. doi: 10.1002/btpr.1664.

source⁷. The experiments performed in literature used CD Opti CHO™ as the media. The K_s value of 0.286 [g/L] and Y value of $1.39 \cdot 10^{10}$ [cell/g substrate] were found for the CD Opti CHO™ and to estimate the media performance. The doubling time of 36 hours given in the design specifications was assumed to be the max cell growth (μ_{max}) to be conservative with estimating design parameters. The peak cell density achievable was $7 \cdot 10^9$ [cell/L], which was based off the peak cell density achieved in literature⁵. The peak cell density and time optimization accounted for cell death.

5.2.2 Design

The Monod model and ODEs used to model cell growth are shown below where X is cell concentration [cells/L], S is substrate concentration [g/L], μ is instantaneous cell growth [1/hr], μ_{max} is the maximum observed cell growth [1/hr], t is time [hr], K_s is a constant [g/L], and Y is substrate yield [cells/g substrate]⁷.

$$\frac{dX}{dt} = \mu X \quad [1]$$

$$\frac{dS}{dt} = -\frac{\mu X}{Y} \quad [2]$$

$$\mu = \frac{\mu_{max} * S}{K_s + S} \quad [3]$$

The differential equations were solved in MATLAB (*Appendix C*). The constant μ_{max} was determined from the 36 hour doubling time to be 0.0193 [1/hr] as a conservative estimate for modeling the cell growth (*Appendix C*), and the K_s and Y were determined from the paper discussed above in *Section 5.2.1*. Eleven reactors were used to produce the peak cell concentration of $7 \cdot 10^9$ [cell/L] in the final reactor of the seed train. The reactor volumes were determined by manually entering different volumes into MATLAB until time and cell production were optimized. The optimized time for each reactor was based off the substrate cell inoculation concentration of 0.96 [g/L] and the max cell density⁷. Once the concentration dipped below 0.96 [g/L] or the cell density was greater than of $7 \cdot 10^9$ [cell/L], it was assumed cell death would occur and growth would stop. *Figure 3* through *Figure 6* are the results of the MATLAB simulation described above.

⁷ Lopez-Meza, Julian, et al. "Using Simple Models to Describe the Kinetics of Growth, Glucose Consumption, and Monoclonal Antibody Formation in Naive and Infliximab Producer CHO Cells." Vol. 68, no. 4, 2016, p. 1287.

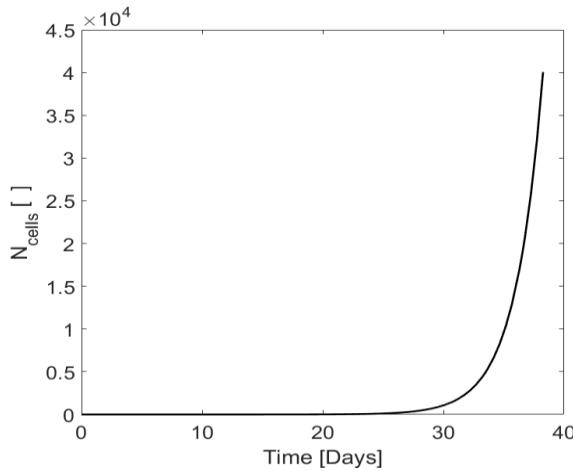


Figure 3: MATLAB simulation of cell population in the seed train. The cells grows exponentially with no death since the seed train reactors provide sufficient substrate concentrations. The transition from one seed train to the next occurs when cells reach their maximum cell density of $7 \cdot 10^9$ [cells/L] or the inoculation substrate concentration dips below 0.96 [g/L].

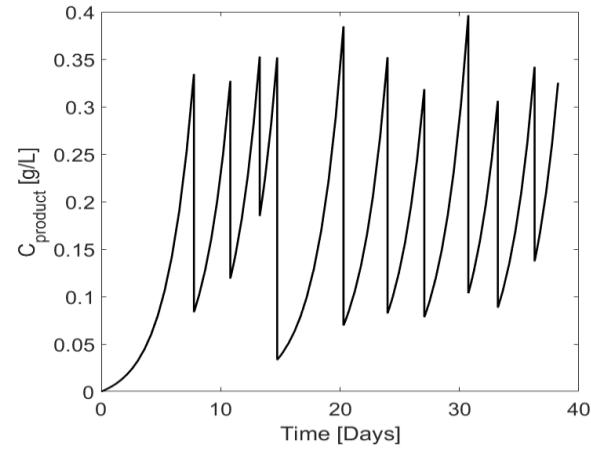


Figure 4: MATLAB simulation of product concentration in the seed train. The product concentration follows the same trend as the cell concentration scaled proportionally to the yield of the cells. The concentration of product instantaneously decreases after transitioning from one seed reactor to the next seed reactor. The titer of CHO cells cannot exceed a product concentration higher than 2 [g/L], which is higher than the max concentration seen in this figure.

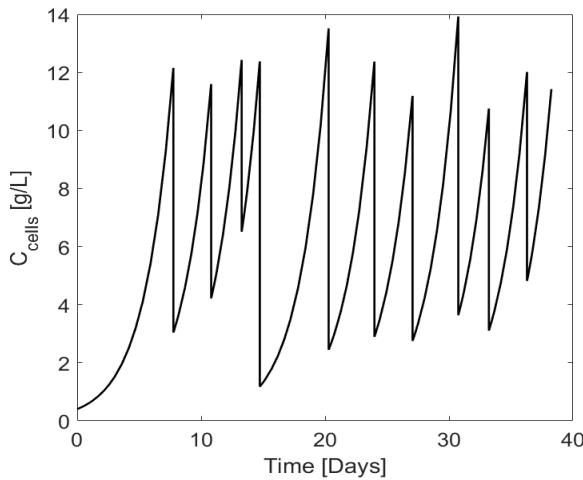


Figure 5: MATLAB simulation of cell concentration in the seed train. The exponential increase of cells in the reactor was a result of the Monod equation for growth parameters of CHO cells. The concentration of cells instantaneously decreases after transitioning from one seed reactor to the next seed reactor.

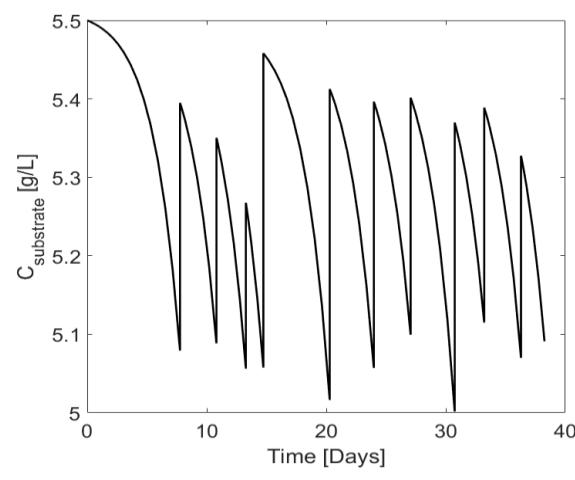


Figure 6: MATLAB simulation of substrate concentration in the seed train. The exponential decrease of substrate concentration is due to cell concentration increasing as time continues. The concentration increases every seed train transition from the original volume entering a fresh media solution.

The seed train is designed with a mixture of disposable and CIP reactors. A high-level PFD of the seed train can be viewed below:

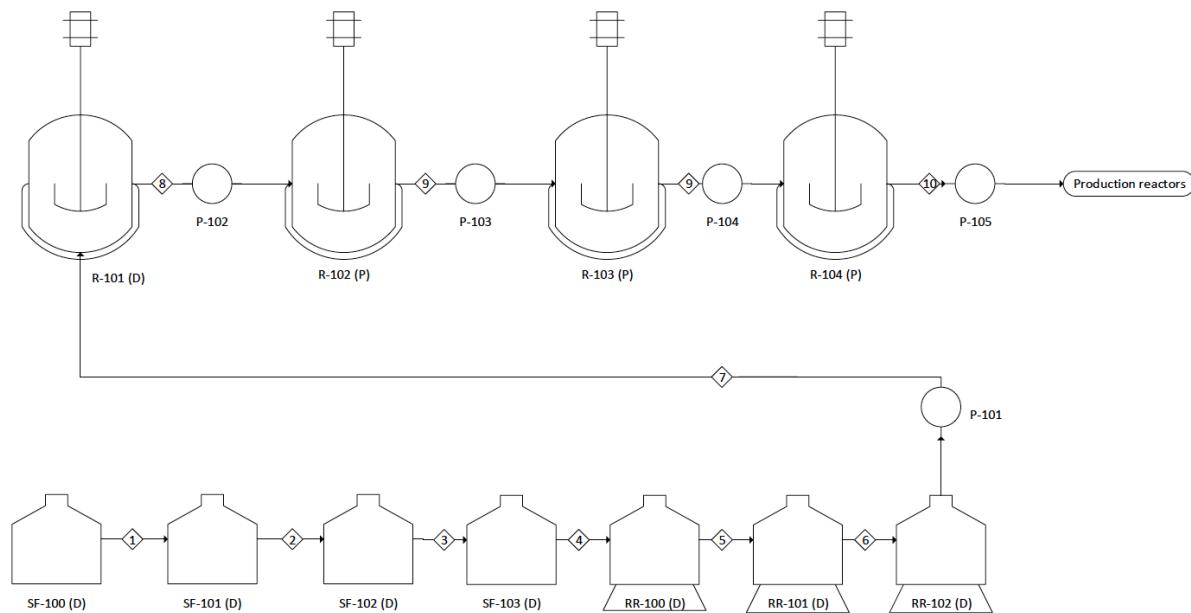


Figure 7: SF 100-103 are disposable shake flask reactors. RR 100-102 are disposable rocking reactors. R 101 is a disposable stirred bag reactor. R 102-104 are permanent stirred batch reactors. “P” designates a permanent reactor while “D” designates disposable.

SF 100-103 are disposable shake flask reactors of 0.01, 0.05, 0.1, and 0.15 L, respectively. RR 100-102 are disposable rocking reactors of 1.5, 10, and 30 L, respectively. R-101 is a disposable agitated bag reactor of 200 L. R 102-104 are permanent agitated batch reactors of 500, 1500, and 4000 L. R 102-104 will be cleaned and steamed in place while the other reactors in the seed train will be replaced. The volume occupied by the cell mixtures of each reactor are 0.005, 0.02, 0.055, 0.105, 0.105, 6.1, 26.1, 106, 406, 1400 and 3500 L, respectively. The initial amount of substrate will be charged into the seed reactors before the cell broth is charged. The cell broth will be poured by operators for the first seven reactors and pumped with peristaltic pumps for the next four. The peristaltic pumps are used to prevent cell damage.

The reactors are kept at 33°C to optimize cell growth. The shake flask reactors will be kept in a 30 L warming bath and the rocking reactors will be heated by the rocker hot plate. The bag and permanent agitated reactors will be heated by heating jackets. The heating fluid will be the neutralized kill tank water at 80°C. The amount of heating water need for the agitated reactor will be determined by a feedback control loop. The estimated heating water is $2.71 \cdot 10^4$ [kg/yr] (*Appendix B Calculation 1*). During start-up procedures, kill tank water will not be available for heating, so city water will be heated and used. The agitators in the agitated batch reactors will be run at 140 rpm per recommendation from *Growth and death kinetics of CHO cells cultivated in*

continuous bioreactor at various agitation rates. The diameter of the agitator blades will be a third of the tank⁸. The shaker and agitator speeds will be further optimized onsite to produce the best cell growth. A low-level diagram of one permanent seed train reactor can be seen below in *Figure 8* that illustrates controls and safety precautions:

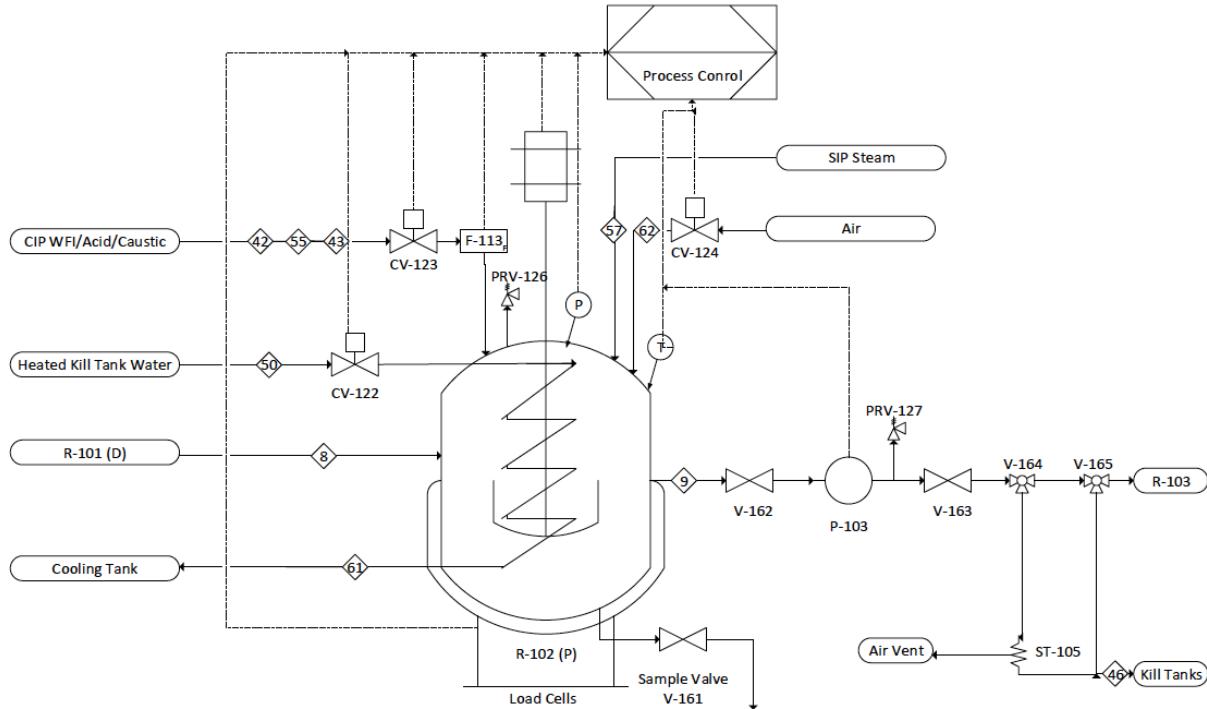


Figure 8: A low level diagram of seed train batch reactor R-102. The CIP/SIP system is shown as well as process control. R-101 is fed to R-102 then R-102 is fed to R-103.

Nomenclature to help understand the PFD exists in *Appendix A*. The CIP/SIP systems are discussed more in depth in *Section 5.11*. CV-122 is regulated off the temperature probe inside the reactor and will allow the heated kill tank water of 80°C to heat the reactor contents. P-103 can be regulated off the load cells under the reactor. V-162 and V-163 are block and bleed valves to replace and perform maintenance on P-103. V-161 is used as a sample port to test cell broth properties such as cell and protein concentration. PRV-126 and PRV-127 are pressure relief valves in case V-162 or V-163 became blocked during SIP. Each permanent seed train reactor follows a similar design. Cleaning protocols can be found in *Section 5.11* and a detailed timeline can be found in *Section 4*.

5.3 Production Reactors

5.3.1 Assumptions

The cell concentration does not exceed $7 \cdot 10^9$ [cell/L] for similar reasons discussed above in *Section 5.2.1*. The max achievable protein concentration was 1.5 [g/L] to be conservative. The media allows for cell growth and protein production and cannot be below 2 [g/L]. Cell death was

⁸ Balandras, Frédérique, et al. "Growth and Death Kinetics of CHO Cells Cultivated in Continuous Bioreactor at Various Agitation Rates." *BMC Proceedings*, vol. 5, no. Suppl 8, 2011, p. P101.

accounted for in the growth parameter as well as the cell concentration maximum. Protein A operated at 90% and polishing operated at 95% protein recovery efficiency making it necessary to produce 1200 [kg/yr] out of the production reactors.

5.3.2 Design

Seven 5500 L production reactors were used to achieve the 1200 [kg/yr]. At the start of production, 500 L from the seed train were added to each production reactor. The initial substrate concentration was 2.9 [g/L] which is within the range of optimal substrate concentrations described in literature⁵. Further optimization of initial substrate concentrations can be performed on site. A high-level diagram of the production reactors can be seen below in *Figure 9*.

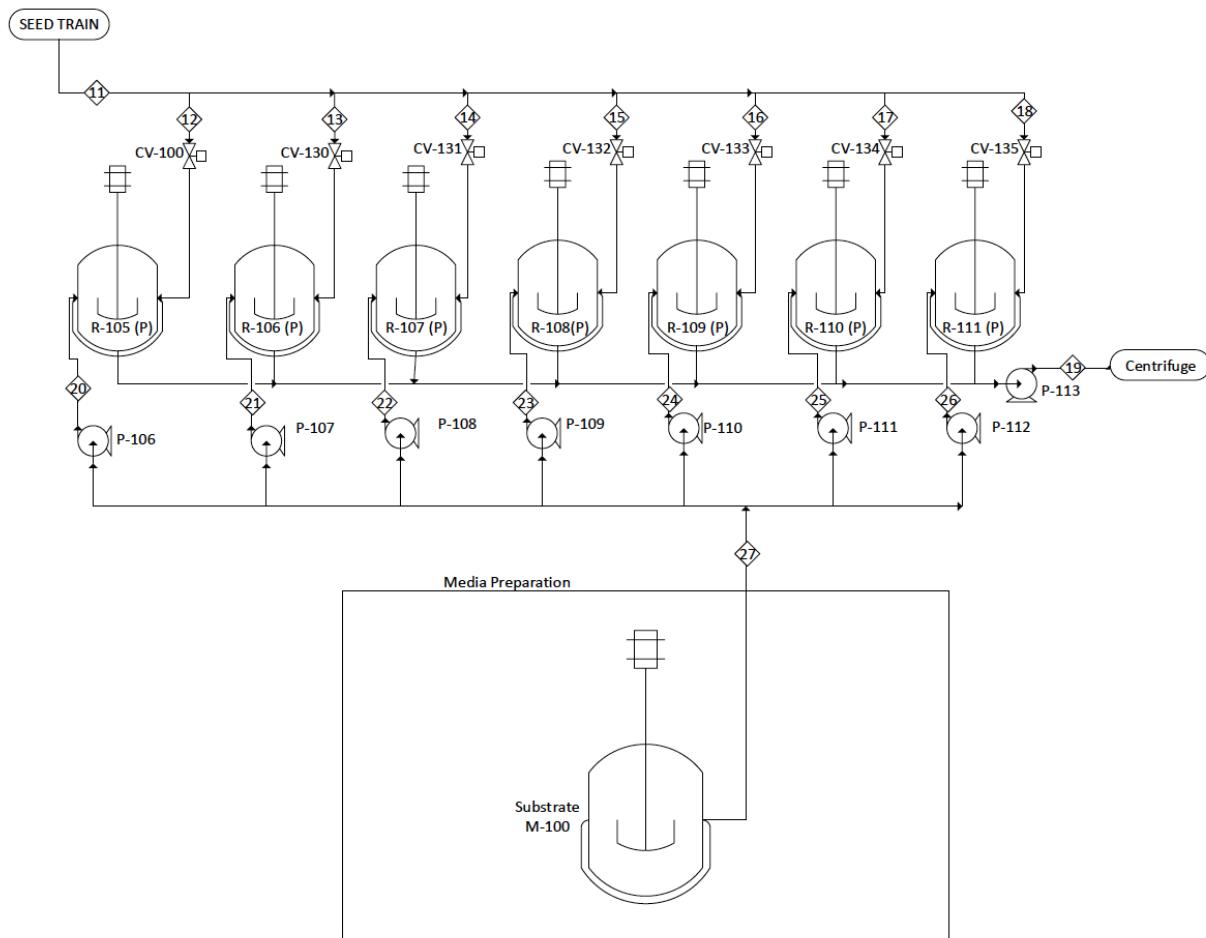


Figure 9: Seven production reactors are used and charged with 500 L of fluid from the seed train. Media is continuously fed to the production reactors from the media preparation area. Once the desired protein concentration is reached, the production reactors are emptied into the storage tank before harvesting.

In *Figure 9*, the feed to the reactors is 25 [L/hr] with 2.9 [g/L] of media. The final volumes of liquid in the reactors are 5000 L, leaving 500 L of excess space in each reactor. Each batch run lasts 7.5 days allowing for 25 runs a year with cleaning time and 75% on-stream efficiency.

MATLAB was used to model and optimize the protein production (*Appendix C*). *Figure 10 to 13* below shows the protein concentration, substrate concentration and volume change over time modelled in MATLAB.

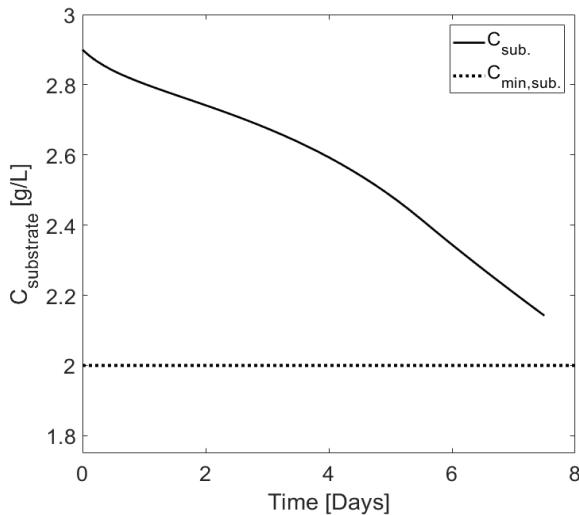


Figure 12: MATLAB simulation of substrate concentration in a production reactor vs. time. The concentration of substrate (solid) lies above the minimum substrate concentration (dashed) at the final time of reactor operation. The trend of the line is a proportional response of cell concentration and cell productivity.

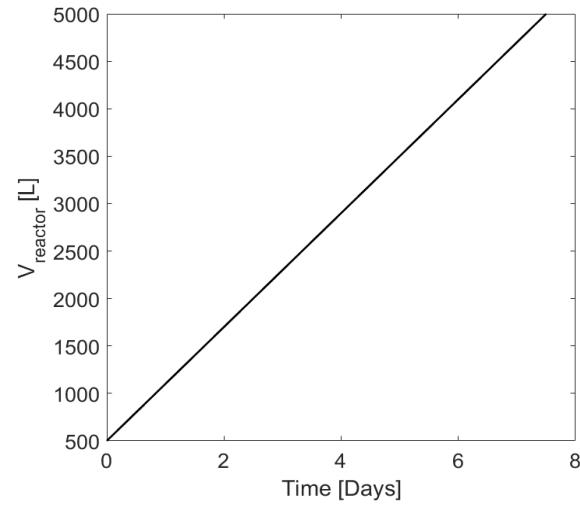


Figure 13: MATLAB simulation of volume in a production reactor vs time. The trend is linear because the fed stream flowrate is fixed at 25 [L/hr] for the total process time of 180 hours.

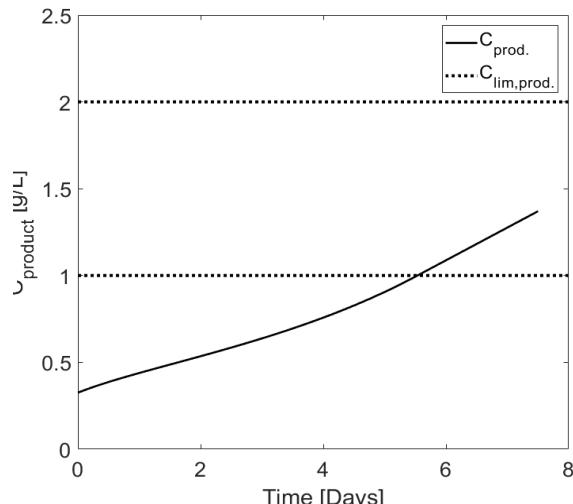


Figure 10: MATLAB simulation of product concentration in a production reactor vs. time. The final product concentration (solid) lies between minimum and maximum concentrations of product (dashed) as specified by the titer specifications of 1[g/L] and 2 [g/L].

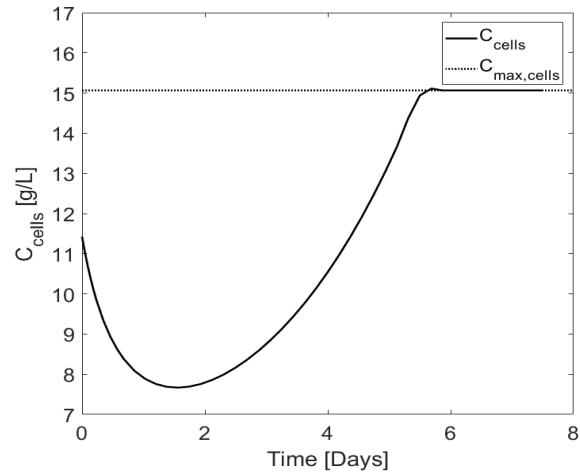


Figure 11: MATLAB simulation of cell concentration in a production reactor vs. time. The concentration of cells decreases initially because the rate of production of cells is less than the rate of fluid entering the reactor. At the time of 1.56 days, the cell concentration increases exponentially until it reaches maximum of 15.11 [g_{cells}/L]. This occurs because the concentration of cells in solution has reached the theoretical maximum.

The media concentration is at the limit of 2 [g/L] at the end of the process to make separation easier and the protein concentration reaches 1.4 [g/L]. The number of reactors was determined by the protein concentration restraint; however, if the titers drastically improve to 5 – 10 [g/L] only one or two reactors would be needed.

The reactors will be fitted with pumping, monitoring and safety systems illustrated below in *Figure 14*.

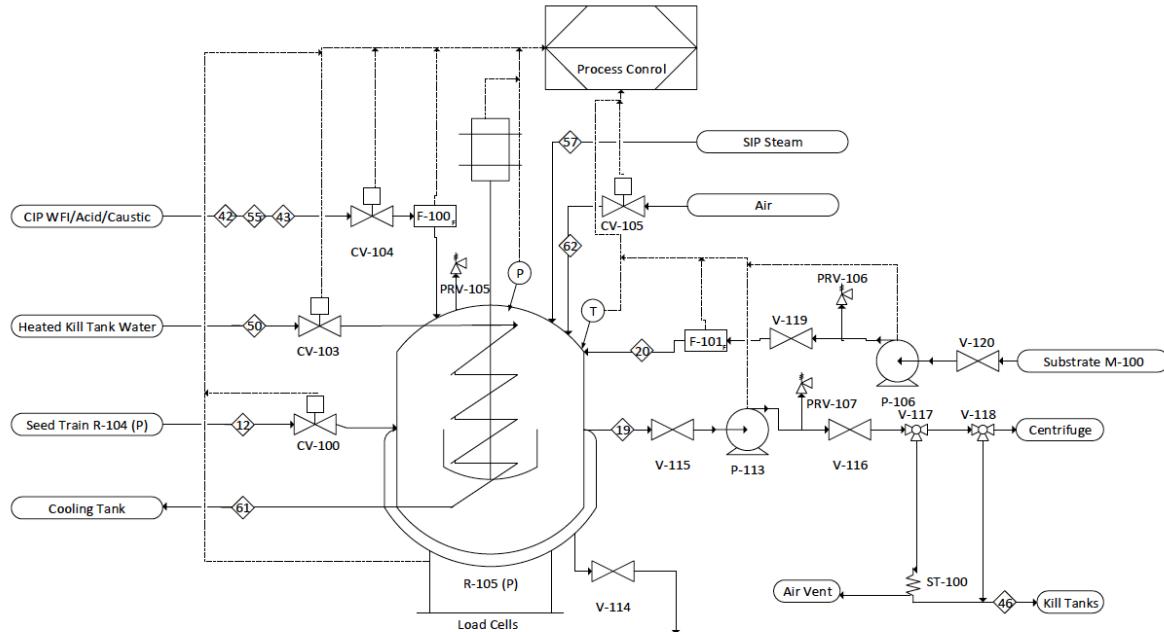


Figure 14: A low-level PFD of a single production reactor. All the production reactors are fed batch and identical.

Figure 14 is identical to the seed train reactor PFD (*Figure 8*) except for an additional feed stream of media (Stream 20). V-120 and V-119 are block and bleed valves for P-106. P-106 is a centrifugal pump unlike the peristaltic pumps used in the seed train because there are no cells in the media. P-106 is regulated by F-101 using a Variable Frequency Drive (VFD). P-113 is also a centrifugal pump because after production cell damage is no longer a concern. V-117, V-118, and ST-100 are used for the SIP/CIP system for every production reactor. The reactor is maintained at 33°C by the kill tank water and CV-103 via a feedback loop from the temperature probe.

5.4 Primary Recovery: Harvest

5.4.1 Assumptions

Centrifugation design assumes complete separation of solids from liquids when the residence time is equal to the settling time. The CHO broth contains media, cells, and product. CHO cells are assumed to be the only solid within this matrix while other components are aqueous in the broth mixture. All the CHO cells will be separated during the centrifugation and filtration unit operations.

Centrifugation design is dependent on the physical properties of the solid and liquid within a system. CHO broth was assumed to be the solid while the liquid was assumed to have the properties of water at 25°C. The CHO broth had a density of 1.06 [g/mL] and a particle size of 12.9 μm ⁹.

5.4.2 Design

The centrifuge was designed to process the 35000 L of CHO broth in 12 hrs. Settling velocity was determined by balancing the drag and buoyancy forces experienced by a particle. This results in the governing equation for the complete separations of solids from liquids (*Equation 4*) where the ratio of flowrate (Q) and equivalent area (Σ) is related to the particle diameter (d), solid density (ρ_s), liquid density (ρ_l), viscosity (μ), and gravitational acceleration (g)¹⁰.

$$\frac{Q}{\Sigma} = \frac{d^2(\rho_s - \rho_l)}{18\mu} g \quad [4]$$

The ratio of flowrate and equivalent area was $4.16 \cdot 10^{-6}$ [m/s] (*Appendix B Calculation 39*). High rotational speeds and flow rates can potentially damage product due to shear stresses. The flowrate through the centrifuge was 2905 [L/hr] which would make the equivalent area 194 m² for these processing conditions. Pilot scale testing is recommended to further optimize the centrifuge.

A disposable depth filter system can be just as economical as permanent systems that would require CIP and SIP¹¹. The Millipore POD System is an example of a disposable depth filter that takes up less volume and can eliminate remaining solid particulates. *Figure 5.4.1* below shows a low-level PFD of the centrifugation process:

⁹ Pan, Xiao, et al. "Metabolic Characterization of a CHO Cell Size Increase Phase in Fed-Batch Cultures." *Applied Microbiology and Biotechnology*, vol. 101, no. 22, 2017, pp. 8101–8113.

¹⁰ Shukla, Abhinav A., Mark R. Etzel, Shishir Gadham, *Process Scale Bioseparations for the Biopharmaceutical Industry*. Florida: CRC Press, 2007.

¹¹ Millipore, "Processing Economics of the Millipore Pod System Versus Millistak + HC Lenticular Stacks," [http://www.millipore.com/publications.nsf/a73664f9f981af8c852569b9005b4eee/6111b56c265876ca8525733c005daf6e/\\$FILE/tb1076en00.pdf](http://www.millipore.com/publications.nsf/a73664f9f981af8c852569b9005b4eee/6111b56c265876ca8525733c005daf6e/$FILE/tb1076en00.pdf), Obtained 28 June 2009.

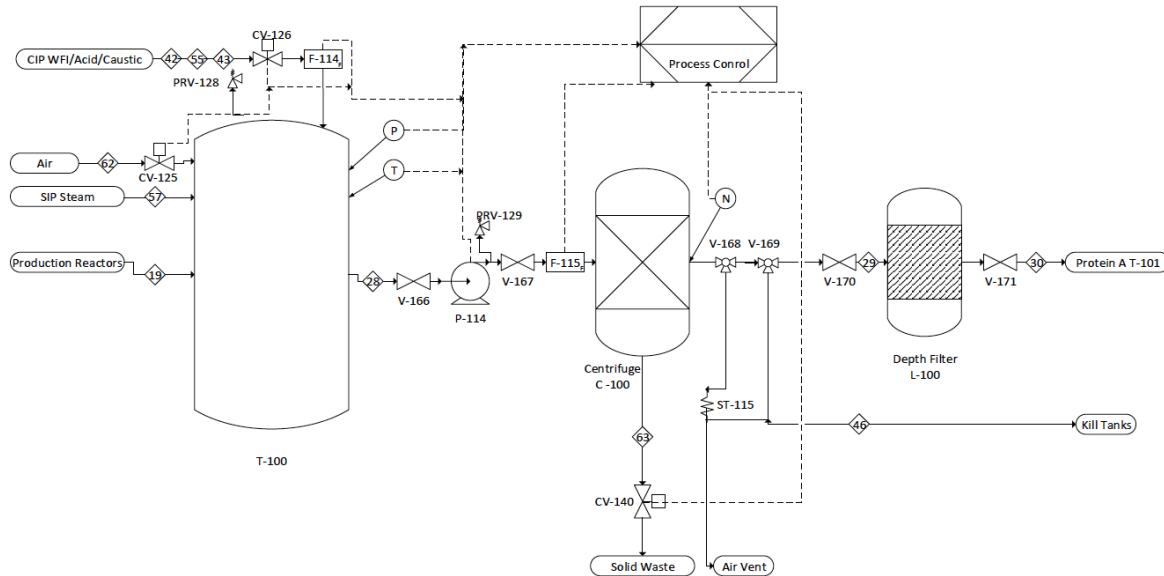


Figure 15: The solid removal phase of the process including a holding tank, centrifuge, depth filter, and accompanying process equipment.

T-100 is a 40000 L tank to hold the product from the production reactors for centrifugation. The production reactors are not directly fed into the centrifuge to allow for cleaning of the production reactors during centrifugation. V-166 and V-167 are block and bleed valves for P-114. P-114 is a centrifugal pump controlled by the flow meter (F-115) and a VFD. The turbidity meter (N) regulates when the solid waste control valve (CV-140) is opened to release the solids built up. The depth filter (L-100) is not under CIP/SIP so the CIP/SIP ends before V-170. L-100 is single use so V-170 and V-171 are used to facilitate filter replacement.

5.5 Purification: Protein A

The order of operation of the column can be seen below in *Figure 5.5.1*.

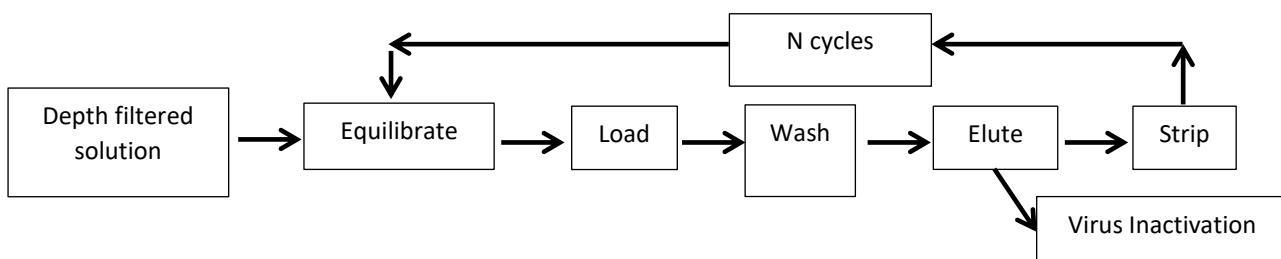


Figure 5.5.1: Stages of operation for a Protein A column. The equilibration fluid prepares the column for extracting the protein from the broth in the loading stage. The wash fluid will rinse out any unwanted particulates before an eluting fluid strips the protein off of the resin. Final stripping will be carried out to regenerate the column before the next cycle.

5.5.2 Assumptions

This unit operation will undergo equilibrating, loading, washing, eluting, and stripping steps every cycle. The stripping and equilibrating solutions are urea, the washing fluid is an acetic acid buffer (pH 5), and the eluting fluid is another acetic acid buffer (pH 4)¹². The washing fluid must be a pH of 5 to remove any non-binding material from the resin, but not below pH 5 as to elute the MAb product. Values from SuperPro Designer were used to determine an adequate column area and velocities. The volume of product was assumed to be 80% the column volume per cycle. Resin will be replaced once a year. It is also assumed that 90% of the MAb will be lost during this process due to non-binding and loss of protein during washes.

5.5.3 Design

The liquid from the depth-filtration will be processed through a Protein A chromatography column of 0.80 m wide and 0.89 m tall for eight cycles. A MATLAB script was developed to optimize the column size for the lowest price of resin and yearly operation. Two equations were analyzed to determine the fastest operation (*Equation 5*) and lowest cost (*Equation 6*), as seen below.

$$P = \frac{1}{L \cdot \left(\frac{1}{C_0 \cdot u_L} + \frac{N}{Q_d \cdot u_N} \right)} \left[\frac{W_{prod}}{V_{col} \cdot t} \right] \quad [5]$$

The loading step enters at a velocity (u_L) and the elution step enters at a velocity (u_N). The optimal length (L) was determined by using the *Equation 5* with constants of initial product concentration (C_0), and binding capacity (Q_d).

$$Price = V \cdot \frac{\$resin}{volume} + N_{batches} \cdot N_{cycles} \cdot \left(\frac{\$equilibrate}{cycle} + \frac{\$wash}{cycle} + \frac{\$elute}{cycle} \right) \quad [6]$$

The volume was iterated from 0.1 to 2 m³ and the number of cycles was found from the binding capacity and total amount of MAb. The volume was used to determine the amount of resin needed for each iterated value. The number of cycles(N_{cycles}) was used to determine the cost of equilibrium, wash, and elution fluids. The price of fluids and resin was added, and a visible trend is seen in *Figure 16*:

¹²Gottschalk, Uwe. *Process Scale Purification of Antibodies*. Second ed., Wiley, 2017.

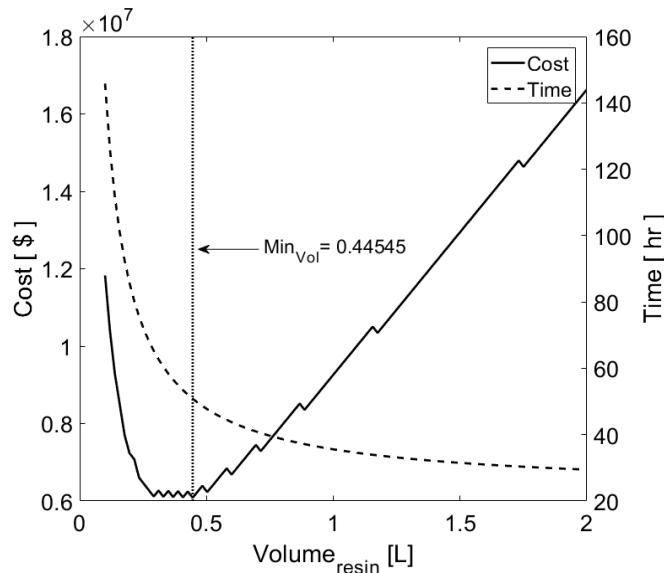


Figure 16: MATLAB iteration of cost vs. Protein A MABselect resin volume. The column area was set at 0.5m^2 and the amount of resin in the column was determined through optimizing cost. The unit operation costs include yearly resin replacement and pH 4, pH 5, and pH 8 buffer volumes per cycle. The optimized column includes a length of 0.89m with eight cycles to process the cell broth in 50.8 hours of operation.

Figure 17 shows the protein A process. The product is held in the 40000 L T-101. P-115 is used to regulate the flow of product into protein A by the feedback loop from F-105. The load cells on T-101 are also used to regulate how much product moves through the protein A column to ensure cycle efficiency is high. V-143 and V-144 are block and bleed valves for P-115. The buffers used for protein A chromatography are also shown in the buffer preparation area. The pH 5 solution is controlled by P-116 and F-106 through a feedback loop. The pH 4 solution is controlled by P-117 and F-108 through a feedback loop. The 8 M urea solution used for regeneration is controlled by P-118 and F-109 through a feedback loop. CV-118 and CV-119 control whether the protein A column effluent is fed into viral inactivation or the kill tanks, respectively.

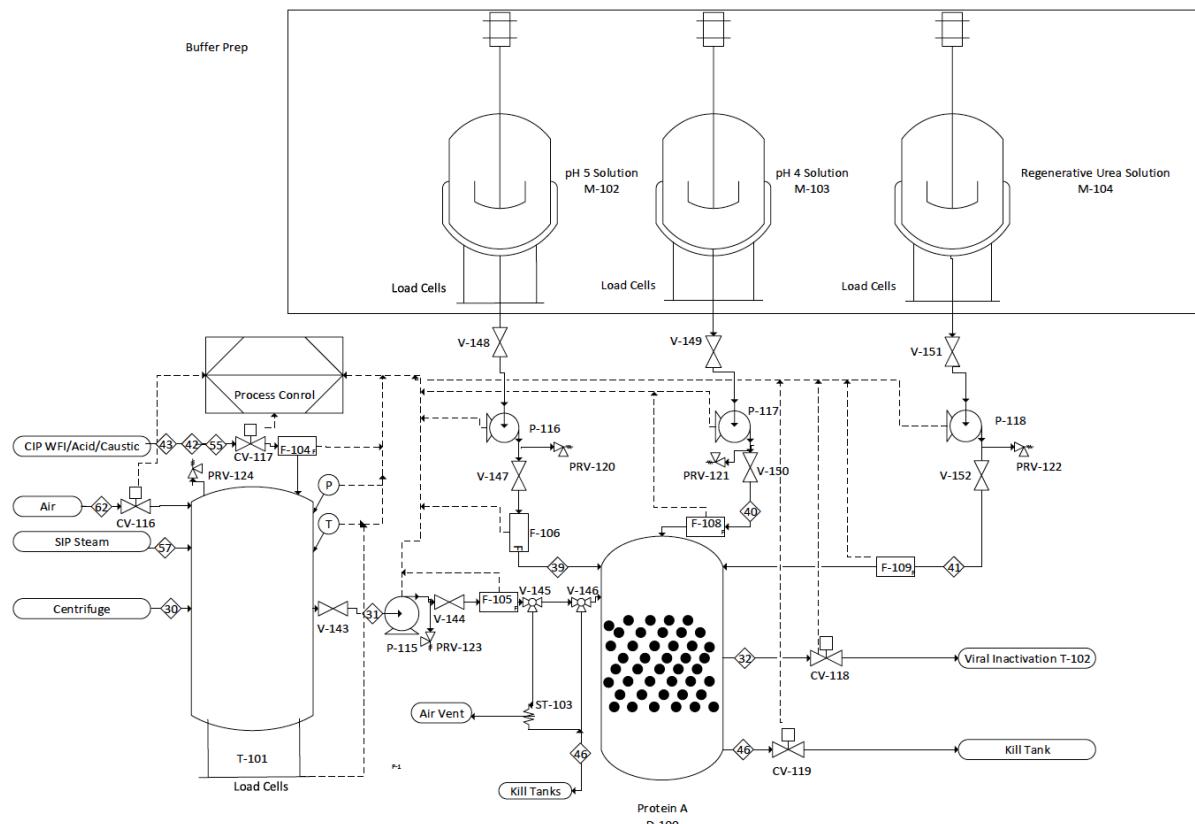


Figure 17: The low-level PFD of the protein A process.

5.6 Viral Inactivation

5.6.1 Assumptions

A pH of 4 has been shown to remove viruses while not denaturing a large amount of the protein with a 0.5 – 2 hours holding time^{Error! Bookmark not defined.}. The MAb is predicted to denature slightly in the acidification process which was accounted for in the protein A efficiency. The pore size for the filter will need to be 19 nm to achieve an LRV of 3-4 for viruses commonly found in MAb production¹³. The inactivation will be done in batch phases based on the 2800 L elution volume from the protein A chromatography.

5.6.2 Design

The elution leaving protein A chromatography will be held in a 4000 L stainless steel holding tank for 0.5 – 2 hours. The optimal time will be determined onsite based on achieving an LRV value between 3 – 4. The pH of the solution should be approximately 4 to achieve the proper viral inactivation. The MAb protein has been shown to not denature and unfold at pHs above 3.5¹⁴, therefore it is extremely important the pH remains above 4 and should never drop below

¹³ Marques, Bruno F., et al. "Virus Filtration of High-Concentration Monoclonal Antibody Solutions." *Biotechnology Progress*, vol. 25, no. 2, 2009, pp. 483–491.

¹⁴ Latypov, Ramil F., et al. "Elucidation of Acid-Induced Unfolding and Aggregation of Human Immunoglobulin IgG1 and IgG2 Fc." *The Journal of Biological Chemistry*, vol. 287, no. 2, 2012, pp. 1381–96.

3.5. The incubated low pH solution will then be pumped through a filter in a normal flow filtration with constant flow rate shown below in *Figure 18*.

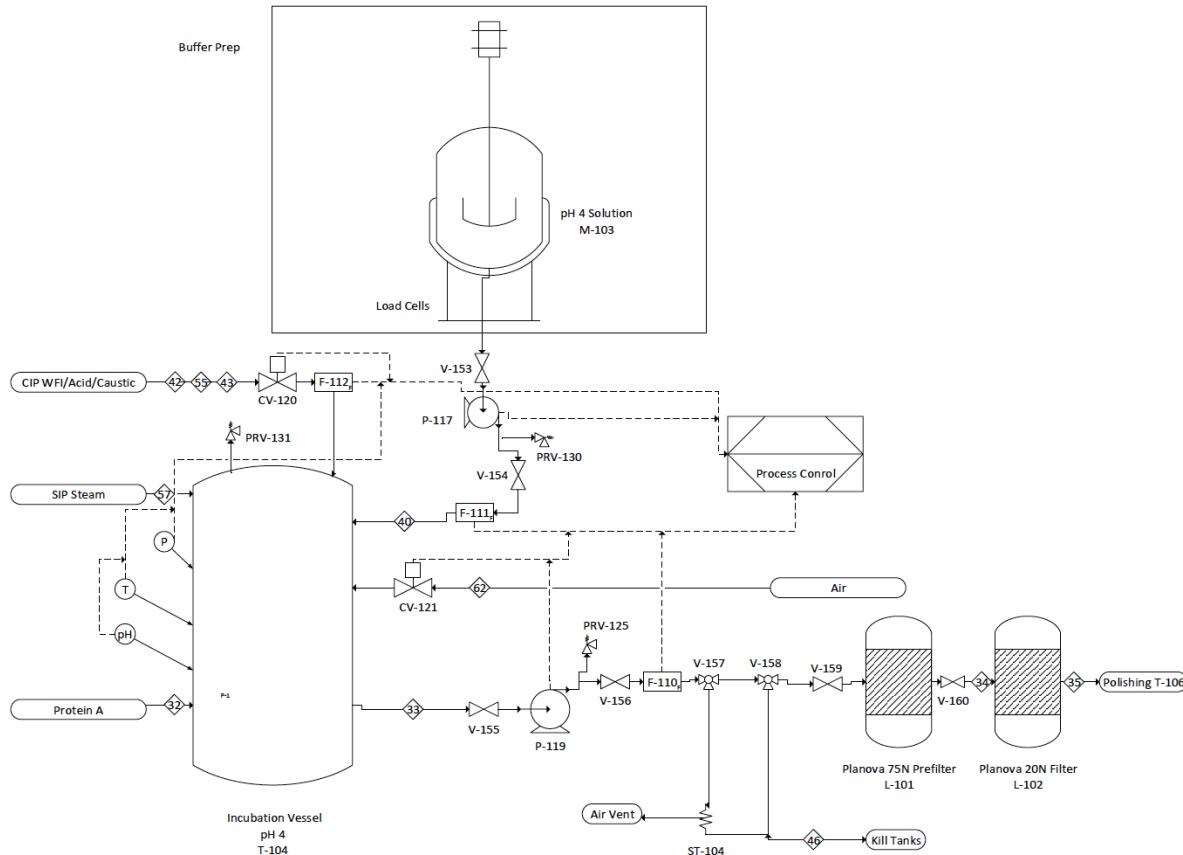


Figure 18: The above figure shows the PFD for viral inactivation. The incubation vessel holds the product at a pH of 4. The product is then pumped at constant flow through a pre-filter and then a filter to remove the viruses.

T-104 is used as the holding vessel for the product from protein A. The pH probe is used to control P-117 and whether or not the pH needs to be reduced further to confirm viral inoculation. The product is pumped through filters L-101 and L-102. F-110 with an accompanying VFD is used to regulate flow rate. V-155 and V-156 are block and bleed valves for P-119. V-159 and V-160 are used for replacing and testing the filters. V-157 and V-158 are the CIP/SIP outlet for the viral inactivation process. The filters are not cleaned to avoid damages and are replaced once deemed ineffective by the test methods described below.

Table 1: The below table shows the filter area and corresponding process time and price for the Asahi Planova 20N viral filter. Calculation 51 was used to calculate time.

Filter Area (m^2)	Filtration Time (hr)	Filter Price \$
0.3	150	1,200
1	50	4,000
4	11	16,000

The times were chosen from the recommended flow rates from the manufacturer and based on the conservative estimate of 3000 L of product. The 1 m^2 filter was chosen to intermediate time

and price. P-119 in *Figure 18* will be used to maintain the 60 [L/hr] recommended flow rate for this area. An Asahi Planova 75N prefilter will be used to increase the life time of the viral filter. The 20 nm pore diameter of the Asahi Planova 20N will be used to remove parvoviruses as recommended by *Virus Filtration of high-concentration MAb solutions*. The Asahi Planova 20N filter achieves the required LRV of 4.3 for parvoviruses. The larger viruses, such as retroviruses, will be filtered out of the product stream by the 20N filter. Integrity testing such as leak testing or gold particle testing will be used to determine filter viability. The filter manufacturer recommends using the Planova Leak Tester to ensure the filters are still viable.

5.7 Polishing

5.7.1 Assumptions

The impurities in the product stream are unknown so it is difficult to determine the binding capacity of these impurities to the resin. To deal with this unknown, it is assumed that for 100 g of MAb per 1 L of resin is needed¹⁵. The MAb binding capacity to the resin is unknown so a conservative 5% of the MAb will bind to the resin. The regenerative solution flow rate is the same as the product stream flow rate for a similar time frame.

5.7.2 Design

The effluent of viral inactivation will be stored in a 3000 L vessel. The product liquid will be titrated using 0.02–0.05 M Tris solution to achieve a pH of 7.5. NaCl will then be added to the product until the conductivity is 12 mS. Four cycles of the resin will be used to process the total 3000 L of product. The first step of the cycle involves pumping 700 L of the product through the ion exchange column. Once the product stream has been processed, the resin will be washed with WFI. The time for washing is unknown so an estimation of one hour will be used with further time optimization occurring on site. The resin will be regenerated with 0.02–0.05 M Tris solution containing 1 M NaCl for a similar process time as the product stream. This sequence is designed per the recommendation in *Process Scale Purification of Antibodies*¹². *Table 2* lists possible resins, subsequent dimensions and process times:

Table 2: column dimensions, process time and resin cost for three different resins. Option 1 has 100% recovery of resin while option 2 have 99% recovery.

Resin Type	Diameter [cm]	Height [m]	Time [hrs]	Resin Cost [\$]
Option 1: Unosphere Q	38.3	1.48	13.64	\$370,000
Option 2: Q Sepharose FF	51.7	0.54	10.52	\$176,000

Option 1 is the optimal choice because it can be regenerated to 100% its capacity while option 2 can only be regenerated to 99%. Although option 2 is cheaper, option 1 will never have to be replaced while option 2 will be regenerated to only 50% of its capacity by the 70th cycle. The plant will run 100 cycles a year, therefore it is optimal to have a resin that does not need frequent replacement. The recommended flow rate for the anion exchange column is 615 [cm/hr]¹⁶.

¹⁵ Liu, Hui F., et al. "Recovery and Purification Process Development for Monoclonal Antibody Production." *MAbs*, vol. 2, no. 5, 2010, pp. 480–499.

¹⁶ "A High-Throughput Flow Cytometer Built for Automation." Bio, www.bio-rad.com/.

MATLAB was used with manufacturing recommendations to size the column to have a diameter of 30.3 cm and height of 166 cm (*Appendix C*). The process time including product loading, washing and regeneration will last 13.64 hours. One cycle will require an estimated 1772 L of WFI and 2800 L of Tris buffer. *Figure 19* below shows the low-level PFD for the polishing step:

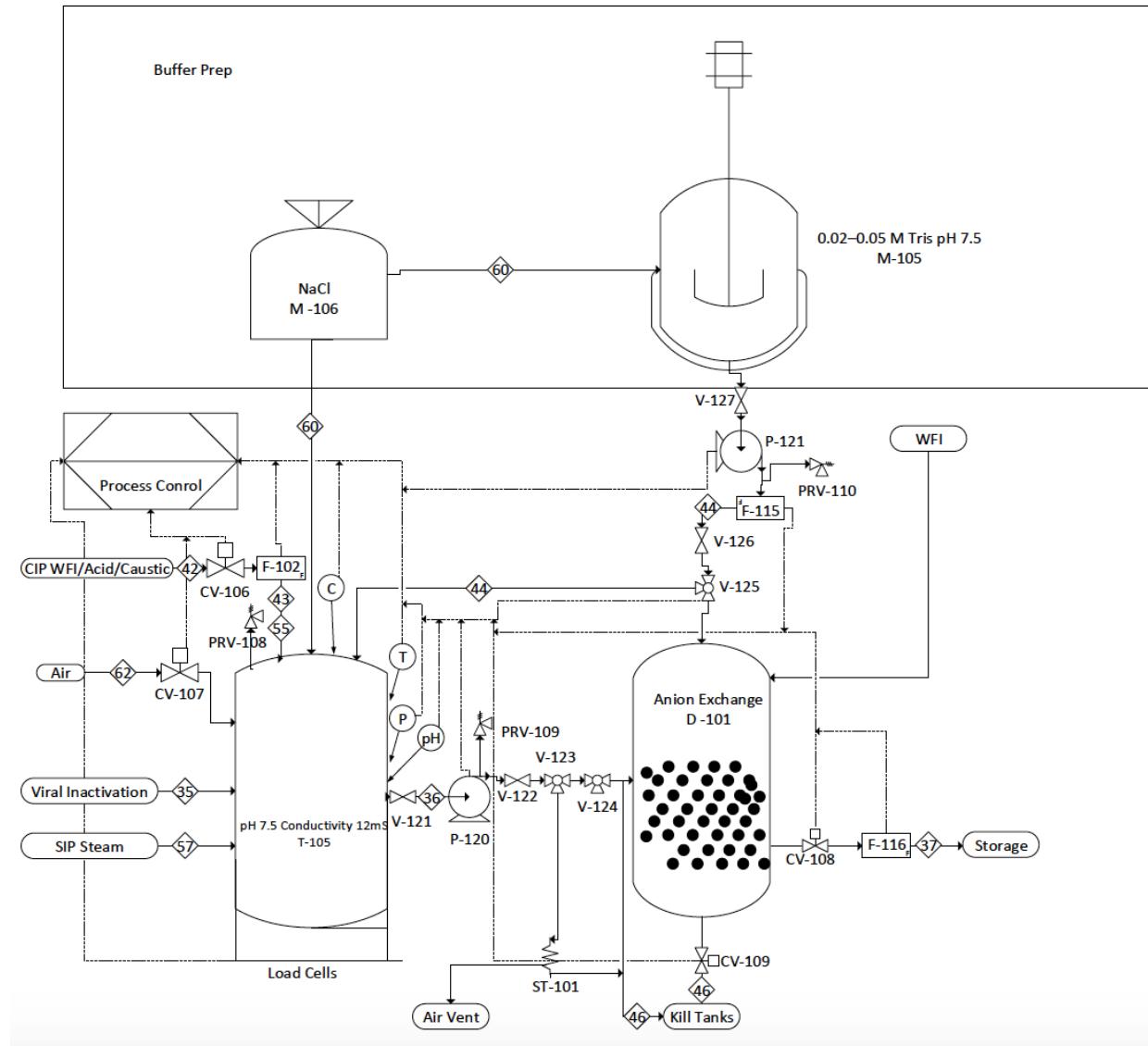


Figure 19: The low-level diagram of polishing. The viral inactivation product is moved through the anion exchange column. The column is washed with WFI and regenerated with a NaCl buffer solution.

T-105 is used for the holding tank as well as a mixing unit. The product is at a pH of approximately 4 and is titrated with the Tris buffer to reach a pH of 7.5 via P-121 controlled by the pH probe. NaCl is then poured into the mixer until the conductivity reaches the 12mS controlled via the conductivity meter. Once the desired pH and conductivity are achieved, NaCl is then added to the buffer to reach a conductivity of 1M. P-120 is controlled in response to the load cells to pump 750 L through the column. V-121 and V-122 are block and bleed valves for

P-120, P-121 is controlled in response to F-115 to pump the regenerative buffer through the column. CV-109 and CV-108 control whether the effluent from D-101 is sent to storage or to the kill tanks. V-123 and V-124 are used for CIP/SIP which does not include D-101 because D-101 is cleaned via regeneration and WFI.

5.8 Buffer Preparation

5.8.1 Design

The buffer design area had to facilitate the mixing of a pH 3, pH 4, pH 5, pH 7.5, detergent, and urea buffers for use in the stages of production. These buffers used acetic acid, detergent, sodium acetate, urea, sodium chloride, and Tris as the base chemicals. The individual formulations are shown in *Table 3-5*. The amount of each solution was also determined using the equation and treating the solution that needed buffer addition as water. The total amounts of each buffer per batch needed is shown in *Table 6*. This was used as a basis for sizing the mixing tanks for making the buffer solutions. Six mixing tanks will be needed since this is the maximum amount of processes that can be running at one time that require buffers. A 48000, 58000, 30000, 2000, 45000, and 90000 L mixing tank will be used for pH 4, pH 5, urea, tris, pH 3, and detergent, respectively.

Table 3: pH 3 formulation

Compound	Volume Percent (%)
0.1 M Acetic Acid	98.23
0.1 M Sodium Acetate	1.77

Table 4: pH 4 formulation

Compound	Volume Percent (%)
0.1 M Acetic Acid	84.7
0.1 M Sodium Acetate	15.3

Table 5: pH 5 formulation

Compound	Volume Percent (%)
0.1 M Acetic Acid	35.7
0.1 M Sodium Acetate	64.3

Table 6: Total amount of each buffer needed in the process

Type	Amount (L/batch)
pH 4	47000
pH 5	56500
pH 3	44000
Urea	28200
Tris buffer w NaCl	2800
Tris	100
Detergent	88200

5.9 Storage of Product

5.5.4 Assumptions

The solution coming from the anion exchange polishing step contains mostly salt, WFI, MAb, and trace amounts of buffer and fine particulates. The final product will be stored at -20°C, therefore glycerol will be added to reduce the solution freezing temperature well below the freezing limit. Studies have shown that the mixture can withstand room temperature for at most a

week without degradation^{5,17}. The time to add glycerol and to transfer the liquid to storage tanks will not affect the quality of the product. Lastly, the final product must be kept in a dark plastic container to prevent UV catalyzed degradation.

5.5.5 Design

The storage will be cooled to -20°C via a refrigeration cycle using Freon™ (R-23) refrigerant. *Figure 20* below shows a PFD of the storing method:

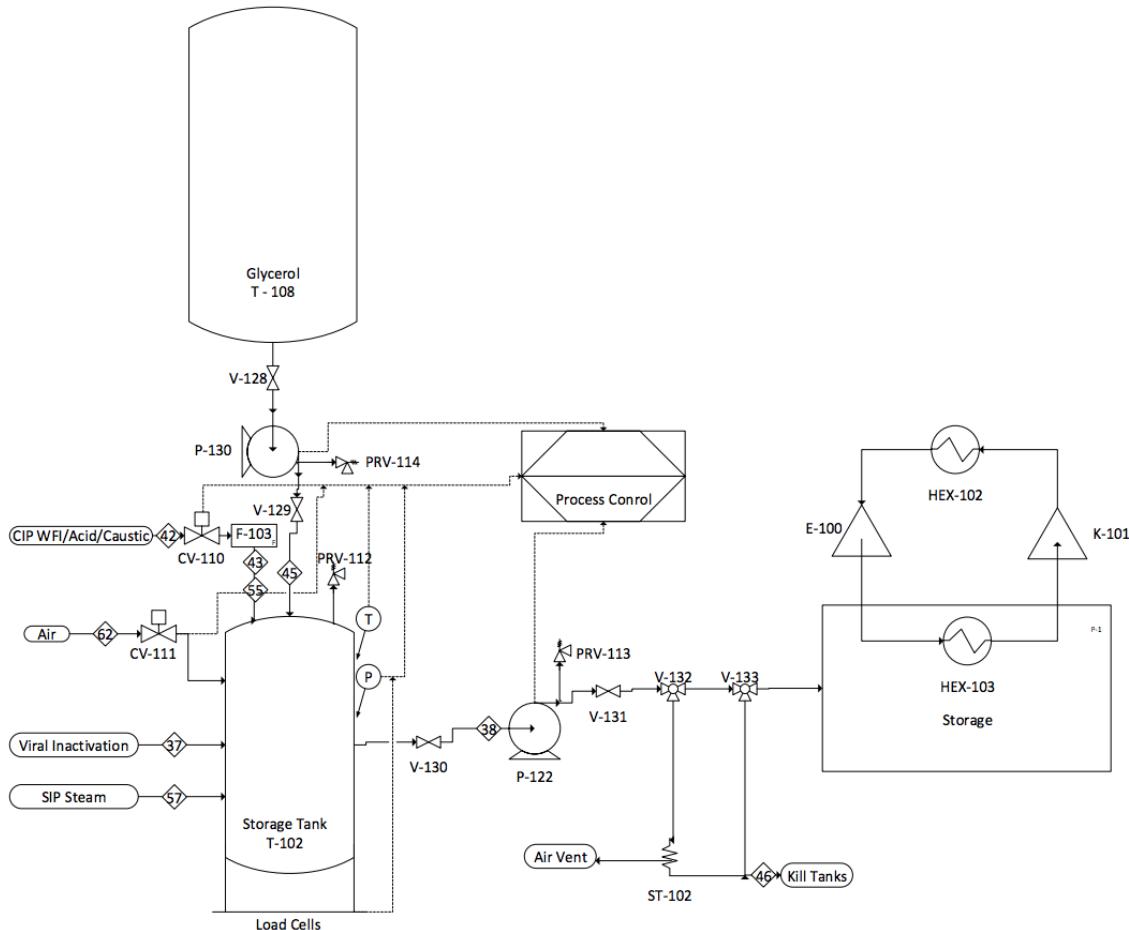


Figure 20: The PFD for the storage process. The product is pumped into the storage tank (T-102) from the polishing step where it is mixed with glycerol. The glycerol mixture is then stored in a cold room, which is cooled by a refrigeration cycle.

The solution from the anion exchange polishing storage flows through piping to T-102. The solution has a MAbs concentration of 14.6 [g/L] and needs to be 5 [g/L] to meet storage requirements. Glycerol in T-108 is added to the solution to reduce the concentration and to

¹⁷ Ciocca, D R, D J Adams, R J Bjercke, G W Sledge, D P Edwards, G C Chamness, and W L McGuire. "Monoclonal Antibody Storage Conditions, and Concentration Effects on Immunohistochemical Specificity." *The Journal of Histochemistry and Cytochemistry : Official Journal of the Histochemistry Society* 31.5 (1983): 691-96. Web.

lower the freezing point from 0°C to -42°C which is well below -20°C¹⁸. The amount of glycerol added to the holding tank is 5200 L, thus increasing the volume of final product to 8000 L. Glycerol is added via P-130, which is controlled by T-102's load cells. The 8000 L is sent to storage via P-122, where the final product is stored in plastic 200 L storage drums. The drums are placed into a storage facility held at -20°C and can be stored for up to a year before protein decomposition occurs. The processing facility can produce up to 40 drums per cycle, meaning 1000 drums per year. The storage facility is designed to hold up to 520 drums of product. It is cooled via the refrigeration cycle shown by HEX-103, HEX-102, E-100 and K-101 (*Appendix B Calculations 58-64*). The process is CIP/SIP via similar mechanisms described in previous sections and *Section 5.11*.

5.10 Kill Tanks

5.10.1 Assumptions

The waste is a mixture of buffers, deactivated virus, water and biological contaminants. The primary component in the waste stream is water and so the physical properties of water will be used for heat exchanger design. Aspen HYSIS will be used as a design tool. HYSYS used the NRTL fluid package and an overall heat transfer coefficient of 130 [W/m²K]. The waste is currently at an unknown pH. The waste is assumed to be the temperature of the combined components at each of their operating temperatures. The heat exchanger hot fluid will be saturated steam from Boiler-100 so the heat of vaporization will be the only source of energy. Adequate processing occurs when the wastewater is pH 6-9 and has been heated to 80°C for 60 seconds.¹⁹ The process will be continuous since waste will be continuously accumulating.

5.10.2 Design

The yearly waste was determined by adding the waste from each unit operation as well as the waste from CIP and SIP procedures. The waste is very dilute and contained over 95% water. The total waste per batch was 430000 L.

The yearly waste was separated into the detergent stream, which is at 60 °C, and the other waste streams, which are at 25°C. The detergent waste and other waste combine and equilibrate into a 32.17°C stream flowing at 1635 kg/hr into two large 250000 L tanks. These tanks are then neutralized to a pH of 7 via a pH feedback loop using a pH 7 buffer. The water then flows into a heat exchanger where 143.2 kg/hr of 100 °C saturated steam is used to sufficiently heat the wastewater to 80 °C (*Appendix B Calculation 74-75*). The required shell and tube heat exchanger will be 19.8 m² which is the conservative number from HYSYS (*Appendix B Calculation 73*). The water will then be cooled to in an additional holding tank before being released to the sewer system. The process is detailed in *Figure 21* below:

¹⁸ Lane, Leonard B. "Freezing Points of Glycerol and Its Aqueous Solutions." *Industrial & Engineering Chemistry* 17.9 (1925): 924. Web.

¹⁹ Gregoriades, Niki, et al. "Heat Inactivation of Mammalian Cell Cultures for Biowaste Kill System Design." *Biotechnology Progress*, vol. 19, no. 1, 2003, pp. 14–20.

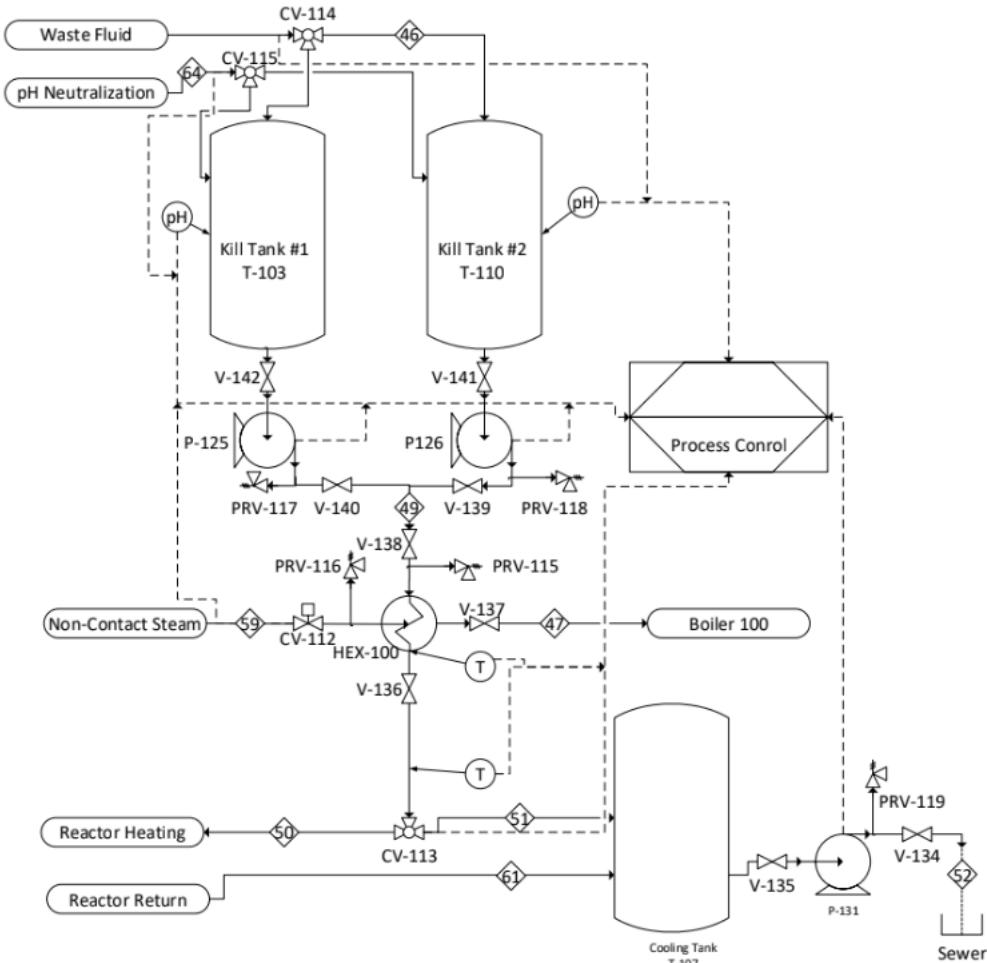


Figure 21: A low level PFD of the kill tank process. Waste from the process is fed into the kill tanks then neutralized. The waste is then pumped through a heat exchanger and either used to heat the reactors or directly pumped into the cooling tank.

T-103 and T-110 are the kill tanks in Figure 21. CV-114 is used to determine which tank to fill based on load cell feedback. CV-115 is used to control which tank requires pH neutralization based on feedback from the pH probes. The pH neutralization can either be an acetic acid buffer or the urea base depending on the pH. P-125 and P-126 are used to pump the waste water through the heat exchanger. They are controlled by a VFD and the load cells for T-110 and T-103. Block and bleed valves surround the pumps and heat exchanger. CV-112 is used to control the steam flow rate based on the feedback from the temperature probe. CV-113 controls whether the upstream processes need heating or if the heated fluid should be moved directly into the cooling tank. The fluid is then pumped into the sewer via P-131.

5.11 Cleaning Procedure

5.11.1 Design

CIP/SIP is used to sterilize the process equipment between batches. The CIP procedure for vessels will consist of six steps. The first step is to flow WFI through the equipment via spray nozzles and pumps for 10 – 15 minutes. The flow rate will be determined by vessel size. Next, a 0.5% chlorinated alkaline detergent at 60°C will be run for 20–30 minutes. Another WFI wash

will be performed for 10 - 15 minutes. An acid wash of pH 3 will then flow for 10 – 15 minutes. A final WFI wash will then be performed. The flow rates of the washes will be determined by the ratio recommended by Sanimatic of 3 gpm per foot of vessel circumference (*Appendix B Calculation 76*)²⁰. Steam at 150°C will be run through the equipment for 40 minutes during SIP. The equipment is assumed to take 10 minutes to reach the sterilization temperature of 121°C and is then sterilized for another 30 minutes. A flow rate of 0.3 [kg/s] of steam per vessel is assumed based on an optimal velocity for low pressure steam of 30 [m/s]²¹. During the steaming process, the steam will be condensed via a steam trap. The existing air in the equipment will be vented to the atmosphere by the steam trap air vent during steaming. Filtered air will be used to pressurize the vessel to counteract implosion risk from steam condensation. The acid, detergent, and water wash will be drained to the waste water treatment. This is the general procedure and will vary for different equipment²². The PFD in *Figure 22* is an overview of the CIP/SIP production:

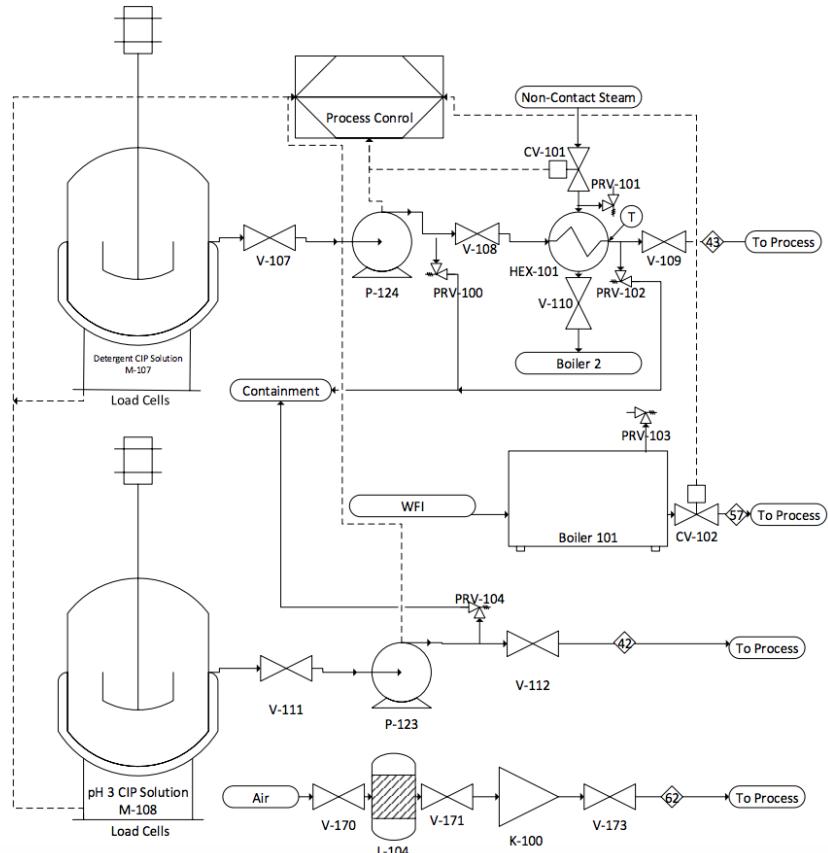


Figure 22: The overview of how CIP/SIP species are produced and distributed.

M-107 contains the detergent solution used in CIP. P-124 is used to pump the solution based on load cell and process feedback. The detergent solution is heated by HEX-101 via non-contact

²⁰ “CIP System Design Considerations for Cleaning Pharmaceutical Equip.” Sanimatic, 9 Aug. 2017, sanimatic.com/cip-system-design/.

²¹ Engineering ToolBox, (2003). *Recommended Velocities in Steam Systems*. [online] Available at: https://www.engineeringtoolbox.com/flow-velocity-steam-pipes-d_386.html [Accessed Day Mo. Year].

²² Susan Featherstone, 8 - Cleaning and sanitising, Editor(s): Susan Featherstone, In Woodhead Publishing Series in Food Science, Technology and Nutrition, A Complete Course in Canning and Related Processes (Fourteenth Edition), Woodhead Publishing, 2015, Pages 149-171, ISBN 9780857096777,

steam. CV-101 controls the steam flow via the temperature probe to ensure the detergent is 60°C. M-108 contains the acidic solution used for CIP. The acidic solution is pumped via P-123 through the process responding to load cell and process feedback. The boiler and the air used for vessel pressurization is shown. The air is filtered via L-104 to meet clean requirements and then compressed in K-100 to 3 bar. The 3 bar allows for proper pressurization from the 2 bar steam used in SIP. Boiler-101 uses WFI to produce the pure steam used for SIP. The steam flow is controlled via CV-102 from cleaning demands. The PRV are sent to containment in the kill tanks because they are working at higher pressure than the other systems and contain deadlier acids and bases.

5.11.2 Seed Train CIP/SIP

The first eight reactors of the seed train will be disposable. The first six seed reactors will be disposable flasks and the next two will be disposable bag reactors. The final three reactors will be permanent and need to be cleaned. The last three reactors take a total of eight days, allowing two days for cleaning before the next batch. The process discussed in the design will be used. The fluids will be flown at 19 gpm for R-102, 27 gpm for R-103, and 37 gpm for R-104 based off the recommendation for vessel circumference to flow rate by Sanimatic and reactor dimensions found in *Section 8²⁰*. The process will use a maximum of 3800 gallons of WFI, 2500 gallons of detergent solution, 1250 gallons of pH 3 buffer, and 2160 kg of steam.

5.11.3 Production Reactor CIP/SIP

The design has allotted three days to clean the production reactor. A similar flow rate for the seed train of 42 gpm will be used for the 5500 L production reactors. The 2.5 days provide more than enough time for cleaning to account for unforeseen issues. The process will use a maximum of 15000 gallons of WFI, 10000 gallons of detergent solution, 5000 gallons of pH 3 buffer, and 5800 kg of steam.

5.11.4 Centrifuge CIP/SIP

The holding tank for the product and centrifuge will be cleaned by the SIP/CIP system. The depth filter is single use and will be replaced instead of cleaned. The fluid flow rate will be 100 gpm based on the 40000 L holding tank. The process has 9.5 days to be cleaned, providing more than enough time. The process will use a maximum of 4500 gallons of WFI, 3000 gallons of detergent solution, 1500 gallons of pH 3 buffer, and 720 kg of steam.

5.11.5 Protein A Chromatography CIP/SIP

The resin column itself is cleaned during the elution and stripping process. The 40000 L holding tank before protein A will be cleaned in a similar method to the holding tank of the centrifuge. A flow rate of 100 gpm will be used with 7.9 days available for cleaning. The process will use a maximum of 4500 gallons of WFI, 3000 gallons of detergent solution 5 wt%, 1500 gallons of pH 3 buffer, and 720 kg of steam.

5.11.6 Viral Inactivation CIP/SIP

The viral filters will undergo integrity testing described in the viral inactivation to ensure they are still effective. The filter will be replaced instead of cleaned if it is deemed ineffective. The 4000 L incubation tank before the step will be cleaned at 45 gpm. The process will use a

maximum of 2000 gallons of WFI, 1400 gallons of detergent solution, 700 gallons of pH 3 buffer, and 720 kg of steam. The process has an allotted 7.9 days for cleaning.

5.11.7 Polishing CIP/SIP

The cleaning for the polishing step is similar to protein A. The ion exchange column cleans itself through the regenerative and wash cycles. The 4000 L holding tank will be cleaned by the same method as the viral inactivation storage tank with a flow rate of 45 gpm. The process will use a maximum of 2000 gallons of WFI, 1400 gallons of detergent solution, 700 gallons of pH 3 buffer, and 720 kg of steam. The process has an allotted 9.4 days for cleaning.

5.11.8 Storage CIP/SIP

The storage system will be cleaned with the SIP/CIP procedure. The tank is 9000 L and will be cleaned with a fluid flow rate of 62 gpm. The process will use a maximum of 2800 gallons of WFI, 1900 gallons of detergent solution, 930 gallons of pH 3 buffer, and 720 kg steam. The process has an allotted 6 days for cleaning.

5.11.9 Yearly SIP/CIP Requirements

Table 7: The below table shows the yearly amount of CIP/SIP material needed for the plants yearly 25 cycles based on flow rates and time discussed above

Cleaning Species	Yearly Amount
WFI	3287000 L
Chlorinated Alkaline Detergent 0.5 wt%	2204000 L
pH 3 Solution	1100000 L
Steam	289000 kg

5.12 Boilers

5.12.1 Overview

Boilers are used to generate steam for the process. Two types of steam will be utilized: pure steam for cleaning and non-contact steam for heating. The pure steam is used for the CIP/SIP and will be generated from WFI and the non-contact steam will be generated from city water.

5.12.2 SIP Boiler

The SIP boiler will produce steam (150°C , 2 bar) to clean the process. The steam will be generated at a total rate of 289000 [kg/yr]. The steam is used in a batch processes and does not need to be continuously flowing in the plant. The max demand for steam was assumed to be 3.5 [kg/s] based on simultaneous cleaning of the production reactors and two other processes. The energy requirement is $7.52 \cdot 10^8$ [kJ/yr] with a maximum heating requirement of 9109 kW (Section 7.12). The boiler is made of stainless steel to ensure the steam remains pure. A blow down of 5% will be used to ensure any contamination is removed from the boiler. A methane stream with four parts air is fed into the boiler to model natural gas to ensure complete combustion. The boiler was designed in HYSYS as an incinerator with a heat exchanger area of 92.5 m^2 (Appendix B Calculation 88).

5.12.3 Non-Contact Boiler

The non-contact boiler is used for the two process heat exchangers (HEX-100 and HEX-101) to heat the detergent solution for CIP and to heat the waste water to kill all the biomass. The max demand for this boiler is $2.68 \cdot 10^{10}$ [kJ/yr] based on the max CIP and waste water demand. The boiler does not come in direct contact with the process, so city water was used for the steam. The saturated steam will be produced at 100°C. The boiler was designed in HYSYS using methane as natural gas and a heat exchanger incinerator combination to model the boiler. The resulting area for the boiler is 17.6 m² (*Appendix B Calculation 90*).

5.13 WFI

5.13.1 Design

WFI is used in every process either directly or indirectly. WFI is available to purchase from a third party or can be produced on site. In this design, WFI is purchased from an outside party. The high-level WFI system is shown below in *Figure 23*:

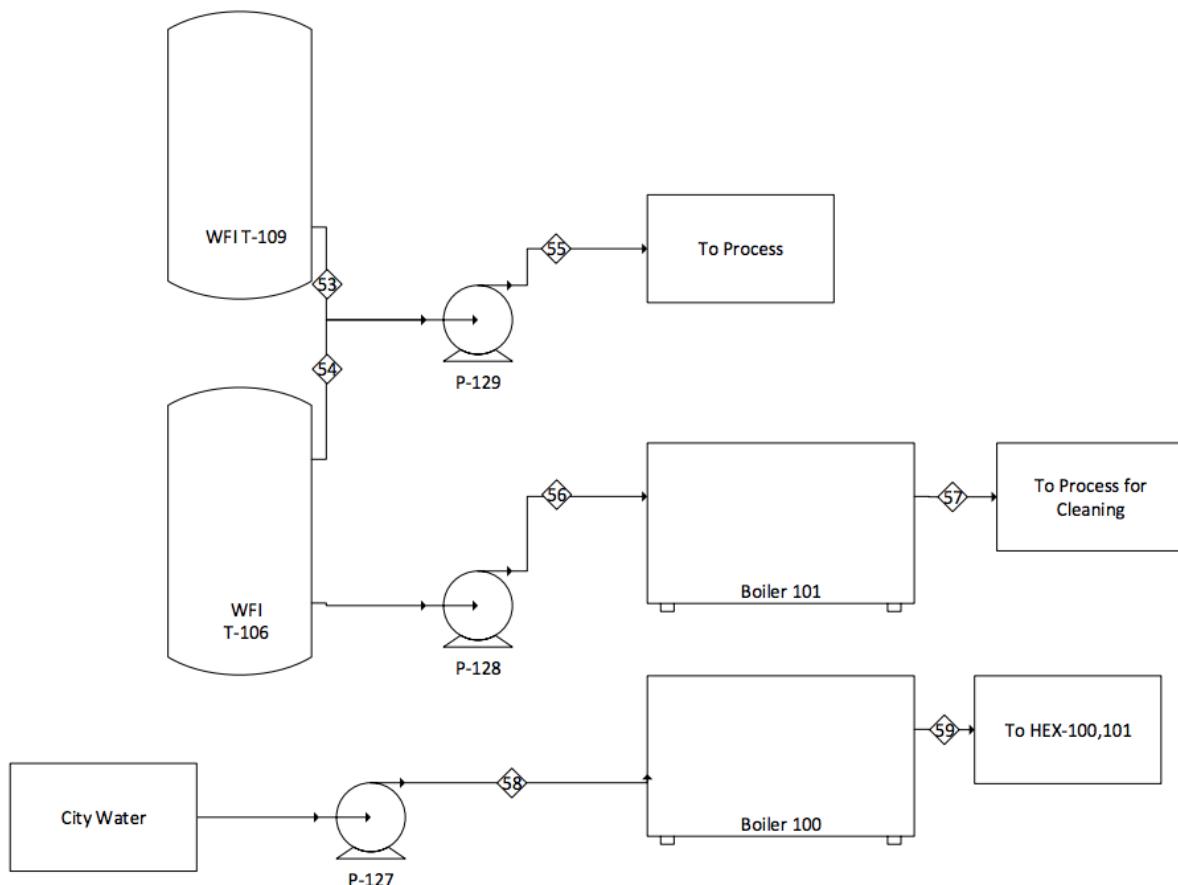


Figure 23: The high-level view of the WFI system along with Boiler 100 & 101.

T-109 and T-106 are 250000 L tanks used to hold the WFI for the process. P-129 is used to pump the WFI to different parts of the process such as CIP. P-128 is used to pump the water into

Boiler 101 for pure steam production. P-127 shows how the city water is used for Boiler 100 for non-contact steam.

6 Material Balances

The process for producing MAbs is carried out in batch systems which are time dependent. The processes have simple input and output mass balances. The bio reactions follow the Monod kinetics discussed in (*Section 5.2*), which require solving ODEs. All the mass balances were performed in MATLAB. This section shows summaries of the mass balances with references to hand calculations and MATLAB files.

6.1 Seed Train Material Balance

The inlets for the seed train include the contents from the previous reactor and materials already charged to the reactor such as substrate. The stream number is not denoted for inlet because of this.

6.1.1 Reactor 1 (SF-100)

Table 8: The below table shows the mass balance summary for reactor 1 (SF-100). The values were determined in MATLAB using Monod kinetics with an optimized batch time of 7.8 days.

Species	In	Out (Stream 1)
Substrate	$2.75 \cdot 10^{-2}$ g	$2.54 \cdot 10^{-2}$ g
Cells	10^6 cells	$3.04 \cdot 10^7$ cells
MAb	0 g	$1.7 \cdot 10^{-3}$ g
WFI	5 g	5 g

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. *Figure 7* shows the inlets and outlets.

6.1.2 Reactor 2 (SF-101)

Table 9: The below table shows the mass balance summary for reactor 2 (SF-101). The values were determined in MATLAB using Monod kinetics with an optimized batch time of 3.05 days.

Species	In	Out (Stream 2)
Substrate	0.11 g	0.10
Cells	$3.04 \cdot 10^7$ cells	$1.16 \cdot 10^8$ cells
MAb	0.0017 g	0.0064 g
WFI	20 g	20 g

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. *Figure 7* shows the inlets and outlets.

6.1.3 Reactor 3 (SF-102)

Table 10: The below table shows the mass balance summary for reactor 3 (SF-102). The values were determined in MATLAB using Monod kinetics with an optimized batch time of 2.5 days.

Species	In	Out (Stream 3)
Substrate	0.3 g	0.27 g
Cells	$1.16 \cdot 10^8$ cells	$3.42 \cdot 10^8$ cells
MAb	0.0064 g	0.019 g
WFI	55 g	55 g

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. *Figure 7* shows the inlets and outlets.

6.1.4 Reactor 4 (SF-103)

Table 11: The below table shows the mass balance summary for reactor 4 (SF-103). The values were determined in MATLAB using Monod kinetics with an optimized batch time of 1.5 days.

Species	In	Out (Stream 4)
Substrate	0.58 g	0.53 g
Cells	$3.42 \cdot 10^8$ cells	$6.5 \cdot 10^8$ cells
MAb	0.019 g	0.037 g
WFI	105 g	105 g

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. *Figure 7* shows the inlets and outlets.

6.1.5 Reactor 5 (RR-100)

Table 12: The below table shows the mass balance summary for reactor 5 (RR-100). The values were determined in MATLAB using Monod kinetics with an optimized batch time of 5.55 days.

Species	In	Out (Stream 5)
Substrate	6.08 g	5.54 g
Cells	$6.5 \cdot 10^8$ cells	$7.5 \cdot 10^9$ cells
MAb	0.037 g	0.425 g
WFI	1105 g	1105 g

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. *Figure 7* shows the inlets and outlets.

6.1.6 Reactor 6 (RR-101)

Table 13: The below table shows the mass balance summary for reactor 6 (RR-100). The values were determined in MATLAB using Monod kinetics with an optimized batch time of 3.7 days.

Species	In	Out (Stream 6)
Substrate	33.6 g	30.9 g
Cells	$7.5 \cdot 10^9$ cells	$3.8 \cdot 10^{10}$ cells
MAb	0.425 g	2.15 g
WFI	6105 g	6105 g

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. *Figure 7* shows the inlets and outlets.

6.1.7 Reactor 7 (RR-102)

Table 14: The below table shows the mass balance summary for reactor 7 (RR-100). The values were determined in MATLAB using Monod kinetics with an optimized batch time of 3.1 days.

Species	In	Out (Stream 7)
Substrate	144 g	133 g
Cells	$3.8 \cdot 10^{10}$ cells	$1.46 \cdot 10^{11}$ cells
MAb	2.15 g	8.3 g
WFI	26.1 kg	26.1 kg

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. *Figure 7* shows the inlets and outlets.

6.1.8 Reactor 8 (R-101)

Table 15: The below table shows the mass balance summary for reactor 8 (R-101). The values were determined in MATLAB using Monod kinetics with an optimized batch time of 3.7 days.

Species	In	Out (Stream 8)
Substrate	584 g	531 g
Cells	$1.46 \cdot 10^{11}$ cells	$7.4 \cdot 10^{11}$ cells
MAb	8.3 g	42 g
WFI	106 kg	106 kg

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. *Figure 7* shows the inlets and outlets.

6.1.9 Reactor 9 (R-102)

Table 16: The below table shows the mass balance summary for reactor 9 (R-102). The values were determined in MATLAB using Monod kinetics with an optimized batch time of 2.5 days.

Species	In	Out (Stream 9)
Substrate	2233 g	2077 g
Cells	$7.4 \cdot 10^{11}$ cells	$2.18 \cdot 10^{12}$ cells
MAb	42 g	124 g
WFI	406 kg	406 kg

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. *Figure 7* shows the inlets and outlets.

6.1.10 Reactor 10 (R-103)

Table 17: The below table shows the mass balance summary for reactor 10 (R-103). The values were determined in MATLAB using Monod kinetics with an optimized batch time of 3.1 days.

Species	In	Out (Stream 10)
Substrate	7733 g	7129 g
Cells	$2.18 \cdot 10^{12}$ cells	$8.45 \cdot 10^{12}$ cells
MAb	124 g	481 g
WFI	1406 kg	1406 kg

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. *Figure 7* shows the inlets and outlets.

6.1.11 Reactor 11 (R-104)

Table 18: The below table shows the mass balance summary for reactor 11 (R-104). The values were determined in MATLAB using Monod kinetics with an optimized batch time of 2.0 days.

Species	In	Out (Stream 11)
Substrate	19.3 kg	17.8 kg
Cells	$8.45 \cdot 10^{12}$ cells	$2.0 \cdot 10^{13}$ cells
MAb	481 g	1140 g
WFI	3506 kg	3506 kg

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. *Figure 7* shows the inlets and outlets.

6.2 Production Reactors

The mass balances for the production reactors were performed in MATLAB (*Appendix C*) under fed batch conditions described in (*Section 5.3*). The fed batch consisted of a media solution of 2.9 (g/L) with a flow rate of 25 (L/hr).

Table 19: Summary of the material balance for all seven production reactors (R -105-111) over the 6.7 day reactor time. Figure 14 shows the inlet and outlet streams.

Species	In (Stream 11 & 20-27)	Out (Stream 19)
Substrate	99 kg	73.5 kg
Cells	$2.0 \cdot 10^{13}$ cells	$2.45 \cdot 10^{14}$ cells
MAb	1.14 kg	49 kg
WFI	35000 kg	35000 kg

The mass balances for substrate, cells, and MAb were performed in MATLAB using an ODE solver (*Appendix C*). The WFI was not consumed in the process. Sample calculations for outlet masses can be found in (*Appendix B Calculations 28-32*).

6.3 Centrifugation Material Balance

The mass balances for the centrifugation is simple as it was assumed that 100% of the solids (cells) were removed in a waste stream. The process is continuous with a 12 hour time period. *Table 20* below summarizes the inlet and outlets surrounding the centrifuge unit operation:

Table 20: A material balance summary of the centrifuge unit operation. The waste out is the mas sent to waste disposal, while product out is sent to downstream production. Figure 15 shows the inlet and outlet streams.

Species	In (Stream 19)	Waste Out (Stream 63)	Production Out (Stream 30)
Substrate	6.13 kg/hr	0	6.13 kg/hr
Cells	$2.04 \cdot 10^{13}$ cells/hr	$2.04 \cdot 10^{13}$ cells/hr	0
MAb	4 kg/hr	0	4 kg/hr
WFI	3000 kg/hr	0	3000 kg/hr

6.4 Protein A Material Balance

Protein A requires cycles, so the mass balance summarized in the *Table 21* is the mass in and out for the eight cycles to process one batch over 50 hours. Two outlets exist: one to downstream production and one to a set of kill tanks. 10% of the proteins will be lost in this process as a conservative value. Buffers of different pH are used in the cycle, yet *Table 21* shows the total mass of acid and bases used for all buffers (*Appendix B Calculations 42-45*).

Table 21: A material balance summary of the protein A unit operation. The waste out is the mass sent to wastewater treatment, while product out is the mass sent to downstream production. Figure 17 shows the inlet and outlet streams.

Species	In (Stream 30 & 39-41)	Waste Out (Stream 46)	Production Out (Stream 32)
Substrate	73.5 kg	73.5 kg	0
Urea	13500 kg	13500 kg	0
MAb	49 kg	4.9 kg	44.1 kg
WFI	166120 kg	163320 kg	2800 kg
Acetic Acid	358 kg	343.8 kg	14.3 kg
Sodium Acetate	437 kg	433.5 kg	3.5 kg

6.5 Viral Inactivation Material Balance

Viral inactivation has no perceptible change in mass from the product of protein A. The only change in mass is the loss of viruses, which is unknown and extremely small.

6.6 Polishing Balance

Similar to protein A, polishing is a cyclical process. The process is estimated to take 13.64 hours per batch and the material balance will reflect one entire batch. The Tris is ignored in the material balance because the amount for titration is unknown and it does not affect the outcome of the exchange column. It is assumed approximately 5% of the product is lost during this step. *Table 22* below summarizes the material balance for polishing:

Table 22: A material balance summary of the polishing unit operation. The waste out is the mass sent to waste water treatment, while product out is the mass sent to downstream production. Figure 19 shows the inlet and outlet streams.

Species	In (Stream 35, 44, & 60)	Waste Out (Stream 46)	Production Out (Stream 37)
MAb	44.1 kg	4.1kg	40 kg
WFI	2800 kg	0	2800 kg
Acetic Acid	14.3 kg	14.3 kg	0
Sodium Acetate	3 kg	3 kg	0

Polishing is the final unit operation of consequence to the MAb. This produces 1000 kg of MAb for 25 batches.

7 Energy Balance and Utility Requirement

7.1 Seed Train Rocking Reactors Energy Balance and Utility Requirement

The energy for the seed train rocking reactors comes from heating the fluid and maintaining a 33°C operating temperature. It is assumed the bulk of the culture is at room temperature of 25°C. The heat capacity and insulation are unknown, so the culture mixture is assumed to have the same properties as water with no heat loss to surroundings. These assumptions are most likely invalid, but they are used for a utility cost estimate. The actual temperature will be regulated through a feedback control loop via a hot plate. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 3-27*)

7.1.1 Reactor 5 (RR-100) Energy Balance and Utility Requirement

Table 23: The yearly utility/energy requirements for reactor 5 (RR-100). The energy needed to heat and mix the reactor are listed as well as the WFI required for making the solution inside the reactor.

Yearly Utility/Energy Requirement	Value
Heating Requirement	923.8 kJ/yr
Mixing Electricity (Rocker)	$3.55 \cdot 10^7$ kJ/yr
WFI Requirement	25L/yr

7.1.2 Reactor 6 (RR-101) Energy Balance and Utility Requirement

Table 24: The yearly utility/energy requirements for reactor 6 (RR-101). The energy needed to heat and mix the reactor are listed as well as the WFI required for making the solution inside the reactor.

Yearly Utility/Energy Requirement	Value
Heating Requirement	5104 kJ/yr
Mixing Electricity (Rocker)	$3.55 \cdot 10^7$ kJ/yr
WFI Requirement	125 L/yr

7.1.3 Reactor 7 (RR-102) Energy Balance and Utility Requirement

Table 25: The yearly utility/energy requirements for reactor 7 (RR-102). The energy needed to heat and mix the reactor are listed as well as the WFI required for making the solution inside the reactor.

Yearly Utility/Energy Requirement	Value
Heating Requirement	21823 kJ/yr
Mixing Electricity (Rocker)	$3.55 \cdot 10^7$ kJ/yr
WFI Requirement	625 L/yr

7.2 Seed Train Bag Reactor

The energy for the seed train bag reactor comes from heating the fluid and maintaining a 33°C operating temperature. It is assumed the bulk of the culture is at room temperature of 25°C. The heat capacity and insulation are unknown, so the culture mixture is assumed to have the same properties as water with no heat loss to surroundings. These assumptions are most likely invalid, but they are used for a utility cost estimate. The actual temperature will be regulated through a feedback control loop via a heat exchanger unit in the reactor.

7.2.1 Reactor 8 (R-101) Energy Balance and Utility Requirement

Table 26: The yearly utility/energy requirements for reactor 8 (R-101). The energy needed to heat and mix the reactor are listed as well as the WFI required for making the solution inside the reactor.

Yearly Utility/Energy Requirement	Value
Heating Requirement	88704 kJ/yr
Mixing Electricity (Agitator)	113 kJ/yr
WFI Requirement	2000 L/yr
Pumping Electricity	11.23 kJ/yr

7.3 Permanent Seed Train Reactors Energy Balance and Utility Requirement

The energy for the permanent seed train reactors comes from heating the fluid and maintaining a 33°C operating temperature. It is assumed the bulk of the culture is at room temperature of 25°C. The heat capacity and insulation are unknown, so the culture mixture is assumed to have the same properties as water with no heat loss to surroundings. These assumptions are most likely invalid, but they are used for a utility cost estimate. The actual temperature will be regulated

through a feedback control loop via heating coils in the reactors. The heating fluid will be 80°C fluid from the kill tank. This is to reduce energy demand but may cause build up in the heating coils.

7.3.1 Reactor 9 (R-102) Energy Balance and Utility Requirement

Table 27: The yearly utility/energy requirements for reactor 9 (R-102). The energy needed to heat and mix the reactor are listed as well as the WFI required for making the solution inside the reactor.

Yearly Utility/Energy Requirement	Value
Heating Requirement	339504 kJ/yr
Mixing Electricity (Agitator)	350 kJ/yr
WFI Requirement	7500 L/yr
Pumping Electricity	61.9 kJ/yr

7.3.2 Reactor 10 (R-103) Energy Balance and Utility Requirement

Table 28: The yearly utility/energy requirement for reactor 10 (R-103). The energy needed to heat and mix the reactor are listed as well as the WFI required for making the solution inside the reactor.

Yearly Utility/Energy Requirement	Value
Heating Requirement	1175503 kJ/yr
Mixing Electricity (Agitator)	2725 kJ/yr
WFI Requirement	25000 L/yr
Pumping Electricity	342 kJ/yr

7.3.3 Reactor 11 (R-104) Energy Balance and Utility Requirement

Table 29: The yearly utility/energy requirements for reactor 11 (R-104). The energy needed to heat and mix the reactor are listed as well as the WFI required for making the solution inside the reactor.

Yearly Utility/Energy Requirement	Value
Heating Requirement	2931104 kJ/yr
Mixing Electricity (Agitator)	9207 kJ/yr
WFI Requirement	77500 L/yr
Pumping Electricity	1650 kJ/yr

7.4 Production Reactors Energy Balance and Utility Requirement

The energy for the production reactors comes from heating the fluid and maintaining a 33°C operating temperature. It is assumed the bulk of the culture is at room temperature of 25°C. The heat capacity and insulation are unknown, so the culture mixture is assumed to have the same properties as water with no heat loss to surroundings. The energy will also be based on the final feed volume. These assumptions are most likely invalid, but they are used for a utility cost estimate. The actual temperature will be regulated through a feedback control loop via heating coils in the reactors. The heating fluid will be non-contact steam. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 33-38*).

Table 30: The yearly utility/energy requirements for the seven production reactors. The energy needed to heat and mix the reactors are listed as well as the WFI required for making the solution inside the reactor.

Yearly Utility/Energy Requirement	Value
Heating Requirement	$2.926 \cdot 10^7$ kJ/yr
Mixing Electricity (Agitator)	$8.75 \cdot 10^6$ kJ/yr
WFI Requirement	787500 L/yr
Pumping Electricity	101168 kJ/yr

7.5 Centrifuge Energy Balance and Utility Requirement

The energy for the centrifuge comes from running the equipment and pumping the fluid. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 40-41*).

Table 31: The yearly utility/energy requirements for the centrifuge. The energy needed to run the centrifuge and pump the fluid is shown in the table.

Yearly Utility/Energy Requirement	Value
Centrifuge power	$5.4 \cdot 10^7$ kJ/yr
Pumping Electricity	101168 kJ/yr

7.6 Protein A Energy Balance and Utility Requirement

The energy for protein A comes from pumping different solutions through the column. The pressure drop along the column is deemed negligible when compared to the pressures required to pump fluids to the next sequential holding tank. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 46-50*).

Table 32: The yearly utility/energy requirements for Protein A chromatography. The energy needed to run the various pumps is shown.

Yearly Utility/Energy Requirement	Value
Pumping Electricity	324276 [kJ/yr]

7.7 Viral Inactivation Energy Balance and Utility Requirement

The energy requirements for viral inactivation is pumping the fluid through the unit operation and mixing the fluid in the holding tank. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 52-53*).

Table 33: The yearly utility/energy requirements for viral inactivation chromatography. The energy needed to run the pump and agitator.

Yearly Utility/Energy Requirement	Value
Pumping Electricity	3453 [kJ/yr]
Mixing Electricity (Agitator)	14291 [kJ/yr]

7.8 Polishing Energy Balance and Utility Requirement

The energy for polishing comes from pumping different solutions through the column. The pressure drop along the column is deemed negligible when compared to the pressures required to

pump fluids to the next sequential holding tank. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 54-57*).

Table 34: The yearly utility/energy requirements for viral inactivation chromatography. The energy needed to run the pump and agitator.

Yearly Utility/Energy Requirement	Value
Pumping Electricity	8382.2 [kJ/yr]
Mixing Electricity (Agitator)	14291 [kJ/yr]

7.9 Storage Energy Balance and Utility Requirement

The energy required for storage is due to the massive cooling requirements, mixing the storage and glycerol, and pumping the fluid. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 65-71*).

Table 35: The yearly utility/energy requirements for storage. The energy needed to run the pumps, agitators and cooling processes.

Yearly Utility/Energy Requirement	Value
Pumping Electricity	10040 kJ/yr
Mixing Electricity (Agitator)	$4.79 \cdot 10^8$ kJ/yr
Compressor	$2.83 \cdot 10^9$ kJ/yr

7.10 Kill Tanks Energy Balance and Utility Requirement

The energy for waste water comes from heating the waste water to 80°C and pumping the water through the process for heating. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 74-75*).

Table 36: The yearly utility/energy requirements for the waste water treatment. The energy needed to pump and heat the waste fluid is listed.

Yearly Utility/Energy Requirement	Value
Heating Requirement	$2.12 \cdot 10^9$ kJ/yr
Mixing Electricity (Agitator)	$2.6 \cdot 10^9$ kJ/yr
City Water Requirement	$9.4 \cdot 10^5$ kg/yr
Pumping Electricity	$8.16 \cdot 10^6$ kJ/yr

7.11 CIP Energy Balance and Utility Requirement

The energy requirement for the CIP system is in heating the detergent solution and pumping the cleaning fluid through the process. The detergent solution is assumed to have the properties of water, start at 25°C and end at 60°C. The pumps are sized to clean the tallest tower (T-100) for a conservative value across all vessels. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 77-81*).

Table 37: The yearly utility/ energy requirements for the CIP process. The energy needed to pump and heat the CIP fluid is listed. The compressor energy used for vessel pressurization is also listed

Yearly Utility/Energy Requirement	Value
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Heating Requirement	$3.22 \cdot 10^8$ kJ/yr
Mixing Electricity (Agitator)	$2.6 \cdot 10^9$ kJ/yr
City Water Requirement	142569 kg/yr
Pumping Electricity	$1.65 \cdot 10^7$ kJ/yr
WFI Requirements	$3.287 \cdot 10^6$ L/yr
Compressor (K-100)	$9.97 \cdot 10^7$ kJ/yr

7.12 SIP (Boiler 101) Energy Balance and Utility Requirement

WFI water at room temperature (25°C) will be heated to saturated steam at 121°C . The boiler must produce 289000 kg steam/ yr at a maximum rate of 3.5 kg/s based on reasons discussed in *section 5.11*. The system will be closed loop so the WFI will be reused except for the blow down and initial start-up. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 83-87*).

Table 38: The yearly utility/ energy requirements for the SIP process. The energy needed to pump and boil the steam.

Yearly Utility/Energy Requirement	Value
Heating Requirement	$7.52 \cdot 10^8$ kJ/yr
Maximum Energy Requirement	9109 kW
Steam Requirement	142569 kg/yr
Pumping Electricity	$9.1 \cdot 10^6$ kJ/yr
WFI Requirements	26010 kg/yr
Natural Gas Requirement	225351 kg/yr

7.13 Heat (Boiler 100) Energy Balance and Utility Requirement

The energy required to heat the processes that require heat exchangers is acquired by boiling city water in a natural gas boiler. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 89,91*).

Table 39: The yearly utility/ energy requirements for the heat boiler process. The energy needed to pump and boil the steam.

Yearly Utility/Energy Requirement	Value
Maximum Heating Requirement	$2.68 \cdot 10^{10}$ kJ/yr
Pumping Electricity	$9.85 \cdot 10^7$ kJ/yr
City Water Requirements	26010 kg/yr
Natural Gas Requirement	49222 kg/yr

7.14 Media Preparation Energy Balance and Utility Requirement

The energy and utilities for media prep include mixing and the need for WFI. The WFI is considered in the production reactor section (*Section 7.4*). The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 92*).

Table 40: The yearly utility/ energy requirements for media preparation. The energy needed is to mix the media preparation vessel.

Yearly Utility/Energy Requirement	Value
Mixing Electricity (Agitator)	$1.15 \cdot 10^8 \text{ kJ/yr}$

7.15 Buffer Preparation Energy Balance and Utility Requirement

The energy and utilities for buffer preparation include mixing and the need for WFI. The WFI is considered in the various sections where buffer is used. The energies and utilities are listed in table format, sample calculations can be found in (*Appendix B Calculations 93-98*).

Table 41: The yearly utility/ energy requirements for buffer preparation. The energy needed is to mix the various buffers.

Yearly Utility/Energy Requirement	Value
Mixing Electricity (Agitator)	$1.0 \cdot 10^9 \text{ kJ/yr}$

8 Equipment Specification Sheets

Table 42: List of equipment refers to figures in unit operations in Section 5 and Unit Site Plan in Section 12.3.

<i>Media Preparation</i>							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]
ST	N	MBP	M-100	36750 L	3.15	4.75	1.15E+08
<i>Buffer Preparation</i>							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]
ST - pH4 (AA)	N	MBP	M-103	47500 L	3.43	5.14	1.76E+08
ST - pH5 (AA)	N	MBP	M-102	58500 L	3.68	5.51	2.49E+08
ST - pH8 (Urea)	N	MBP	M-104	30000 L	2.94	4.41	8.18E+07
ST - pH8 (Tris)	N	MBP	M-105	2500 L	1.29	1.93	1.30E+06
pH 3 Tank	N	MBP	M-108	45000L	3.37	5.05	1.61E+08
Detergent Tank	N	MBP	M-107	90000L	4.24	6.36	3.40E+08
NaCl Tank	Y	MBP	M-106	-	-	-	-
<i>Seed Train</i>							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]
Shake Flask	Y	LAB	SF-100(D)	0.01 L	Standard	Standard	-
Shake Flask	Y	LAB	SF-101(D)	0.05 L	Standard	Standard	-
Roller Flask	Y	LAB	SF-102(D)	0.1 L	Standard	Standard	-
Roller Flask	Y	LAB	SF-103(D)	0.15 L	Standard	Standard	-
Heat Bath	N	LAB	-	30 L	Standard	Standard	8.28E+06
Bag Rocker	Y	LAB	RR-100(D)	1.5 L	Standard	Standard	3.55E+07

Bag Rocker	Y	LAB	RR-101(D)	10 L	Standard	Standard	3.55E+07
Bag Rocker	Y	LAB	RR-102(D)	30 L	0.55	0.83	3.55E+07
Bag Reactor	Y	SEED	R - 101(P)	200 L	0.44	1.32	113
Bioreactor	N	SEED	R - 102(P)	500 L	0.60	1.79	349
Bioreactor	N	SEED	R - 103(P)	1500 L	0.86	2.58	2726
Bioreactor	N	SEED	R - 104(P)	4000 L	1.20	3.60	9207
Pump #1- Peristaltic	N	SEED	P-101	-	-	-	11
Pump #2- Peristaltic	N	SEED	P-102	-	-	-	62
Pump #3- Peristaltic	N	SEED	P-103	-	-	-	342
Pump #4- Peristaltic	N	SEED	P-104	-	-	-	1650
Production							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]
Bioreactor	N	PROD	R-105(P)	5500 L	1.33	4.00	1.25E+06
Bioreactor	N	PROD	R-106(P)	5500 L	1.33	4.00	1.25E+06
Bioreactor	N	PROD	R-107(P)	5500 L	1.33	4.00	1.25E+06
Bioreactor	N	PROD	R-108(P)	5500 L	1.33	4.00	1.25E+06
Bioreactor	N	PROD	R-109(P)	5500 L	1.33	4.00	1.25E+06
Bioreactor	N	PROD	R-110(P)	5500 L	1.33	4.00	1.25E+06
Bioreactor	N	PROD	R-111(P)	5500 L	1.33	4.00	1.25E+06
Pump #1- Peristaltic	N	PROD	P-105	-	-	-	4572
Pump #2- Centrifugal	N	PROD	P-106	-	-	-	6520
Pump #3 - Centrifugal	N	PROD	P-107	-	-	-	6520
Pump #4- Centrifugal	N	PROD	P-108	-	-	-	6520
Pump #5- Centrifugal	N	PROD	P-109	-	-	-	6520
Pump #6- Centrifugal	N	PROD	P-110	-	-	-	6520
Pump #7- Centrifugal	N	PROD	P-111	-	-	-	6520
Pump #8- Centrifugal	N	PROD	P-112	-	-	-	6520
Pump #9- Centrifugal	N	PROD	P-113	-	-	-	55341
Valve #1	N	PROD	V-100	-	-	-	-
Valve #2	N	PROD	V-101	-	-	-	-
Valve #3	N	PROD	V-102	-	-	-	-
Valve #4	N	PROD	V-103	-	-	-	-
Valve #5	N	PROD	V-104	-	-	-	-
Valve #6	N	PROD	V-105	-	-	-	-
Valve #7	N	PROD	V-106	-	-	-	-
Filtration							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]

Storage Tank	N	CDT	T-100	40,000 L	3.25	4.85	-
Centrifuge	N	CDT	Cn-100	0.71 m ²	0.72	-	50 KWATTS
Depth Filter	Y	CDT	L-100	-	-	-	-
Pump #1- Centrifugal	N	CDT	P-114	-	-	-	67007
Protein A							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]
Protein A Column	N	PTA	D-100	(445 L resin)	1.56	0.89	-
Pump #1- Centrifugal	N	PTA	P-115	-	-	-	10430
Pump #2- Centrifugal	N	PTA	P-116	-	-	-	1.89E+05
Pump #3- Centrifugal	N	PTA	P-117	-	-	-	1.57E+05
Pump #4- Centrifugal	N	PTA	P-118	-	-	-	4.29784E+04
Storage Tank	N	CDT	T-101	40,000 L	3.25	4.85	-
Viral Inactivation							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]
Incubation Vessel	N	PTA	T-104	4000 L	1.50	2.25	14292
Pump #1- Centrifugal	N	VIAP	P-119	-	-	-	3454
Planova 75N Filter	N	VIAP	L-101	-	-	-	-
Planova 20N Filter	N	VIAP	L-102	-	-	-	-
Polishing							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]
Titration Tank	N	VIAP	T-105	4000 L	1.50	2.25	14292
Pump #1- Centrifugal	N	VIAP	P-120	-	-	-	2054
Pump #2- Centrifugal	N	VIAP	P-121	-	-	-	9393
Anion Column	N	VIAP	D-101	-	0.28	2.03	-
Packaging							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]
Storage Tank	N	PKG	T-102	8500 L	1.93	4.24	9.99E+06
Glycerol Tank	N	PKG	T-108	20000 L	5.54	8.31	4.69E+08
Pump #1 - Centrifugal	N	PKG	P-122	-	-	-	2.22E+03
Pump #2 - Centrifugal	N	PKG	P-130	-	-	-	7.25E+03
Drums	Y	STOR	DRMS	40x 200L	0.57	0.85	-
Compressor	N	STOR	K-101	-	-	-	2.12E+09
Heat Exchanger #1	N	STOR	HEX-102	13.80	13.8 m ²	-	-
Heat Exchanger #2	N	STOR	HEX-103	64.80	64.8m ²	-	-
CIP							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]

Pump #1-Centrifugal	N	HEAT	P-123	-	-	-	4.62E+06
Pump #2-Centrifugal	N	HEAT	P-124	-	-	-	1.19E+07
Heat Exchanger #1	N	HEAT	HEX - 101	-	-	-	-
Kill Tank							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]
Kill Tank #1	N	KILL	T-103	250000 L	5.56	10.29	1.32E+09
Kill Tank #2	N	KILL	T-110	250000 L	5.56	10.29	1.32E+09
Pump #1-Centrifugal	N	KILL	P-125	-	-	-	7.96E+06
Pump #2-Centrifugal	N	KILL	P-126	-	-	-	7.96E+06
Heat Exchanger #1	N	KILL	HEX-100	-	-	-	-
Cooling Tank	N	KILL	T-107	450000 L	6.96	10.44	-
Pump #3-Centrifugal	N	KILL	P-131	-	-	-	1.99E+05
Heat							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]
Pump #1-Centrifugal	N	HEAT	P-127	-	-	-	9.85E+07
Boiler	N	HEAT	B-100	-	-	-	2.08E+09
WFI							
Unit	Disposable	Location	Number	Size	D [m]	H [m]	Energy [kJ/yr]
WFI Tank #1	N	WFI	T-106	250000 L	5.56	10.29	-
WFI Tank #2	N	WFI	T-109	250000 L	5.56	10.29	-
Pump #1-Centrifugal	N	WFI	P-128	-	-	-	8.28E+06
Pump #2-Centrifugal	N	WFI	P-129	-	-	-	8.24E+05
Boiler	N	WFI	B-101	-	-	-	1.88E+11
Air Compressor	N	WFI	K-100	-	-	-	9.98E+07
Filter	Y	WFI	L-104	-	-	-	-

9 Equipment Cost Summary

Only major pieces of equipment were priced. Two methods were used to price the equipment. The first method used cost curves from the *Tolwer and Sinnott: Chemical Engineering Design* Equation 7 was used in combination with the contents of Appendix D to find the equipment dependent constants a, b, and n²³. The characteristic size (S) was determined for each major

²³ Towler, Gavin Sinnott, Ray K.. (2013). *Chemical Engineering Design - Principles, Practice and Economics of Plant and Process Design* (2nd Edition). Elsevier. Retrieved from <https://app.knovel.com/hotlink/toc/id:kpCEDPPEP4/chemical-engineering/chemical-engineering>

equipment. This method did not have the exact types of equipment and so comparisons were made with similar equipment that could be priced when necessary.

$$Cost = a + b * S^n \quad [7]$$

The second method scaled the prices from *Biopharmaceutical Process Optimization with Simulation and Scheduling Tools* which priced similar equipment in a MAb production facility simulated on SuperPro Designer²⁴. The scaling method produced both more conservative and more accurate prices for process equipment. These prices are predominately used in equipment costing unless no comparison could be made in which case cost curves were used. Table 43 summarizes the major equipment cost for the MAb production facility.

Table 43: Major equipment cost summary.

Quantity	Name	Description	Unit Cost	Total Cost
1	M-100	Stirred Tank Volume = 36750 L	472737	472737
1	M-103	Stirred Tank Volume = 47500 L	551419	551419
1	M-102	Stirred Tank Volume = 58500 L	624825	624825
1	M-104	Stirred Tank Volume = 30000 L	418541	418541
1	M-105	Stirred Tank Volume = 2500 L	94239	94239
1	M-108	Stirred Tank Volume = 45000 L	533817	533817
1	M-107	Stirred Tank Volume = 90000 L	809116	809116
25	RR-100(D)	Bag Rocker Volume = 1.5 L	30931	773275
25	RR-101(D)	Bag Rocker Volume = 10 L	96547	2413677
25	RR-102(D)	Bag Rocker Volume = 30 L	186643	4666077
1	R - 101(P)	Seed Bioreactor Volume = 200 L	435741	435741
1	R - 102(P)	Seed Bioreactor Volume = 500 L	755080	755080
1	R - 103(P)	Seed Bioreactor Volume = 1500 L	1459707	1459707
1	R - 104(P)	Seed Bioreactor Volume = 4000 L	1287429	1287429
7	R-105 to 111(P)	Production Bioreactor Volume = 5500 L	984308	6890155
2	T-100/101	Storage Tank Volume = 40000 L	30514	61028
1	Cn -100	Centrifuge Throughput = 2600 L/hr	583600	583600
1	T-104	Storage Tank Volume = 4000 L	19240	19240
4	L-101	Planova 75N Filter	18108	72431
4	L-102	Planova 20N Filter	18108	72431
1	D-101	Ion Exchange Column Volume = 120 L	287827	287827
1	T-102	Storage Tank Volume = 8500 L	14664	14664
1	T-108	Storage Tank Volume = 20000 L	21307	21307
1	HEX - 101	Heat Exchanger Area = 142 m ²	55071	55071

²⁴ Demetri Petrides, Doug Carmichael, Charles Siletti, and Alexandros Koulouris. "Biopharmaceutical Process Optimization with Simulation and Scheduling Tools." *Bioengineering* 1.4 (2014): 154-87. Web.

4	T-103,110,106,109	Storage Tank Volume = 250000 L	92945.716	371783
1	T-107	Storage Tank Volume = 500000 L	146891	146891
1	B-100	Boiler Throughput = 150 kg/hr	3097	3097
1	B-101	Boiler Throughput = 12600 kg/hr	276143	276143
1	K-101	Compressor Power=67.22 kW	939082.023	939082
1	HEX-102	Heat Exchanger Area =13.8 m ²	33114.0791	33114
1	HEX-103	Heat Exchanger Area= 64.8 m ²	40809.1802	40809
		Pumps (<i>Appendix D</i>)	196224	196224
			Total Equipment Cost	25380574

10 Fixed Capital Investment Summary

The fixed capital investment was estimated using the equipment cost and the Lang Factor. The Lang Factor is a metric for determining the extra costs associated with facility equipment and is a bridge to relate equipment cost to overall plant cost. *Economic Comparison Between Conventional and Disposables-Based Technology for the Production of Biopharmaceuticals* estimates that the Lang Factor for a biopharmaceutical plant can be as high as 8.13²⁵. The capital investment of the building was negligible since the building already exists and is on land already owned by the company. The overall plant cost was determined to be \$210,000,000 using the Lang Factor of 8.13.

11 Safety, Health, and Environmental Considerations

11.1 PPE

The PPE required for working in biopharmaceutical processes is a full bunny suit and eye protection. If working with powders and acids, respirators may be required. Additional protection against acids while working in the buffer preparation area need to be considered. Working in the lab may require less intensive PPE depending on the lab work. Warm clothes are needed to work in the storage facility.

11.2 Solid Biohazardous Waste

The plant produces solid hazardous waste. This originates from the single use equipment and CHO cell separation from the centrifuge. Several options are available to deal with this waste

²⁵ Novais, J. L., N. J. Titchener-Hooker, and M. Hoare. "Economic Comparison between Conventional and Disposables-based Technology for the Production of Biopharmaceuticals." *Biotechnology and Bioengineering* 75.2 (2001): 143-53. Web.

source: Recycling, incineration, and landfills²⁶. Recycling would be difficult since many disposable items are made from multiple types of plastics and other materials. Incineration could produce energy for the plant but would emit CO₂ to the atmosphere and require additional equipment. Waste could be sent to landfills in two ways: treated and untreated biohazard. Treated biohazard would need to be sterilized in an autoclave and possibly grinded up. This could then be disposed of as regular solid waste for a fraction of the cost of biohazardous waste. The easiest option is to send to landfills designated as biohazard waste. However, biohazardous waste would become more expensive over time. Sending product to a landfill as biohazardous waste will be used but looking into incineration and sterilization could be explored in the future.

11.3 Air Emissions

The only emissions to the atmosphere are from burning of natural gas to produce steam. It is estimated the plant will produce 700 kg CO₂. This amount of CO₂ is reasonable and does not need to be filtered. If any other air emissions are detected such as NO_x or SO_x an air study will need to be conducted.

11.4 Onsite Safety

The main risk for onsite safety is coming into contact with biological waste containing viruses. Operators will need to be properly trained and PPE will be worn when there is a potential to come into contact with viruses. The biological waste is sterilized as described above and should pose no further risk. Acids are also present onsite in liquid form. The acids have containment if process malfunctions were to occur. There is no risk for run-away reactions or flammable mixtures.

11.5 Offsite Safety

The amount of biological material emitted to the environment is heat treated to sterilization. This is under process control and should pose no threat to offsite parties. The waste water is also cooled to limit thermal pollution. There is no risk of explosions or spills that would affect outside parties.

12 Process Safety Considerations

12.1 Inherently Safer Design

The inherently safer design checklist from *Guidelines for Hazard Identification Procedures* was used to gauge the inherent safety of the plant²⁷. The checklist can be viewed below. The pertinent questions and considerations were answered in the following sections.

²⁶ Rawlings, Bruce, and Helene Pora. "A Prescriptive Approach to Management of Solid Waste from Single-Use Systems." BioProcess International, 21 Mar. 2018, bioprocessintl.com/manufacturing/supply-chain/a-prescriptive-approach-to-management-of-solid-waste-from-single-use-systems-183658/.

²⁷ American Institute of Chemical Engineers. Center for Chemical Process Safety. *Guidelines for Hazard Evaluation Procedures*. 3rd ed. New York, N.Y. : Hoboken, N.J.: CCPS, Center for Chemical Process Safety ; Wiley-Interscience, 2008. Web.

1 Intensification / Minimization

- 1.1 Do the following strategies reduce inventories of hazardous raw materials, intermediates, and/or finished products?
 - Improved production scheduling
 - Just-in-time deliveries
 - Direct coupling of process elements
 - Onsite generation and consumption
- 1.2 Do the following actions minimize in-process inventory?
 - Eliminating or reducing the size of in-process storage vessels
 - Designing processing equipment handling hazardous materials for the smallest feasible inventory
 - Locating process equipment to minimize the length of hazardous material piping runs
 - Reducing piping diameters
- 1.3 Can other types of unit operations or equipment reduce material inventories? For example:
 - Wiped film stills in place of continuous still pots
 - Centrifugal extractors in place of extraction columns
 - Flash dryers in place of tray dryers
 - Continuous reactors in place of batch
 - Plug flow reactors in place of continuous-flow stirred tank reactors
 - Continuous in-line mixers in place of mixing vessels
- 1.4 Can thermodynamic or kinetic efficiencies of reactors be improved by design upgrades (e.g., improved mixing or heat transfer) to reduce hazardous material volume?
- 1.5 Can equipment sets be combined (e.g., replacing reactive distillation with a separate reactor and multi-column fractionation train; installing internal reboilers or heat exchangers) to reduce overall system volume?
- 1.6 Can pipeline inventories be reduced by feeding hazardous materials as a gas instead of a liquid (e.g., chlorine)?
- 1.7 Can process conditions be changed to avoid handling flammable liquids above their flash points?
- 1.8 Can process conditions be changed to reduce production of hazardous wastes or by-products?

2 Substitution / Elimination

- 2.1 Is it possible to eliminate hazardous raw materials, process intermediates, or by-products by using an alternative process or chemistry?
- 2.2 Is it possible to eliminate in-process solvents by changing chemistry or processing conditions?
- 2.3 Is it possible to substitute less hazardous raw materials? For example:
 - Noncombustible rather than flammable
 - Less volatile
 - Less reactive
 - More stable
 - Less toxic
- 2.4 Is it possible to use utilities with lower hazards (e.g., low-pressure steam instead of combustible heat transfer fluid)?
- 2.5 Is it possible to substitute less hazardous final product solvents?
- 2.6 For equipment containing materials that become unstable at elevated temperatures or freeze at low temperatures, is it possible to use heating and cooling media that limit the maximum and minimum temperature attainable?

3 Attenuation / Moderation

- 3.1 Is it possible to keep the supply pressure of raw materials lower than the design pressure of the vessels to which they are fed?
- 3.2 Is it possible to make reaction conditions (e.g., pressure or temperature) less severe by using a catalyst or by using a better catalyst?
- 3.3 Can the process be operated at less severe conditions using any other route? For example:
- Improved thermodynamic or kinetic efficiencies of reactors by design upgrades (e.g., improved mixing or heat transfer) to reduce operating temperatures and/or pressures
 - Changes to the order in which raw materials are added
 - Changes in phase of the reaction (e.g., liquid/liquid, gas/liquid, or gas/gas)
- 3.4 Is it possible to dilute hazardous raw materials to reduce the hazard potential? For example, by using the following:
- Aqueous ammonia instead of anhydrous
 - Aqueous HCl instead of anhydrous
 - Sulfuric acid instead of oleum
 - Dilute nitric acid instead of concentrated fuming nitric acid
 - Wet benzoyl peroxide instead of dry
- attainable process temperature (i.e., higher maximum allowable working temperature to accommodate loss of cooling, simplifying reliance on the proper functioning of external systems, such as refrigeration systems, to control temperature such that vapor pressure is less than equipment design pressure)?
- 4.3 Can passive leak-limiting technology (e.g., blowout resistant gaskets and excess flow valves) be utilized to limit potential for loss of containment?
- 4.4 Can process units be located to reduce or eliminate adverse effects from other adjacent hazardous installations?
- 4.5 Can process units be located to eliminate or minimize the following?
- Off-site impacts
 - On-site impacts on employees and other plant facilities
- 4.6 For processes handling flammable materials, is it possible to design the facility layout to minimize the number and size of confined areas and to limit the potential for serious overpressures in the event of a loss of containment and subsequent ignition?
- 4.7 Can the plant be located to minimize the need for transportation of hazardous materials?
- 4.8 Can materials be transported in the following ways?
- In a less hazardous form
 - Via a safer transport method
 - Via a safer route

4 Limitation of Effects

- 4.1 Is it possible to design and construct vessels and piping to be strong enough to withstand the largest overpressure that could be generated within the process, even if the “worst credible event” occurs (eliminating the need for complex, high-pressure interlock systems and/or extensive emergency relief systems)?
- 4.2 Is all equipment designed to totally contain the materials that might be present inside at ambient temperature or the maximum

5 Simplification / Error Tolerance

- 5.1 Is it possible to separate a single, procedurally complex, multipurpose vessel into several simpler processing steps and processing vessels, thereby reducing the potential for hazardous interactions when

- the complexity of the number of raw materials, utilities, and auxiliary equipment is reduced for specific vessels?
- 5.2 Can equipment be designed so that it is difficult to create a potentially hazardous situation due to an operating or maintenance error? For example:
- Simplifying displays
 - Designing temperature-limited heat transfer equipment
 - Lowering corrosion potential by use of resistant materials of construction
 - Lowering operating pressure to limit release rates
 - Using higher processing temperatures (to eliminate cryogenic effects such as embrittlement failures)
 - Using passive vs. active controls (e.g., stronger piping and vessels)
 - Using buried or shielded tanks
 - Using fail-safe controls if utilities are lost
 - Limiting the degree of instrumentation redundancy required
 - Using refrigerated storage vs. pressurized storage
 - Spreading electrical feed over independent or emergency sources
 - Reducing wall area to minimize corrosion/fire exposure
 - Reducing the number of connections and paths
 - Minimizing the number of flanges in hazardous processes
 - Valving/piping/hose designed to prevent connection error
 - Using fewer bends in piping
 - Increasing wall strength
 - Using fewer seams and joints
 - Providing extra corrosion/erosion allowance
- Reducing vibration
- Using double-walled pipes, tanks, and other containers
- Minimizing use of open-ended valves
- Eliminating open-ended, quick-opening valves in hazardous service
- Improving valve seating reliability
- Eliminating unnecessary expansion joints, hoses, and rupture disks
- Eliminating unnecessary sight glasses/glass rotameters
- 5.3 Can procedures be designed so that it is difficult to create a potentially hazardous situation due to an operating or maintenance error? For example:
- Simplifying procedures
 - Reducing excessive reliance on human action to control the process
- 5.4 Can equipment be eliminated or arranged to simplify material handling?
- Using gravity instead of pumps to transfer liquids
 - Siting to minimize hazardous transport or transfer
 - Reducing congestion (i.e., easier to access and maintain)
 - Reducing knock-on effects from adjacent facilities
 - Removing hazardous components early in the process rather than spreading them throughout the process
 - Shortening flow paths
- 5.5 Can reactors be modified to eliminate auxiliary equipment (e.g., by creating a self-regulatory mechanism by using natural convection rather than forced convection for emergency cooling)?
- 5.6 Can distributed control system (DCS) modules be simplified or reconfigured such that failure of one module does not disable a large number of critical control loops?

12.1.1 Intensification/Minimization

- 1.1.The generation of buffers and media onsite reduce the need for hazard materials and intermediates. The timeline (*Section 4*) offers a schedule to reduce onsite hazardous materials.
- 1.2. The plant was laid out (*Section 12.3*) to ensure successive unit operations were directly next to each other to reduce lengthy piping. The storage vessels were designed to minimize size. For example, T-100 is filled to 87.5% of its capacity when the plant is at max capacity instead of 50%.
- 1.3. The process is strictly batch therefore creating continuous unit operations is difficult.
- 1.4. The reactors were designed with agitators to improve mixing as well as heat transfer. Heating fluid was recycled from other energy processes to reduce the need for steam.
- 1.5.The heated waste water was used to heat the reactors instead of producing heating fluid from another source. The WFI tank (T-106 and T-109) distributes WFI throughout the plant instead of having smaller holding tanks by each individual process.
- 1.6.The process is biological and needs to be aqueous. With the minimal heat requirement needed for the reactors, liquid water instead of steam is used to reduce steam as a hazardous material.
- 1.7. No flammable liquids are used in the process.
- 1.8.Disposable reactors were used for small reactor sizes to reduce the use of cleaning waste. The disposable reactors themselves will be disposed of but the disposal is less risky than cleaning the reactors with CIP/SIP protocol.

12.1.2 Substitution/Elimination

- 2.1.The need for steam for heating the reactors was eliminated by recycling the waste water for heating.
- 2.2.The only solvent used in the process is WFI.
- 2.3.Caustic was substituted for a urea solution. Weak acids such as acetic acids were used for cleaning and the process instead of stronger acids.
- 2.4. Low pressure steam is used for SIP and in heat exchangers.
- 2.5. Glycerol is necessary to achieve the proper storage conditions and has minimal health risks.
- 2.6. Glycerol is used to prevent the MAb product from freezing.

12.1.3 Attenuation/Moderation

- 3.1. The design pressure for all the vessels is 10% or 25 psi above the maximum allowable pressure. The highest pressure component is air entering at 3 bar and is limited by the compressor preventing any over-pressurization.
- 3.2. The reactions are bio-based and do not produce excess heat or pressure.
- 3.3. The operating conditions are fairly relaxed. During operations, the process should never exceed more than 3 bar or 80°C. During cleaning, the temperatures might exceed 121°C, however this is necessary to reach FDA cleaning requirements.
- 3.4. The potentially hazardous materials used in the process are acetic acid, sodium acetate, Tris buffer, alkaline detergent, and urea. Acetic acid and sodium acetate never exceed more than 0.1M. Urea is used to negate the use for a caustic solution. Alkaline detergent is present at 0.5 wt% and Tris buffer is only present at 0.02-0.05M. All hazardous materials are either diluted or substituted.

12.1.4 Limitations of Effects

- 4.1. The processes generate little to no pressure, so the vessels being rated for 5 bar is sufficient.
- 4.2. Yes, there is no exotherms present in the process, so the vessels are rated to perform under any process circumstance.
- 4.3. Block and bleed valves are placed around all potential leaking equipment to provide maintenance to prevent loss of containment.
- 4.4. Boilers, buffers, and media are separated from the rest of the process to reduce risk of adverse effects if one of these processes failed.
- 4.5. Buffer preparation, media preparation, and boilers are isolated from other parts of the plant to reduce on-site impacts.
- 4.6. No flammable materials are used in the process.
- 4.7. The plant is being renovated from an existing facility.
- 4.8. Piping is through walls and is protected from human exposure whenever possible.

12.1.5 Simplification/Error Tolerance

- 5.1. The production reactors were separated into smaller reactors to reduce the potential for hazardous interactions.
- 5.2. Stainless steel is used for almost all unit operations. There is no safety risk if utilities are lost because there are no energy producing or consuming reactions. Feedback loops are used to eliminate operator error. The rest of the inherent safety in this section is outside the scope of this report.
- 5.3. The majority of the process is automated via control valves and feedback loops to reduce the potential for operator error.
- 5.4. The site plan was optimized to shorten pipe lengths and reduce operator handling. Gravity could potentially have been utilized for sewer drainage depending on where sewer access is, but that is outside the scope of this report.
- 5.5. The auxiliary equipment for the reactors is minimal and cannot be further reduced otherwise mixing and cleaning would not be possible.
- 5.6. Control systems may need to be configured to prevent domino effect failure, but this is outside the scope of this report.

12.2 Hazards Identification and Risk Analysis

The Hazard identification guidelines from *Guidelines for Hazard Identification Procedures Appendix B* was used to analyze the possible hazards of the plant²². A what if analysis was performed.

12.2.1 Process

12.2.1.1 Materials^{28,29,30,31,32,33}

Material	Associated Hazards	Preventative Action
Acetic Acid	Flammable Toxic (SDS link)	Used in low concentrations (0.1M). Contained in isolated room (Buffer Preparation).
Sodium Acetate	Combustible dust formation	Mixed with liquid. Contained in isolated room (Buffer Preparation). Powdered form is kept in closed container.
Tris	Toxic Health hazard	Used in low concentrations (0.02-0.05M). Contained in isolated room (Buffer Preparation). Stored in sealed environment.
Urea	Non-Hazardous	Keep in isolated sealed container
Sodium Chloride	Non-Hazardous	Keep in isolated sealed container
Water	Non-Hazardous	N/A
Biomass	Potential health issues	Sterilized before disposal or human contact.
CD OptiCHO™	Non-Hazardous	Keep in isolated environment to avoid contamination (Media Preparation)
Viruses	Health Hazard	Sterilized before disposal or human contact. Viruses sterilized via pH treatment and heating. Removed via filtration.
Steam	Pressure Build-up Burns	Contained in piping and insulation. Generation isolated in boiler room.

12.2.1.2 Unit Siting and Layout

Unit	Potential Hazards	Preventative Action
Media Preparation	Transfer of materials to plant and between processing stages; External environmental and political forces	External environmental forces considered in civil design; Material transfer shortest distance possible
Buffer Preparation	Transfer of materials to plant and between processing stages; External environmental and political forces	External environmental forces considered in civil design; Material transfer shortest distance possible; External environmental forces considered in civil design
Seed Train	Biological waste leak; External environmental and political forces	Seed train containment and using mostly non-hazardous materials; External environmental forces considered in civil design;
Production Reactors	Biological waste leak; External environmental and political forces	Seed train containment and using mostly non-hazardous materials; External environmental forces considered in civil design;

²⁸ Acetic Acid; SDS No. S25118 [Online]; Fisher Science Education. 15 Jet View Drive, Rochester, NY 14624, Sep 06, 2015 (accessed May 03, 2019)

²⁹ Sodium Acetate; SDS No. S25530 [Online]; Fisher Science Education. 15 Jet View Drive, Rochester, NY 14624, Sep 06, 2015 (accessed May 03, 2019)

³⁰ Tris; SDS No. 1185-53-1 [Online]; Fisher Science Education. 15 Jet View Drive, Rochester, NY 14624, Sep 26, 2009 (accessed May 03, 2019)

³¹ Urea; SDS No. 57-13-6 [Online]; Fisher Science Education. 15 Jet View Drive, Rochester, NY 14624, Sep 17, 2010 (accessed May 03, 2019)

³² Sodium Chloride; SDS No. S25875 [Online]; Fisher Science Education. 15 Jet View Drive, Rochester, NY 14624, Oct 24, 2014 (accessed May 03, 2019)

³³ CD OptiCHOTM AGTTM; SDS No. A1122201 [Online]; Fisher Science Education. 15 Jet View Drive, Rochester, NY 14624, Jan 11, 2018 (accessed May 03, 2019)

Harvesting	Rapid rotating object; External environmental and political forces	Proper training for operators; External environmental forces considered in civil design
Protein A	Exposure to buffer solutions; Potential exposure to bio-waste; External environmental and political forces	Interlocking systems; Close proximity to buffer prep; External environmental forces considered in civil design; Waste sent to waste treatment
Viral Inactivation	Exposure to buffer solutions; Potential exposure to bio-waste; External environmental and political forces;	Interlocking systems; Close proximity to buffer prep; External environmental forces considered in civil design; Waste sent to waste treatment
Polishing	Exposure to buffer solutions; Potential exposure to bio-waste; External environmental and political forces;	Interlocking systems; Close proximity to buffer prep; External environmental forces considered in civil design; Waste sent to waste treatment
Kill Tanks	Potential exposure to hot elements; External environmental and political forces;	Close to boiler; Insulated pipes; External environmental forces considered in civil design

12.2.2 Equipment

12.2.2.1 Seed Train

Failure Mode	Cause	Effect	Preventative Action
Loss of electricity	Power outage	Loss of electrically powered components: mixing, pumps, valves, process control, temperature control	Manual valves; reaction is not exothermic; loss of product; backup generator
Pump mechanical issue (P-103)	Pump malfunction	Loss of production flow	Preventative maintenance plan; spare parts on hand
Heating water not available; Boiler malfunction; Control valve failure	Loss of reactor heating	Non ideal cell conditions	Preventative maintenance; direct steam injection
Mixing mechanical issues	Agitators, mixers, and shake flask malfunction	Non-uniform heating	Preventative maintenance; Use large reactor with longer time
SIP valve failure (V-162)	Steam buildup	Pressure and heat buildup	Pressure relief valve (PRV-126)
Air compressor or valve failure (CV-124 & K-100)	Fed air loss	Condensing steam creates vacuum	Redundancy in line
Valve blockage during CIP (V-162)	Liquid buildup in reactor	Overflow of buffer solutions	Process control
Valve blockage downstream of pump (V-163 & 164 & 165)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-127)
Over pressurization (ST-105)	Steam trap air vent malfunction	Pressure buildup	Pressure relief valve (PRV-126)
Pressure probe failure	Electrical malfunction and probe ware	Over/under pressurization	Pressure relief valve; Preventative maintenance (PRV-126)
Temperature probe failure	Electrical malfunction and probe ware	Over heating/ underheating;	Preventative maintenance
Load cell failure	Electrical malfunction and probe ware	Insufficient process control	Preventative maintenance

Flow meter failure (F-113)	Electrical malfunction and probe ware	Insufficient process control	Preventative maintenance
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12.2.2.2 Production Reactor

Failure Mode	Cause	Effect	Preventative Action
Loss of electricity	Power outage	Loss of electrically powered components: mixing, pumps, valves, process control, temperature control	Manual valves; reaction is not exothermic; loss of product; backup generator
Pump mechanical issue (P-113)	Pump malfunction	Loss of production flow	Preventative maintenance plan; spare parts on hand
Heating water not available; Boiler malfunction; Control valve failure	Loss of reactor heating	Non ideal cell conditions	Preventative maintenance; direct steam injection
Mixing mechanical issues	Agitators, mixers, and shake flask malfunction	Non-uniform heating	Preventative maintenance; Use large reactor with longer time
SIP valve failure (V-115)	Steam buildup	Pressure and heat buildup	Pressure relief valve (PRV-105)
Air compressor or valve failure (CV-105 & K-100)	Fed air loss	Condensing steam creates vacuum	Redundancy in line
Valve blockage during CIP (V-115)	Liquid buildup in reactor	Overflow of buffer solutions	Process control
Valve blockage downstream of pump (V-116 & 117 & 118)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-107)
Over pressurization (ST-100)	Steam trap air vent malfunction	Pressure buildup	Pressure relief valve (PRV-105)
Pressure probe fails	Electrical malfunction and probe ware	Over/under pressurization	Pressure relief valve; Preventative maintenance (PRV-105)
Temperature probe fails	Electrical malfunction and probe ware	Over heating/ underheating;	Preventative maintenance
Load cell failure	Electrical malfunction and probe ware	Insufficient process control	Preventative maintenance
Flow meter failure (F-100 & 101)	Electrical malfunction and probe ware	Insufficient process control	Preventative maintenance
Pump mechanical issue (P-106)	Pump malfunction	Loss of substrate feed leading to cell death	Preventative maintenance plan; spare parts on hand
Pressure buildup downstream of pump (P-106)	Valve blocked (V-119)	Pressure buildup	Pressure relief valve (PRV-106)

12.2.2.3 Harvest

Failure Mode	Cause	Effect	Preventative Action
Loss of electricity	Power outage	Loss of electrically powered components: mixing, pumps, valves, process control, temperature control	Manual valves; reaction is not exothermic; loss of product; backup generator
Pump mechanical issue (P-114)	Pump malfunction	Loss of production flow	Preventative maintenance plan; spare parts on hand
SIP valve failure (V-166)	Steam buildup	Pressure and heat buildup	Pressure relief valve (PRV-

			128)
Air compressor or valve failure (CV-125 & K-100)	Fed air loss	Condensing steam creates vacuum	Redundancy in line
Valve blockage during CIP (V-166)	Liquid buildup in reactor	Overflow of buffer solutions	Process control
Valve blockage downstream of pump (V-167)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-129)
Over pressurization (ST-115)	Steam trap air vent malfunction	Pressure buildup	Pressure relief valve (PRV-128)
Pressure probe fails	Electrical malfunction and probe ware	Over/under pressurization	Pressure relief valve; Preventative maintenance (PRV-128)
Temperature probe fails	Electrical malfunction and probe ware	Over heating/ underheating;	Preventative maintenance
Flow meter failure (F-114 & 115)	Electrical malfunction and probe ware	Insufficient process control	Preventative maintenance
Solid waste disposal valve failure	Process control/ mechanical malfunction	Solid waste buildup	Depth filter; turbidity meter monitoring
Depth filter fails	Pore blockage	Increase pressure drop; Loss of filtration	Replace filter

12.2.2.4 Protein A Chromatography

Failure Mode	Cause	Effect	Preventative Action
Loss of electricity	Power outage	Loss of electrically powered components: mixing, pumps, valves, process control, temperature control	Manual valves; reaction is not exothermic; loss of product; backup generator
Pump mechanical issue (P-115)	Pump malfunction	Loss of production flow	Preventative maintenance plan; spare parts on hand
Pump mechanical issue (P-116)	Pump malfunction	Loss of washing solution flow	Preventative maintenance plan; spare parts on hand
Pump mechanical issue (P-117)	Pump malfunction	Loss of eluting fluid flow	Preventative maintenance plan; spare parts on hand
Pump mechanical issue (P-118)	Pump malfunction	Loss of regenerative solution flow	Preventative maintenance plan; spare parts on hand
SIP valve failure (V-143)	Steam buildup	Pressure and heat buildup	Pressure relief valve (PRV-124)
Air compressor or valve failure (CV-116 & K-100)	Fed air loss	Condensing steam creates vacuum	Redundancy in line
Valve blockage during CIP (V-143)	Liquid buildup in reactor	Overflow of buffer solutions	Process control
Valve blockage downstream of pump (V-144)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-123)
Valve blockage downstream of pump (V-147)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-120)
Valve blockage downstream of pump (V-150)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-121)
Valve blockage downstream of pump (V-152)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-122)
Over pressurization (ST-103)	Steam trap air vent malfunction	Pressure buildup	Pressure relief valve (PRV-124)

Pressure probe fails	Electrical malfunction and probe ware	Over/under pressurization	Pressure relief valve; Preventative maintenance (PRV-124)
Temperature probe fails	Electrical malfunction and probe ware	Over heating/underheating	Preventative maintenance
Flow meter failure (F-104, 105, 106 108, & 109)	Electrical malfunction and probe ware	Insufficient process control	Preventative maintenance
Control valve to viral inactivation fails (CV-118)	Loss of process control	Downstream productions stop	Preventative maintenance
Valve to kill tank fails (CV-119)	Loss of process control	No waste disposal	Preventative maintenance
Improper mixing of buffer	Operator error	Insufficient protein A chromatography	Detailed SOP and operator training

12.2.2.5 Viral Inactivation

Failure Mode	Cause	Effect	Preventative Action
Loss of electricity	Power outage	Loss of electrically powered components: mixing, pumps, valves, process control, temperature control	Manual valves; reaction is not exothermic; loss of product; backup generator
Pump mechanical issue (P-119)	Pump malfunction	Loss of production flow	Preventative maintenance plan; spare parts on hand
Pump mechanical issue (P-117)	Pump malfunction	Loss of titrant flow	Preventative maintenance plan; spare parts on hand
Mixing mechanical issues	Agitators, mixers, and shake flask malfunction	Non-uniform heating	Preventative maintenance; Use large reactor with longer time
SIP valve failure (V-155)	Steam buildup	Pressure and heat buildup	Pressure relief valve (PRV-131)
Air compressor or valve failure (CV-121 & K-100)	Fed air loss	Condensing steam creates vacuum	Redundancy in line
Valve blockage during CIP (V-155)	Liquid buildup in reactor	Overflow of buffer solutions	Process control
Valve blockage downstream of pump P-119 (V-156, 157, 158, & 159)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-125)
Over pressurization (ST-104)	Steam trap air vent malfunction	Pressure buildup	Pressure relief valve (PRV-131)
Pressure probe fails	Electrical malfunction and probe ware	Over/under pressurization	Pressure relief valve; Preventative maintenance (PRV-131)
Temperature probe fails	Electrical malfunction and probe ware	Over heating/underheating;	Preventative maintenance
Flow meter failure (F-110, 111, & 112)	Electrical malfunction and probe ware	Insufficient process control	Preventative maintenance
Pressure buildup downstream of pump P-117 (V-154)	Valve blocked (V-154)	Pressure buildup	Pressure relief valve (PRV-130)
Prefilter Malfunction (L-101)	Inadequate filtration	Viral contamination	Leak testing performed regularly

Prefilter Malfunction (L-102)	Inadequate filtration	Viral contamination	Leak testing performed regularly
Improper mixing of buffer	Operator error	Insufficient protein A chromatography	Detailed SOP and operator training

12.2.2.6 Polishing

Failure Mode	Cause	Effect	Preventative Action
Loss of electricity	Power outage	Loss of electrically powered components: mixing, pumps, valves, process control, temperature control	Manual valves; reaction is not exothermic; loss of product; backup generator
Pump mechanical issue (P-120)	Pump malfunction	Loss of production flow	Preventative maintenance plan; spare parts on hand
Pump mechanical issue (P-121)	Pump malfunction	Loss of washing solution flow	Preventative maintenance plan; spare parts on hand
SIP valve failure (V-121)	Steam buildup	Pressure and heat buildup	Pressure relief valve (PRV-108)
Air compressor or valve failure (CV-107 & K-100)	Fed air loss	Condensing steam creates vacuum	Redundancy in line
Valve blockage during CIP (V-121)	Liquid buildup in reactor	Overflow of buffer solutions	Process control
Valve blockage downstream of pump (V-122, 123, & 124)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-109)
Valve blockage downstream of pump (V-126 & 125)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-110)
Over pressurization (ST-101)	Steam trap air vent malfunction	Pressure buildup	Pressure relief valve (PRV-108)
Pressure probe fails	Electrical malfunction and probe ware	Over/under pressurization	Pressure relief valve; Preventative maintenance (PRV-108)
Temperature probe fails	Electrical malfunction and probe ware	Over heating/underheating;	Preventative maintenance
Flow meter failure (F-102, 116, & 115)	Electrical malfunction and probe ware	Insufficient process control	Preventative maintenance
Control valve to storage fails (CV-108)	Loss of process control	Downstream productions stop	Preventative maintenance
Valve to kill tank fails (CV-109)	Loss of process control	No waste disposal	Preventative maintenance
Improper mixing of buffer	Operator error	Insufficient protein A chromatography	Detailed SOP and operator training

Valve fails (V-25)	Valve malfunction	Ineffective titration and column washing	Operator training and preventative maintenance
Insufficient sodium chloride mixture	Operator error		
pH probe fails	Electrical malfunction and probe ware	Over acidic solution	Pressure relief valve; Preventative maintenance (PRV-108)
Conductivity probe fails	Electrical malfunction and probe ware	Undesired conductivity	Preventative maintenance

12.2.2.7 Storage

Failure Mode	Cause	Effect	Preventative Action
Loss of electricity	Power outage	Loss of electrically powered components: mixing, pumps, valves, process control, temperature control	Manual valves; reaction is not exothermic; loss of product; backup generator
Pump mechanical issue (P-122)	Pump malfunction	Loss of production flow	Preventative maintenance plan; spare parts on hand
Pump mechanical issue (P-130)	Pump malfunction	Loss of titrant flow	Preventative maintenance plan; spare parts on hand
SIP valve failure (V-130)	Steam buildup	Pressure and heat buildup	Pressure relief valve (PRV-112)
Air compressor or valve failure (CV-111 & K-100)	Fed air loss	Condensing steam creates vacuum	Redundancy in line
Valve blockage during CIP (V-130)	Liquid buildup in reactor	Overflow of buffer solutions	Process control
Valve blockage downstream of pump P-122 (V-131, 132, & 133)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-113)
Over pressurization (ST-102)	Steam trap air vent malfunction	Pressure buildup	Pressure relief valve (PRV-112)
Pressure probe fails	Electrical malfunction and probe ware	Over/under pressurization	Pressure relief valve; Preventative maintenance (PRV-112)
Temperature probe fails	Electrical malfunction and probe ware	Over heating/underheating;	Preventative maintenance
Flow meter failure (F-103)	Electrical malfunction and probe ware	Insufficient process control	Preventative maintenance
Pressure buildup downstream of pump	Valve blocked (V-154)	Pressure buildup	Pressure relief valve (PRV-114)

P-130 (V-129)			
Load cell failure	Electrical malfunction and probe ware	Insufficient process control	Preventative maintenance
Refrigerant leakage	Equipment issues	Refrigerant cycle leakage	Equipment checks
Refrigerant cycle loses circulation	Compressor/ expansion valve failure	Storage room heat up; loss of product	Preventative maintenance

12.2.2.8 Kill Tanks

Failure Mode	Cause	Effect	Preventative Action
Loss of electricity	Power outage	Loss of electrically powered components: mixing, pumps, valves, process control, temperature control	Manual valves; reaction is not exothermic; loss of product; backup generator
Pump mechanical issue (P-126)	Pump malfunction	Loss of production flow	Preventative maintenance plan; spare parts on hand
Pump mechanical issue (P-131)	Pump malfunction	Loss of titrant flow	Preventative maintenance plan; spare parts on hand
Pump mechanical issue (P-125)	Pump malfunction	Loss of titrant flow	Preventative maintenance plan; spare parts on hand
Valve blockage downstream of pump P-126 (V-139 & 138)	Valve malfunction	Pressure buildup	Pressure relief valve (PRV-118)
Temperature probe fails	Electrical malfunction and probe ware	Over heating/underheating;	Preventative maintenance
Pressure buildup downstream of pump P-131 (V-134)	Valve blocked (V-154)	Pressure buildup	Pressure relief valve (PRV-119)
Pressure buildup downstream of pump P-125 (V-140 & 138)	Valve blocked (V-154)	Pressure buildup	Pressure relief valve (PRV-117)
Wastewater distribution failure	Valve Failure (CV-114)	Potential overflow	Operator maintenance
pH neutralization failure	Valve or pH meter failure (CV-115)	Undesired pH	Preventative maintenance, process control
Heat exchanger failure	Valve failure (CV-112)	Lack of treatment before sewage disposal; Insufficient reactor heating	Process control; Temperature probes
Over pressurization of heat exchanger (HEX-100)	Valve blocked (V-137)	Over pressurization	Pressure relief (PRV-116)

Insufficient reactor heating	Valve failure (CV-113)	No reactor heating; Cell death	Operator intervention
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12.2.2.9 SIP/CIP

Failure Mode	Cause	Effect	Preventative Action
Loss of electricity	Power outage	Loss of electrically powered components: mixing, pumps, valves, process control, temperature control	Manual valves; reaction is not exothermic; loss of product; backup generator
Improper mixing of buffer	Operator error	Insufficient protein A chromatography	Detailed SOP and operator training
Mixing mechanical issues	Agitators, mixers, and shake flask malfunction	Non-uniform heating	Preventative maintenance; Use large reactor with longer time
Over pressurization of heat exchanger (HEX-101)	Valve blocked (V-110 & V-109)	Over pressurization	Pressure relief (PRV-102 & 101)
Pump mechanical issue (P-124)	Pump malfunction	Loss of titrant flow	Preventative maintenance plan; spare parts on hand
Pump mechanical issue (P-123)	Pump malfunction	Loss of titrant flow	Preventative maintenance plan; spare parts on hand
Pressure buildup downstream of pump P-124 (V-108)	Valve blocked (V-154)	Pressure buildup; buffer release	Pressure relief valve (PRV-100); Additional containment
Pressure buildup downstream of pump P-123 (V-112)	Valve blocked (V-154)	Pressure buildup; buffer release	Pressure relief valve (PRV-104); Additional containment
Air filter fails (L-104)	Filter blockage	Contaminated air	Filter replacement; Operator intervention
Boiler steam buildup	Excess methane flow	Over pressurization	Pressure relief valve (PRV-103)

12.3 Siting and Layout of Processes and Equipment³⁴

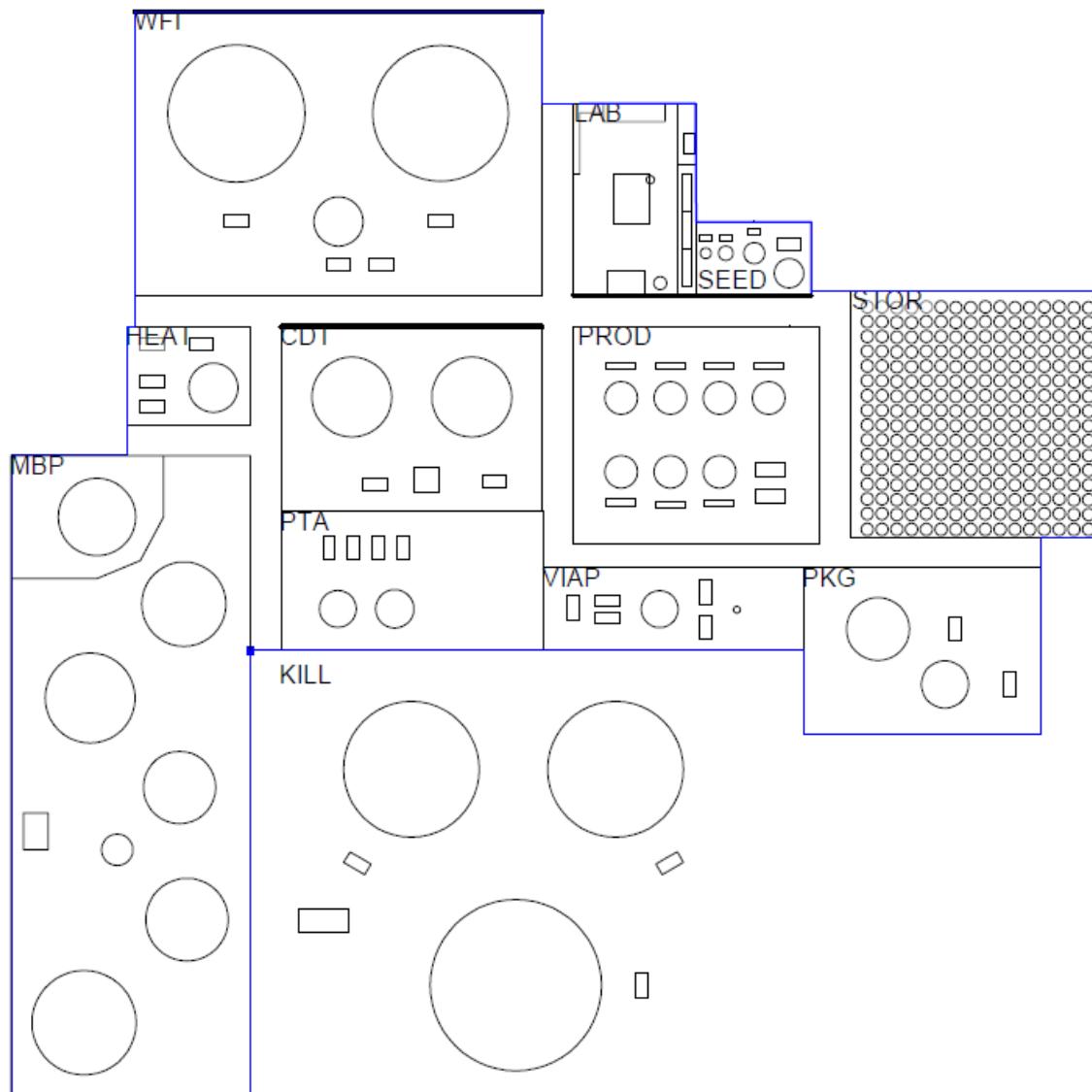


Figure 24: Overall site layout for the MAb production facility. Blue lines represent inside unit operations. Kill tanks are outside. All dimensions comply with Guidelines for facility siting and layout.

³⁴ American Institute of Chemical Engineers. Center for Chemical Process Safety, and Wiley InterScience. *Guidelines for Facility Siting and Layout*. New York: Center for Chemical Process Safety of the American Institute of Chemical Engineers, 2003. Web.

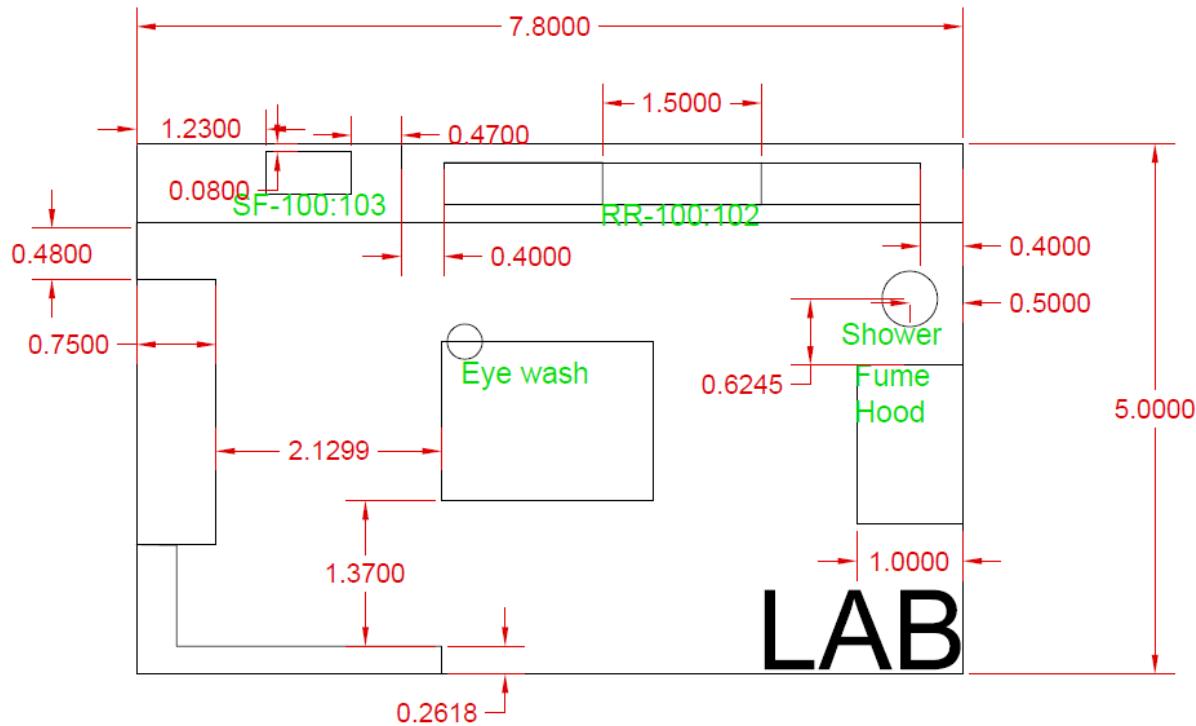


Figure 25: The site layout for the laboratory and smaller seed train reactors (SF 100-103)

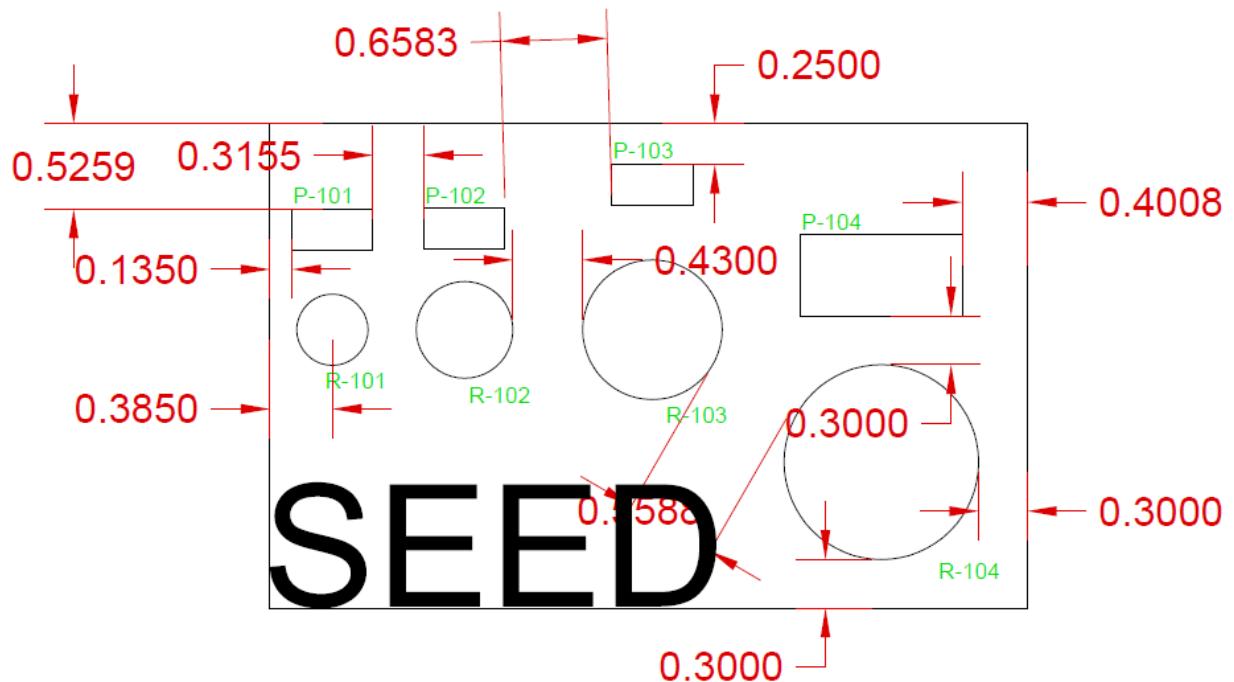


Figure 26: The cite layout for the large seed train reactors (RR 100-102, R 101-104).

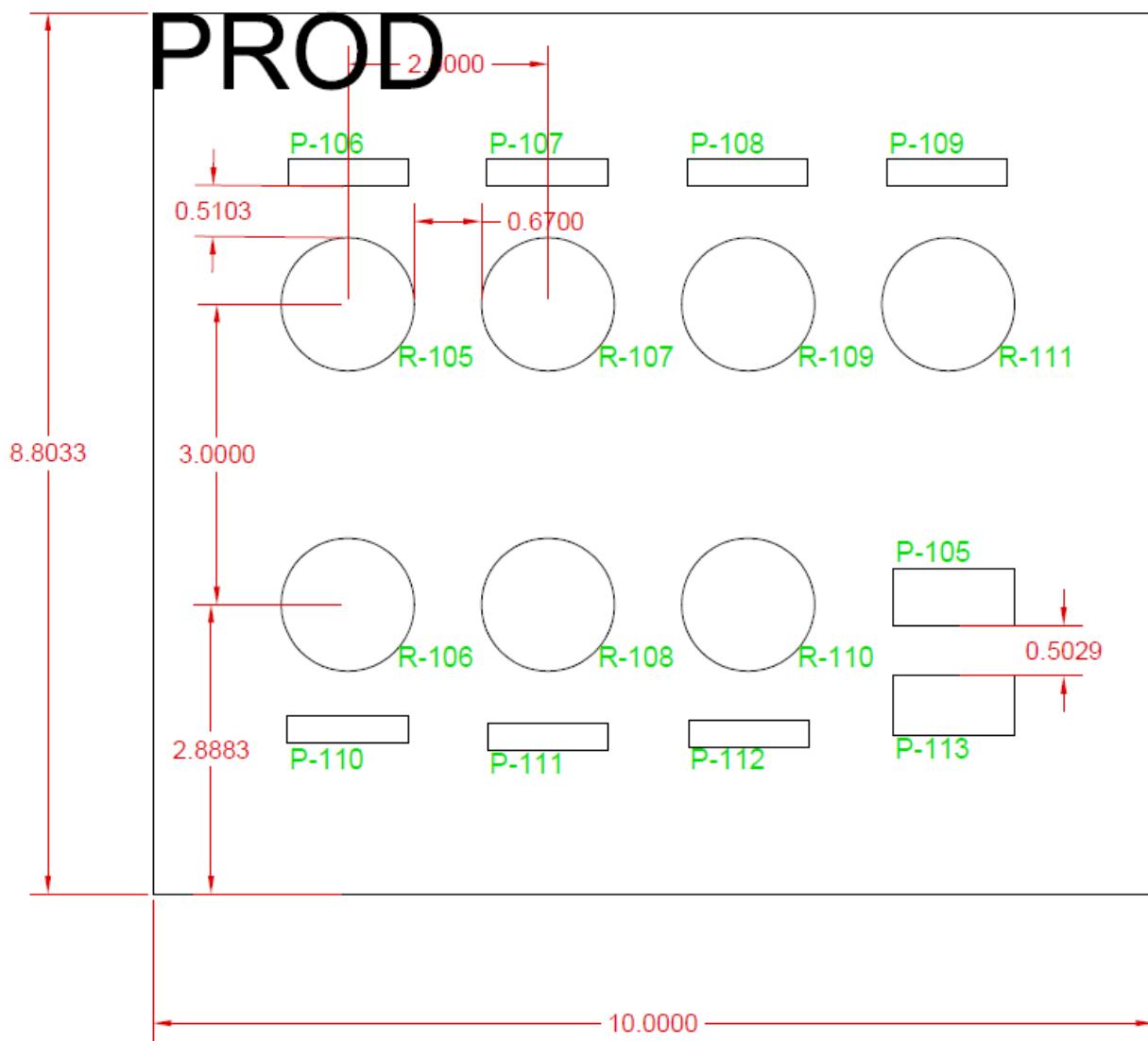


Figure 27: Site layout for production reactors (R 105-111).

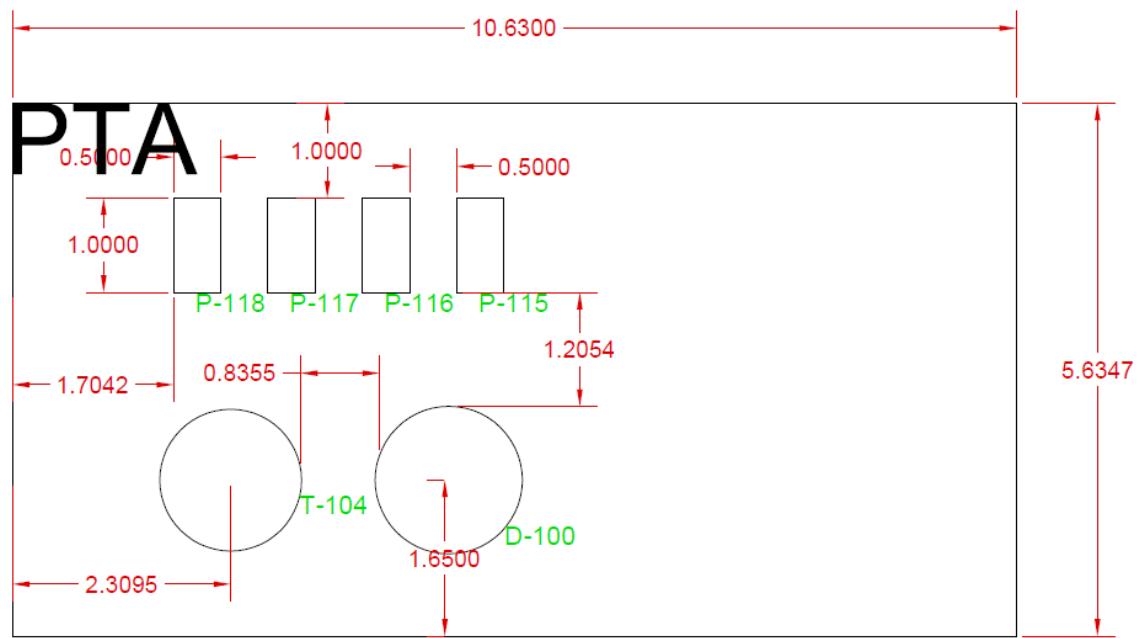


Figure 28: Site layout for Protein A chromatography.

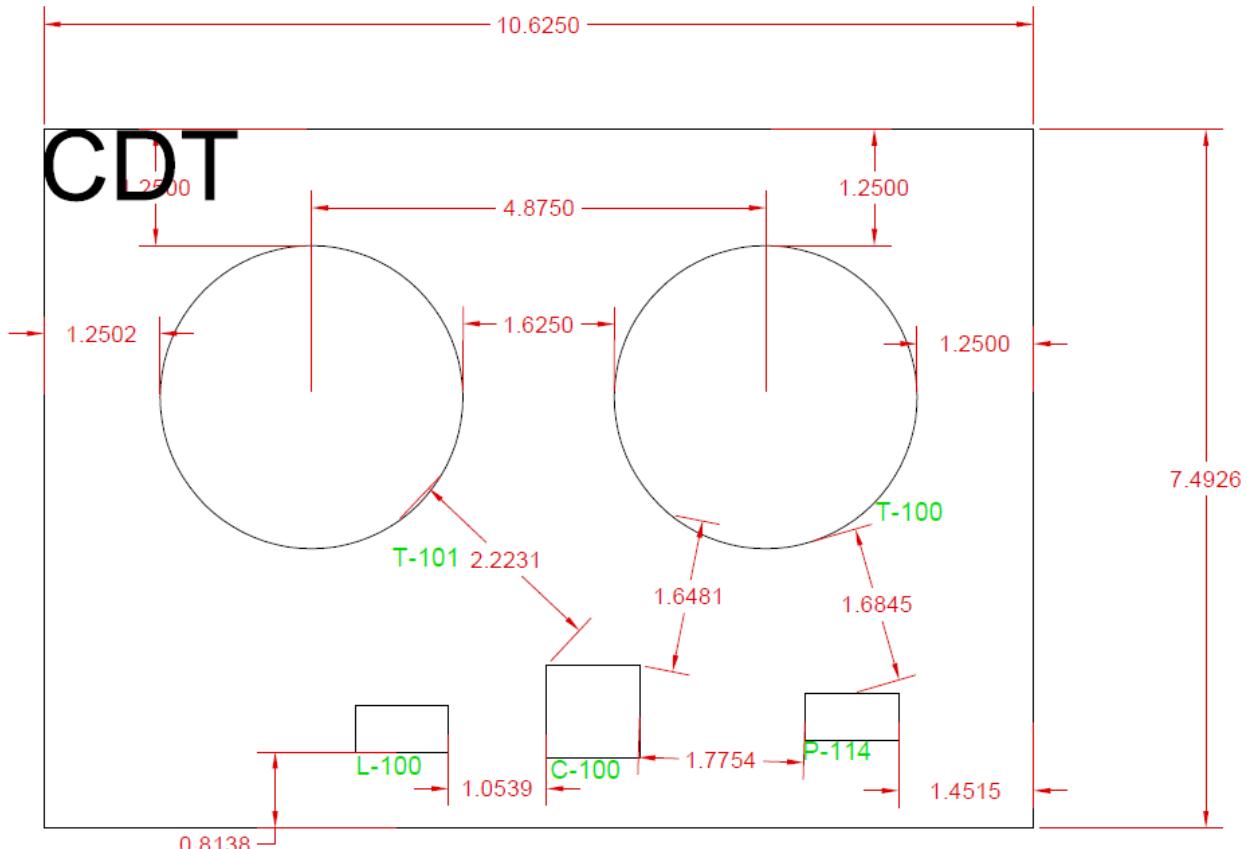


Figure 29: Site layout for the harvesting process (Centrifuge and Depth Filtrartion).

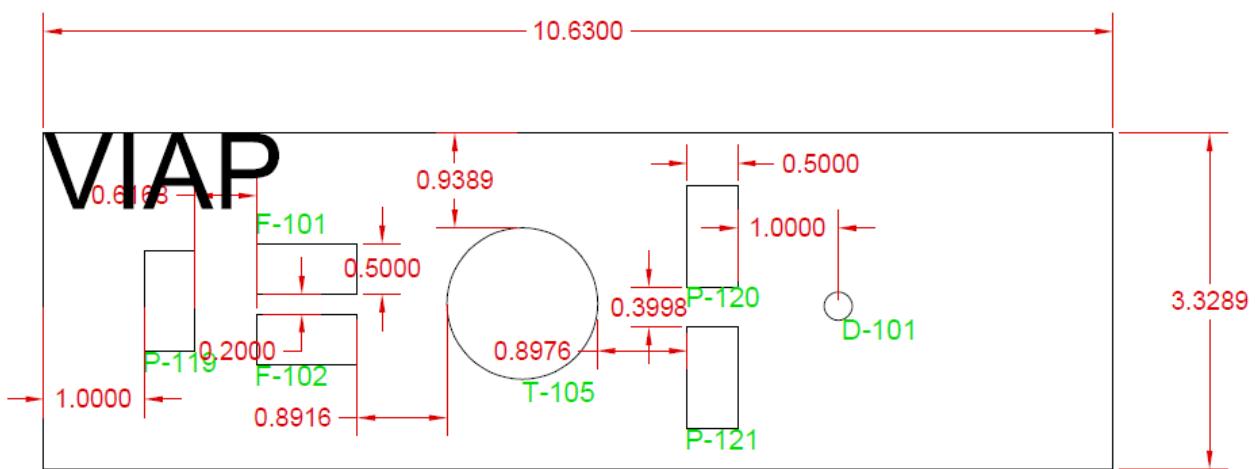


Figure 30: Site layout for viral inactivation and ploishing.

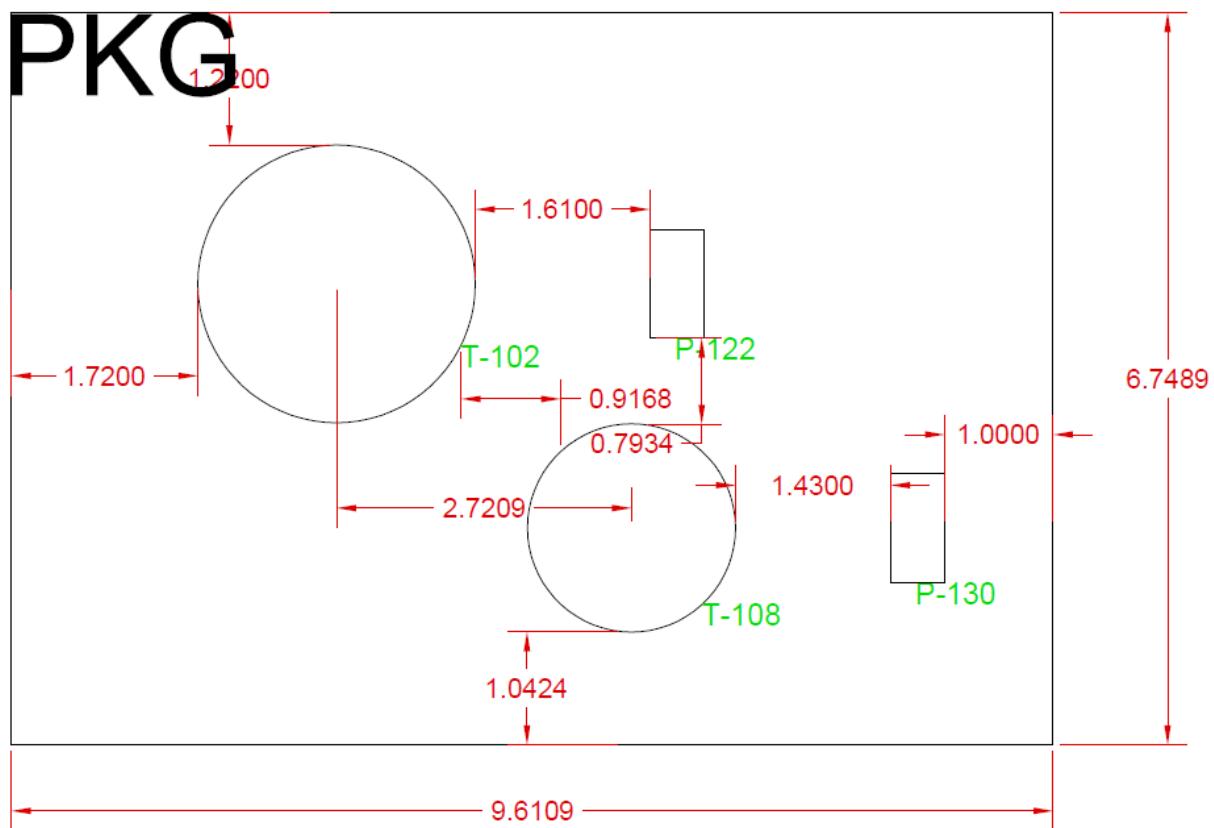


Figure 3I: Site plan for preparing product for storage.

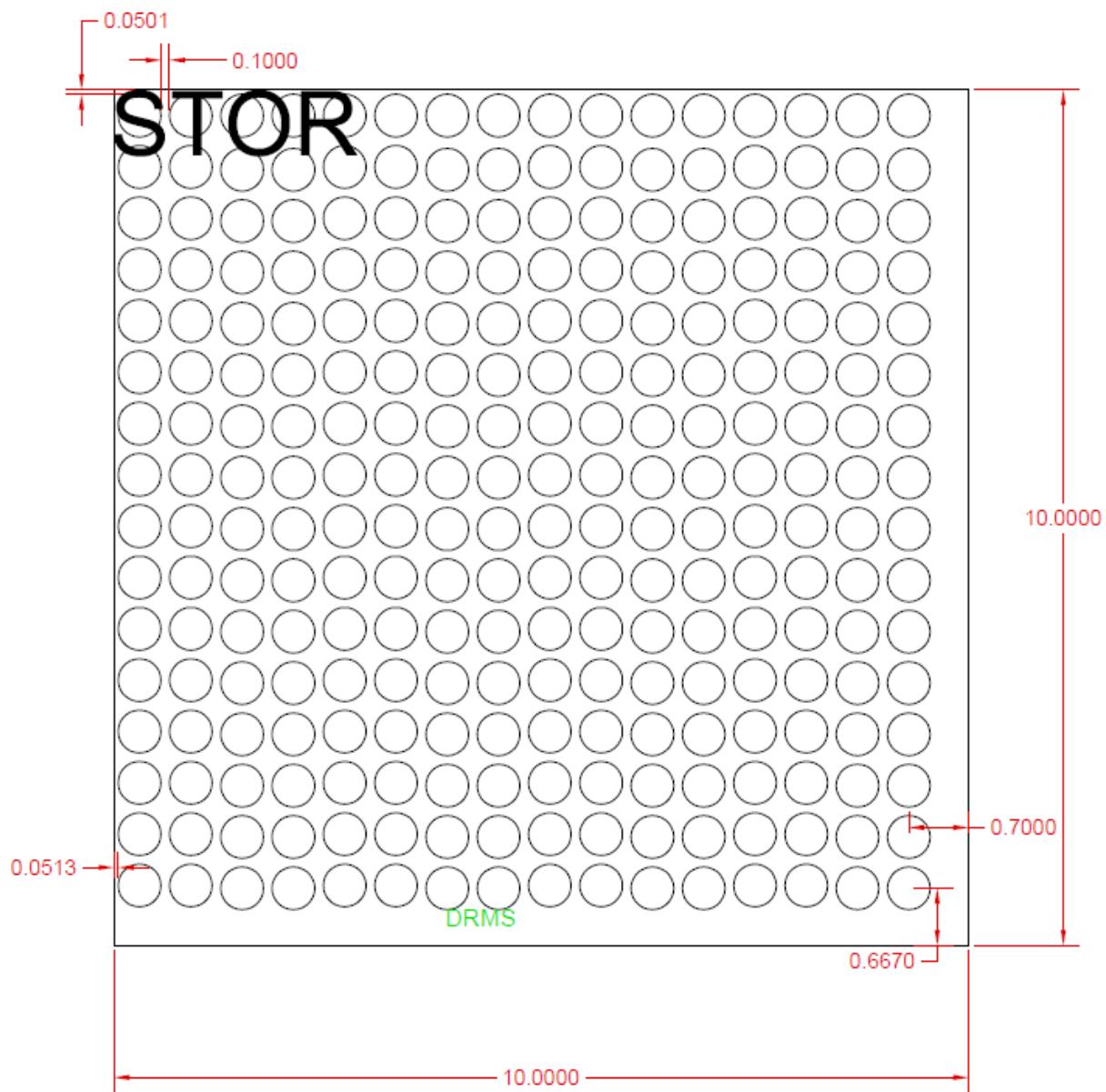


Figure 32: Site layout for storage room.

KILL

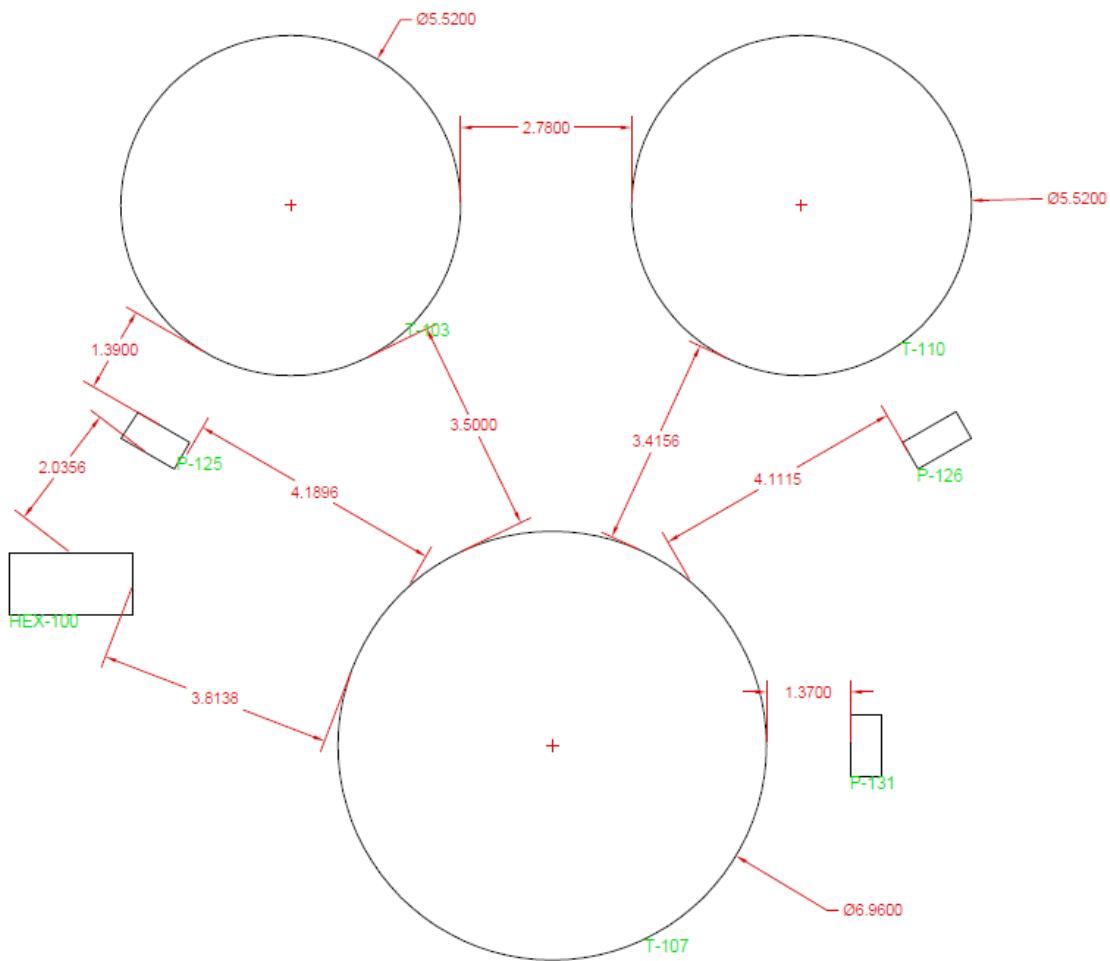


Figure 33: Site layout for kill tanks and cooling tank.

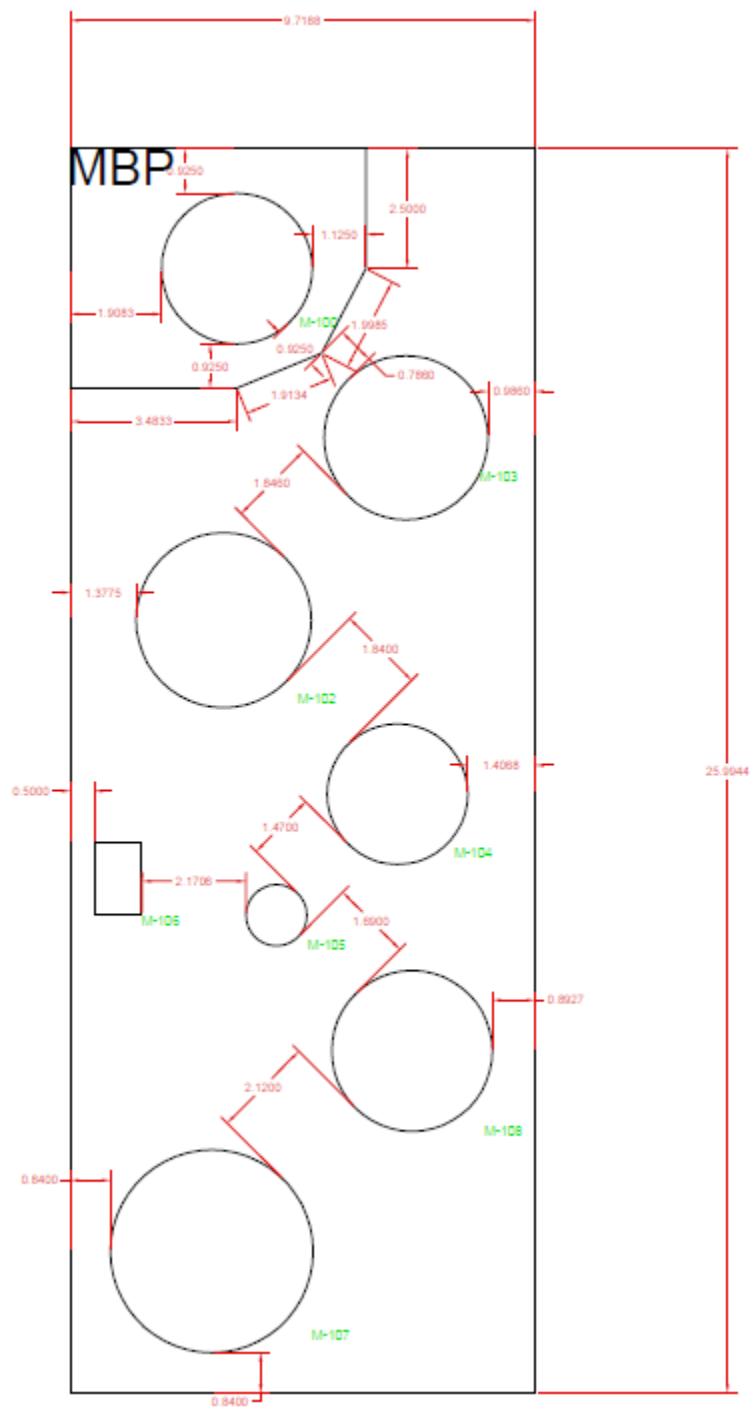


Figure 34: Site layout for media and buffer preparation. Media preparation is in the same building but a different room than buffer preparation.

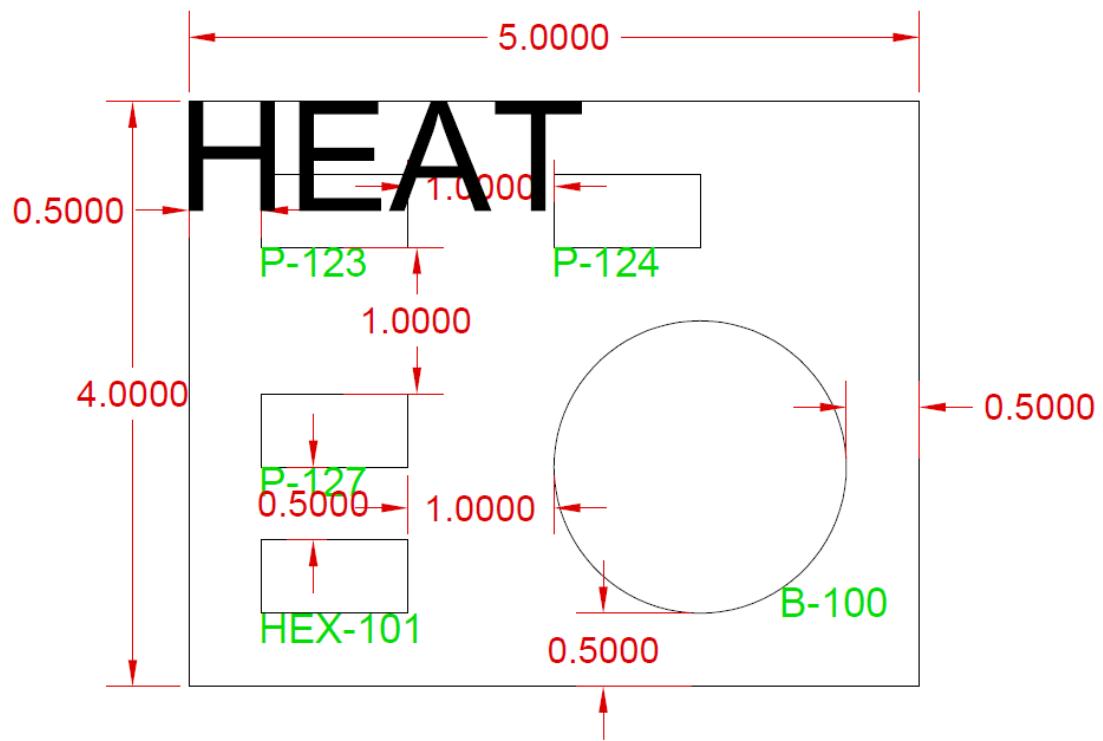


Figure 35: Site layout for heat boiler (Boiler 100).

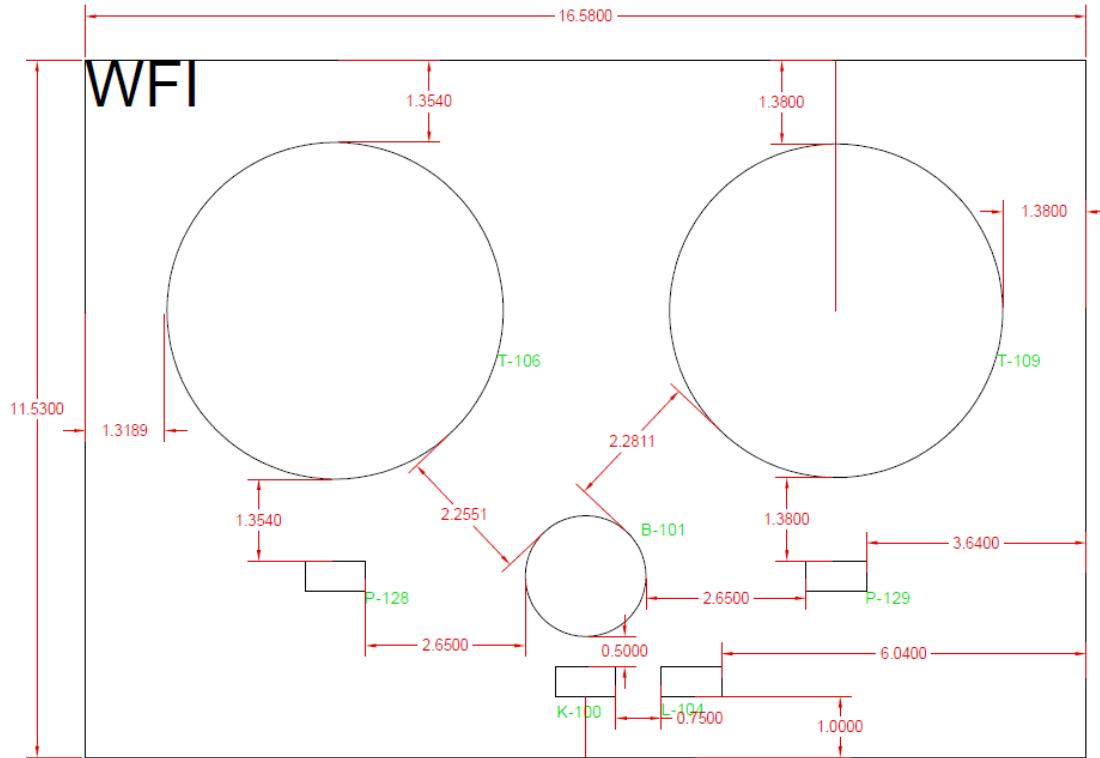


Figure 36: Site layout for WFI and Boiler 101.

13 Other Important Considerations

13.1 Manufacturing WFI Onsite

Water for injection (WFI) is an essential material for biopharmaceutical manufacturing. WFI acts as a solvent and a cleaning agent for the processes. In this design, WFI was purchased from a third party; however, it is possible to manufacture it onsite via vapor compression. It is estimated that onsite manufactured WFI could cost as little as \$3.15 for 1000L compared to purchasing from a third party vendor at \$1000 for 1000 L³⁵. The capital cost for installing this equipment is unknown and is very energy intensive. The process requires a lot of readily available steam, which the plant does not have. The spatial and energy needs are such that the onsite manufacturing of WFI was not considered in this design. A cost analysis of the capital cost of WFI compared to third party purchasing could be performed to make a more informed decision.

13.2 Location

The location for the plant was unknown, so regional utility costs may vary from the pricing used in this report. The land was already purchased, so this was not factored into the costs. Average

³⁵ King, Marita A. "Selection Criteria for WFI Production Equipment, Controlled Environments Magazine, September 2005, http://www.cemag.us/Article_Print.asp?pid=546, Obtained 28 June 2009.).

operational wages were assumed to be \$55,000 a year. Specific state environmental regulations were unknown, so environmental emissions were limited as much as possible. The only possibly toxic emissions the plant produced were from burning natural gas which only produces CO₂ under complete combustion.

13.3 Pressure Safety

The maximum allowable pressure for the plant is 5 bar. Pressure relief valves were placed in the PFDs but were not sized. Wall thicknesses of vessels and pipe diameters were not considered in the scope of this design as well.

13.4 Pressure Drops

The pressure drops the pumps needed to overcome were only due to unit operations and gravity. Pressure drops from valves and piping were not considered. The pumps may need be resized once a more detailed piping and valve layout is produced.

14 Manufacturing/ Operation Costs (Exclusive of Capital Requirements)

Manufacturing costs were separated in terms of raw materials, utilities, operating labor, interest, labor related costs, capital related costs, and sales related costs. All costs were derived from raw materials, operating labor, plant cost, and utilities as shown in *Equation 8*.

$$\text{Manufacturing Cost} = R + U + O + I + L + C + S \quad [8]$$

A breakdown of raw materials, operating labor, and utilities are shown below in *Table 44, 45, and 46*. The interest was assumed to be zero at the start of the plant.

Table 44: Summary of the raw material costs per year.

Raw Material	Amount per year	Total Cost per Year (\$)
WFI	10725000 L	10725000
Acetic Acid	15903 L	12440
Urea	337777440 g	101333
Sodium Acetate	9110815 g	524236
Sodium Chloride	2180000 g	57487
Tris	241070 g	12656
Glycerol	130000 L	9945000
Media	127257 L	7024586
CHO Cells	2500mL	41900
Detergent	11020 L	465564
Protein A Resin	445 L	3560000
Anion Exchange Resin	120 L	370000
	Total	32470203

Table 45: Summary of Utility usage per year

Utility	Amount per year	Unit Cost	Total Cost per Year (\$)
Electricity	$2.24 \cdot 10^7$ kWh	\$0.05/kWh	1122161
Natural Gas	$3.82 \cdot 10^7$ m ³	\$0.005/ft ³	192418
Water	$1.44 \cdot 10^7$ L	\$0.00054/L	7829
Sewer	$1.07 \cdot 10^7$ L	\$0.005/gal	14213
		Total	1336620

Table 46: Summary of operators for each equipment

Equipment Type	Amount	Operators per unit	Total Operators
Stirred Tank	7	0.5	3.5
Bioreactor	14	0.5	7
Centrifuge	1	1	1
Columns	2	0.5	1
Heat Exchanger	4	0.1	0.4
Boilers	2	1	2
Wastewater	1	2	2
		Total	16.9
		Crews	5
		Yearly salary	55000
		Total Cost	4647500

All other manufacturing costs were approximated by raw materials, utilities, operating labor, plant cost (PC), and sales. The manufacturing cost was determined as a function of these costs (*Equation 9*).

$$\begin{aligned} \text{Manufacturing Cost} &= R + U + O + I + L + C + S \\ &= R + U + O + I + 0.6O + 0.26P + 0.2 \text{ (sales)} \end{aligned} \quad [9]$$

Manufacturing cost was determined for a breakeven profit. Sales is a function of profit. To determine the breakeven manufacturing cost the profit was set to zero which yielded a manufacturing cost of \$119000000.

Table 47: Summary of the manufacturing costs

Raw Materials	32470202.9
Utilities	1336619.93
Operating Labor	4647500
Interest	0

Labor related Costs	2788500
Capital Related Costs	53649457.3
Sales related Costs	23723070
Manufacturing Cost	118615350

15 Economic Analysis

The net present value (NPV) for the plant was determined. A 20-year time frame, 20-year MACRS depreciation, 10% interest, and 45% tax were assumed. The revenue for the plant was calculated to be \$1.5 billion assuming the product could be sold at \$2000 per gram. This is a conservative value with other prices reaching as high as \$20,000 per gram³⁶. Over the 20 year time horizon the NPV for the plant was calculated to be 7 billion dollars and the internal rate of return (IRR) was greater than the interest rate. The NPV and IRR both indicate that the plant will be profitable. *Table 48* shows the MACRS analysis.

Table 48: MACRS depreciation and net present value calculation table

Year	MACRS Depreciation	Cash Flow	Depreciation	Taxable Income	Tax	Cash Flow
0.00	0.00E+00	-2.06E+08	0.00E+00	0.00E+00	0.00E+00	-2.06E+08
1.00	2.81E-02	1.51E+09	5.80E+06	1.50E+09	0.00E+00	1.51E+09
2.00	7.29E-02	1.51E+09	1.50E+07	1.49E+09	6.75E+08	8.30E+08
3.00	6.74E-02	1.51E+09	1.39E+07	1.49E+09	6.71E+08	8.35E+08
4.00	6.24E-02	1.51E+09	1.29E+07	1.49E+09	6.71E+08	8.34E+08
5.00	5.79E-02	1.51E+09	1.19E+07	1.49E+09	6.72E+08	8.34E+08
6.00	5.34E-02	1.51E+09	1.10E+07	1.49E+09	6.72E+08	8.33E+08
7.00	4.94E-02	1.51E+09	1.02E+07	1.49E+09	6.72E+08	8.33E+08
8.00	4.57E-02	1.51E+09	9.42E+06	1.50E+09	6.73E+08	8.32E+08
9.00	4.46E-02	1.51E+09	9.20E+06	1.50E+09	6.73E+08	8.32E+08
10.00	4.46E-02	1.51E+09	9.20E+06	1.50E+09	6.73E+08	8.32E+08
11.00	4.46E-02	1.51E+09	9.20E+06	1.50E+09	6.73E+08	8.32E+08
12.00	4.46E-02	1.51E+09	9.20E+06	1.50E+09	6.73E+08	8.32E+08
13.00	4.46E-02	1.51E+09	9.21E+06	1.50E+09	6.73E+08	8.32E+08
14.00	4.46E-02	1.51E+09	9.20E+06	1.50E+09	6.73E+08	8.32E+08
15.00	4.46E-02	1.51E+09	9.21E+06	1.50E+09	6.73E+08	8.32E+08
16.00	4.46E-02	1.51E+09	9.20E+06	1.50E+09	6.73E+08	8.32E+08

³⁶ Kelley, Brian. "Industrialization of MAb Production Technology: The Bioprocessing Industry at a Crossroads." *MAbs*, vol. 1, no. 5, 2009, pp. 443–452.

17.00	4.46E-02	1.51E+09	9.21E+06	1.50E+09	6.73E+08	8.32E+08
18.00	4.46E-02	1.51E+09	9.20E+06	1.50E+09	6.73E+08	8.32E+08
19.00	4.46E-02	1.51E+09	9.21E+06	1.50E+09	6.73E+08	8.32E+08
20.00	4.46E-02	1.51E+09	9.20E+06	1.50E+09	6.73E+08	8.32E+08
21.00	2.79E-02	1.51E+09	5.75E+06	1.50E+09	6.73E+08	8.32E+08
					NPV	\$6,914,043,017.68

16 Conclusions and Recommendations

The Monoclonal Antibody Manufacturing Facility was successful in producing 1000 kg of product a year with using Chinese Hamster Ovary (CHO) suitable for producing titers of 1 g/L to 2 g/L. The plant is recommended to be built based off of an economic analysis yielding a Net Present Value of \$7 billion in twenty years. The facility planning requires more documentation before performing complete construction. Further recommendations to improve the current design are: to perform a cost analysis between imported and on-site production of WFI, to complete low-level development of unit operations, and to develop a pilot plant for complete characterization of unit operations.

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19 Appendix A: Nomenclature

Table 49: PFD notation guide

Variable	Description
R	Reactor
SF	Shake Flask
(P)	Permanent
(D)	Disposable
C	Centrifuge
K	Compressor
V	Valve
CV	Control Valve
PRV	Pressure Relief Valve
P	Pump
D	Exchange Column
T	Tank
M	Mixing Vessel
L	Filter
HEX	Heat Exchanger
RR	Rocking Reactor
	Temperature Probe
	pH Probe
	Conductivity Probe
	Pressure Probe
	Turbidity Probe

Table 50: Variables used in sample calculation in Appendix B

Variable	Description
m	Mass
V	Volume
L	Length
D	Diameter
n	moles
N	Amount (i.e. cycles, disks)
T	Temperature
A	Area
U	Overall Heat Transfer Coefficient
H	Enthalpy
E	Energy
\dot{Q}	Heat Flow
\dot{m}	Mass Flow

\dot{V}	Volumetric Flow
ρ	Density
K_s	Monod Constant
Y	Yield for Monod Kinetics
X	Cell Concentration
S	Substrate Concentration
P	Product Concentration
t	Time
μ	Instantaneous Cell Growth
μ_{max}	Maximum Cell Growth
Q_p	Binding Capacity
d_p	Particle Diameter
η	Efficiency
Σ	Equivalent Area
Q	Centrifuge Flow Rate
ΔP	Pressure

20 Appendix B: Sample Calculations/Simulations

Seed Train

Design Equations:

The seed train reactors were designed in MATLAB consult *Appendix C*.

Material Balances:

ODEs were solved in MATLAB consult *Appendix C*.

Energy Balances and Utilities:

Calculation 1: Total heating water required for seed reactors:

$$\dot{m}_{heating\ water} = \frac{E_{R101} + E_{R102} + E_{R103} + E_{R104}}{Cp_{water}\Delta T} = \frac{4534865 \frac{kJ}{yr}}{4.18 \frac{kJ}{kg\ K} \cdot (80^\circ C - 40^\circ C)} \\ = 2.71 \cdot 10^4 \frac{kg}{yr}$$

Calculation 2: Heat bath for reactors (SF 100-103)

$$E = Power \cdot t_{year} \cdot \eta = 0.35\ kW \cdot 365 \frac{days}{yr} \cdot 24 \frac{hr}{day} \cdot 3600 \frac{s}{hr} \cdot 0.75 = 8.28 \cdot 10^6 \frac{kJ}{yr}$$

Reactor 5 (RR-100):

Calculation 3: Yearly Heating Requirement

$$\begin{aligned} \text{liquid volume} &= 1.105 L \\ m_{cycle} &= \text{liquid volume} \cdot \rho_{water} = 1.105 L \cdot 1000 \frac{g}{L} = 1105 \text{ grams} \\ E &= N_{batches} \cdot \Delta T \cdot m_{cycle} \cdot Cp_{water} = 25 \text{ cycles} \cdot (33^\circ\text{C} - 25^\circ\text{C}) \cdot 4.18 \frac{J}{g^\circ\text{C}} \cdot 1105 g = 923.8 \frac{kJ}{year} \end{aligned}$$

Calculation 4: Yearly Rocking Electricity Requirement

$$E = \text{Power} \cdot t_{year} \cdot \eta = 1.5 \text{ kW} \cdot 365 \frac{\text{days}}{\text{yr}} \cdot 24 \frac{\text{hr}}{\text{day}} \cdot 3600 \frac{\text{s}}{\text{hr}} \cdot 0.75 = 3.55 \cdot 10^7 \frac{kJ}{\text{yr}}$$

Calculation 5: Yearly WFI Requirement

$$\begin{aligned} V_{WFI} &= V_{current \ reactor} - V_{previous \ reactor} = 1.105L - 0.105L = 1L \\ V_{WFI \ yearly} &= V_{WFI} \cdot N_{batches} = 1L \cdot 25 = 25 \frac{L}{\text{yr}} \end{aligned}$$

Reactor 6 (RR-101)

Calculation 6: Yearly Heating Requirement

$$\begin{aligned} \text{liquid volume} &= 6.105 L \\ m_{cycle} &= \text{liquid volume} \cdot \rho_{water} = 6.105 L \cdot 1000 \frac{g}{L} = 6105 \text{ grams} \\ E &= (\text{number of cycles}) \cdot \Delta T \cdot m_{cycle} \cdot Cp_{water} = 25 \text{ cycles} \cdot (33^\circ\text{C} - 25^\circ\text{C}) \cdot 4.18 \frac{J}{g^\circ\text{C}} \cdot 6105 g \\ &= 5104 \frac{kJ}{year} \end{aligned}$$

Calculation 7: Rocking Electricity Requirement

$$E = \text{Power} \cdot t_{year} \cdot \eta = 1.5 \text{ kW} \cdot 365 \frac{\text{days}}{\text{yr}} \cdot 24 \frac{\text{hr}}{\text{day}} \cdot 3600 \frac{\text{s}}{\text{hr}} \cdot 0.75 = 3.55 \cdot 10^7 \frac{kJ}{\text{yr}}$$

Calculation 8: Yearly WFI Requirement

$$\begin{aligned} V_{WFI} &= V_{current \ reactor} - V_{previous \ reactor} = 6.105L - 1.105L = 5L \\ V_{WFI \ yearly} &= V_{WFI} \cdot N_{batches} = 5L \cdot 25 = 125 \frac{L}{\text{yr}} \end{aligned}$$

Reactor 7 (RR-102)

Calculation 9: Yearly Heating Requirement

$$\begin{aligned} \text{liquid volume} &= 26.105 L \\ m_{cycle} &= \text{liquid volume} \cdot \rho_{water} = 26.105 L \cdot 1000 \frac{g}{L} = 26105 \text{ grams} \end{aligned}$$

$$E = (\text{number of cycles}) \cdot \Delta T \cdot m_{cycle} \cdot Cp_{water} = 25 \text{ cycles} \cdot (33^\circ\text{C} - 25^\circ\text{C}) \cdot 4.18 \frac{\text{J}}{\text{g}^\circ\text{C}} \cdot 26105 \text{ g}$$

$$= 21823 \frac{\text{kJ}}{\text{year}}$$

Calculation 10: Rocking Electricity Requirement

$$E = \text{Power} \cdot t_{year} \cdot \eta = 1.5 \text{ kW} \cdot 365 \frac{\text{days}}{\text{yr}} \cdot 24 \frac{\text{hr}}{\text{day}} \cdot 3600 \frac{\text{s}}{\text{hr}} \cdot 0.75 = 3.55 \cdot 10^7 \frac{\text{kJ}}{\text{yr}}$$

Calculation 11: Yearly WFI Requirement

$$V_{WFI} = V_{current \ reactor} - V_{previous \ reactor} = 26.105L - 6.105L = 5L$$

$$V_{WFI \ yearly} = V_{WFI} \cdot N_{batches} = 25L \cdot 25 = 625 \frac{L}{\text{yr}}$$

Reactor 8 (R-101)

Calculation 12: Yearly Heating Requirement

$$\text{liquid volume} = 106.105 \text{ L}$$

$$m_{cycle} = \text{liquid volume} \cdot \rho_{water} = 106.105 \text{ L} \cdot 1000 \frac{\text{g}}{\text{L}} = 106105 \text{ grams}$$

$$E = (\text{number of cycles}) \cdot \Delta T \cdot m_{cycle} \cdot Cp_{water}$$

$$= 25 \text{ cycles} \cdot (33^\circ\text{C} - 25^\circ\text{C}) \cdot 4.18 \frac{\text{J}}{\text{g}^\circ\text{C}} \cdot 106105 \text{ g} = 88704 \frac{\text{kJ}}{\text{year}}$$

Calculation 13: Agitator Electricity Requirement^{37,38}

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot N_{batches} \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{\text{kg}}{\text{m}^3} \cdot (2.3 \frac{\text{rev}}{\text{s}})^3 \cdot (0.15 \text{ m}^3)^5 \cdot 25 \text{ batches} \cdot 13282 \text{ s} = 113 \frac{\text{kJ}}{\text{year}}$$

Calculation 14: Pump P-101 Electricity Requirement

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{\text{kg}}{\text{m}^3} \cdot 9.8 \frac{\text{m}}{\text{s}^2} \cdot (1.32 \text{ m} - 0 \text{ m}) = 12910 \text{ Pa}$$

$$\text{Energy}(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches}$$

$$E = \frac{12910 \text{ Pa}}{0.75} \cdot 26.105 \text{ L} \cdot \frac{1 \text{ m}^3}{1000 \text{ L}} \cdot 25 = 11.23 \frac{\text{kJ}}{\text{year}}$$

³⁷ Mirro, Rich, and Kevin Voll. "Which Impeller Is Right for Your Cell Line?" *BioProcess International*, 30 July 2014, bioprocessintl.com/analytical/cell-line-development/which-impeller-is-right-for-your-cell-line-183538/

³⁸ Hasnaes, Frederik. Experimental Report CellVessel: Motor Sizing . CerCell Aps, 2013, pp. 1–3, Experimental Report CellVessel: Motor Sizing

Calculation 15: Yearly WFI Requirement

$$V_{WFI} = V_{current\ reactor} - V_{previous\ reactor} = 106.105L - 26.105L = 80L$$

$$V_{WFI\ yearly} = V_{WFI} \cdot N_{batches} = 80L \cdot 25 = 2000 \frac{L}{yr}$$

Reactor 9 (R-102)

Calculation 16: Yearly Heating Requirement

$$\text{liquid volume} = 406.105 L$$

$$m_{cycle} = \text{liquid volume} \cdot \rho_{water} = 406.105 L \cdot 1000 \frac{g}{L} = 406105 \text{ grams}$$

$$E = (\text{number of batch}) \cdot \Delta T \cdot m_{cycle} \cdot Cp_{water} = 25 \text{ batch} \cdot (33^\circ\text{C} - 25^\circ\text{C}) \cdot 4.18 \frac{J}{g^\circ\text{C}} \cdot 406105 g$$

$$= 339504 \frac{kJ}{year}$$

Calculation 17: Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot N_{batches} \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot \left(2.3 \frac{rev}{s}\right)^3 \cdot (0.19 m^3)^5 \cdot 25 \text{ batches} \cdot 8882s = 350 \frac{kJ}{year}$$

Calculation 18: Pump P-102 Electricity Requirement

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (1.79m - 0m) = 17507 Pa$$

$$\text{Energy}(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches}$$

$$E = \frac{17507 Pa}{0.75} \cdot 106.105 L \cdot \frac{1m^3}{1000L} \cdot 25 = 61.9 \frac{kJ}{yr}$$

Calculation 19: Yearly WFI Requirement

$$V_{WFI} = V_{current\ reactor} - V_{previous\ reactor} = 406.105L - 106.105L = 300L$$

$$V_{WFI\ yearly} = V_{WFI} \cdot N_{batches} = 300L \cdot 25 = 7500 \frac{L}{yr}$$

Reactor 10 (R-103)

Calculation 20: Yearly Heating Requirement

$$\text{liquid volume} = 1406.105 L$$

$$m_{cycle} = \text{liquid volume} \cdot \rho_{water} = 1406.105 L \cdot 1000 \frac{g}{L} = 1406105 \text{ grams}$$

$$\begin{aligned}
E &= (\text{number of cycles}) \cdot \Delta T \cdot m_{cycle} \cdot C p_{water} \\
&= 25 \text{ cycles} \cdot (33^\circ\text{C} - 25^\circ\text{C}) \cdot 4.18 \frac{\text{J}}{\text{g}^\circ\text{C}} \cdot 1406105 \text{ g} = 1175503.4 \frac{\text{kJ}}{\text{year}}
\end{aligned}$$

Calculation 21: Agitator Electricity Requirement

$$\begin{aligned}
E &= N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot N_{batches} \cdot t_{operation} \\
E &= 0.3 \cdot 998 \frac{\text{kg}}{\text{m}^3} \cdot \left(2.3 \frac{\text{rev}}{\text{s}}\right)^3 \cdot (0.28 \text{ m}^3)^5 \cdot 25 \text{ batches} \cdot 11083 \text{ s} = 2725 \frac{\text{kJ}}{\text{year}}
\end{aligned}$$

Calculation 22: Pump P-103 Electricity Requirement

$$\begin{aligned}
\Delta P &= \rho \cdot g \cdot \Delta h = 998 \frac{\text{kg}}{\text{m}^3} \cdot 9.8 \frac{\text{m}}{\text{s}^2} \cdot (2.58 \text{ m} - 0 \text{ m}) = 25233 \text{ Pa} \\
\text{Energy}(E) &= \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches} \\
E &= \frac{25,233 \text{ Pa}}{0.75} \cdot 406.105 \text{ L} \cdot \frac{1 \text{ m}^3}{1000 \text{ L}} \cdot 25 = 342 \frac{\text{kJ}}{\text{yr}}
\end{aligned}$$

Calculation 23: Yearly WFI Requirement

$$\begin{aligned}
V_{WFI} &= V_{\text{current reactor}} - V_{\text{previous reactor}} = 1406.105 \text{ L} - 406.105 \text{ L} = 1000 \text{ L} \\
V_{WFI \text{ yearly}} &= V_{WFI} \cdot N_{batches} = 1000 \text{ L} \cdot 25 = 25000 \frac{\text{L}}{\text{yr}}
\end{aligned}$$

Reactor 11 (R-104)

Calculation 24: Yearly Heating Requirement

$$\begin{aligned}
\text{liquid volume} &= 3506.105 \text{ L} \\
m_{cycle} &= \text{liquid volume} \cdot \rho_{water} = 3506.105 \text{ L} \cdot 1000 \frac{\text{g}}{\text{L}} = 3506105 \text{ grams} \\
E &= (\text{number of cycles}) \cdot \Delta T \cdot m_{cycle} \cdot C p_{water} \\
&= 25 \text{ cycles} \cdot (33^\circ\text{C} - 25^\circ\text{C}) \cdot 4.18 \frac{\text{J}}{\text{g}^\circ\text{C}} \cdot 3506105 \text{ g} = 2931104 \frac{\text{kJ}}{\text{year}}
\end{aligned}$$

Calculation 25: Agitator Electricity Requirement

$$\begin{aligned}
E &= N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot N_{batches} \cdot t_{operation} \\
E &= 0.3 \cdot 998 \frac{\text{kg}}{\text{m}^3} \cdot \left(2.3 \frac{\text{rev}}{\text{s}}\right)^3 \cdot (0.4 \text{ m}^3)^5 \cdot 25 \text{ batches} \cdot 7077 \text{ s} = 9207 \frac{\text{kJ}}{\text{year}}
\end{aligned}$$

Calculation 26: Pump P-104

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (3.6m - 0m) = 35209 Pa$$

$$Energy(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches}$$

$$E = \frac{17507 Pa}{0.75} \cdot 1406.105 L \cdot \frac{1m^3}{1000L} \cdot 25 = 1650 \frac{kJ}{yr}$$

Calculation 27: Yearly WFI Requirement

$$V_{WFI} = V_{current\ reactor} - V_{previous\ reactor} = 3506.105L - 1406.105L = 3100L$$

$$V_{WFI\ yearly} = V_{WFI} \cdot N_{batches} = 3100L \cdot 25 = 77500 \frac{L}{yr}$$

Production Reactors

Design Equations:

The seed train reactors were designed in MATLAB consult *Appendix C*.

Material Balances:

Calculation 28: Calculates the total mass of substrate added to the production reactors including the initial and fed batch amount.

$$m_{substrate,in} = m_{substrate,initial} + (t \cdot \dot{V}_{fed\ batch} \cdot C_{substrate} \cdot N_{reactors})$$

$$= 17.8 kg + \left(160 hr \cdot 25 \frac{L}{hr} \cdot 0.0029 \frac{kg}{L} \cdot 7 reactors \right) = 99 kg$$

Calculation 29: Calculates the total mass of water added to the production reactors including the initial and fed batch amount.

$$m_{WFI,in} = m_{WFI,initial} + m_{WFI,added} + (t \cdot \dot{V}_{fed\ batch} \cdot N_{reactors} \cdot \rho_{WFI})$$

$$= 3506 kg + 3500 kg + \left(160 hr \cdot 25 \frac{L}{hr} \cdot 7 reactors \cdot 1 \frac{kg}{L} \right) = 35000$$

Calculation 30: Calculates the final number of cells at the end of production. MATLAB was used to determine final cell concentration (*Appendix C*).

$$n_{cells,out} = V_{final} \cdot N_{reactors} \cdot C_{cell\ final} = 5000L \cdot 7 reactors \cdot 7 \cdot 10^9 \frac{cells}{L} = 2.45 \cdot 10^{14} cells$$

Calculation 31: Calculates the final mass of MAb at the end of production. MATLAB was used to determine final MAb concentration (*Appendix C*).

$$m_{mab,out} = V_{final} \cdot N_{reactors} \cdot C_{mab\ final} = 5000L \cdot 7 reactors \cdot 1.4 \frac{g}{L} = 49 kg$$

Calculation 32: The final mass of MAb at the end of production. MATLAB was used to determine final MAb concentration (*Appendix C*).

$$m_{substrate,out} = V_{final} \cdot N_{reactors} \cdot C_{substrate\ final} = 5000L \cdot 7\ reactors \cdot 2.1 \frac{g}{L} = 73.5\ kg$$

Energy Balances and Utilities::

Calculation 33: Yearly Heating Requirement

$$\text{liquid volume} = 5000L$$

$$m_{batch} = \text{liquid volume} \cdot N_{reactors} \cdot \rho_{water} = 5000 L \cdot 7 \cdot 1000 \frac{g}{L} = 3.5 \cdot 10^7 g$$

$$E = N_{batches} \cdot \Delta T \cdot m_{batch} \cdot C_p_{water} = 25\ batches \cdot (33^\circ\text{C} - 25^\circ\text{C}) \cdot 4.18 \frac{J}{g\ ^\circ\text{C}} \cdot 3.5 \cdot 10^7 g$$

$$= 2.926 \cdot 10^7 \frac{kJ}{yr}$$

Calculation 34: Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot N_{batches} \cdot t_{operation} \cdot N_{reactors}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot \left(2.3 \frac{rev}{s}\right)^3 \cdot (0.44 m^3)^5 \cdot 25\ batches \cdot 576000s \cdot 7 = 8.75 \cdot 10^6 \frac{kJ}{yr}$$

Calculation 35: Pump P-105 Electricity Requirement

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (4m - 0m) = 39121 Pa$$

$$\text{Energy}(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches}$$

$$E = \frac{39121 Pa}{0.75} \cdot 3506.105 L \cdot \frac{1m^3}{1000L} \cdot 25 = 4572 \frac{kJ}{yr}$$

Calculation 36: Pump P 106-112 Electricity Requirement

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (4m - 0m) = 39122 Pa$$

$$\text{Energy}(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches}$$

$$E = \frac{39121 Pa}{0.75} \cdot 5000 L \cdot \frac{1m^3}{1000L} \cdot 25 = 6520 \frac{kJ}{yr*pump}$$

Calculation 37: Pump P-113 Electricity Requirement

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (4.85m - 0m) = 47435 Pa$$

$$\text{Energy}(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches}$$

$$E = \frac{47435 Pa}{0.75} \cdot 35000 L \cdot \frac{1m^3}{1000L} \cdot 25 = 55340 \frac{kJ}{yr}$$

Calculation 38: Yearly WFI Requirement

$$V_{WFI} = N_{reactors} \cdot (V_f - V_i) = 7 \cdot (5000L - 500L) = 31500L$$

$$V_{WFI \text{ yearly}} = V_{WFI} \cdot N_{batches} = 31500L \cdot 25 = 787500 \frac{L}{yr}$$

Centrifuge

Design Equations:

Calculation 39: Centrifuge sizing

$$\frac{Q}{\Sigma} = \frac{d^2(\rho_s - \rho_l)}{18\mu} g$$

$$Q = (12.9 \cdot 10^{-6} m)^2 \cdot \frac{1060 \frac{kg}{m^3} - 1000 \frac{kg}{m^3}}{18 \cdot 0.001308 Pa \cdot s} \cdot 9.81 \frac{m}{s^2} = 4.16 \cdot 10^{-6} \frac{m}{s}$$

Solver was used to determine an equivalent area (Σ) that yielded a 12 hr processing time.

$$\sum = 178 m^2$$

$$Q = 2665 \frac{L}{hr}$$

Energy Balances and Utilities::

Calculation 40: Pump P-114 Electricity Required

The Depth Filter (*F-100*) has 10kPa pressure drop along the membrane

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (4.85m - 0m) + 10kPa = 57434 Pa$$

$$Energy(E) = \frac{\Delta P \cdot Q}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches}$$

$$E = \frac{57434 Pa}{0.75} \cdot 35000 L \cdot \frac{1m^3}{1000L} \cdot 25 = 67007 \frac{kJ}{yr}$$

Calculation 41: Centrifuge Electricity Requirement³⁹

$$E = (\text{Average Power Consumption}) \cdot t_{operation}$$

$$50 kW \cdot 12 \frac{hrs}{batch} \cdot 25 batches \cdot 3600 \frac{s}{hr} = 5.4 \cdot 10^7 \frac{kJ}{yr}$$

Protein A

Design Equations:

³⁹ H.M. Amaro, I. Sousa-Pinto, F.X. Malcata, A. Catarina Guedes, 16 - Microalgal fatty acids—From harvesting until extraction, Editor(s): Cristina Gonzalez-Fernandez, Raúl Muñoz, In Woodhead Publishing Series in Energy, Microalgae-Based Biofuels and Bioproducts, Woodhead Publishing, 2017, Pages 369-400, ISBN 9780081010235,

The protein A unit operation was sized in MATLAB consult *Appendix C*.

Material Balances:

Calculation 42: The inlet and outlet masses for acetic acid. Volumes were calculated in MATLAB (*Appendix C*).

$$\begin{aligned} m_{aa,in} &= (V_{pH5} \cdot \% 0.1M_{aa}) + (V_{pH4} \cdot \% 0.1M_{aa}) \\ &= (56200 L \cdot 0.357 \cdot 0.1 \frac{mol}{L}) + (46800 L \cdot 0.847 \cdot 0.1 \frac{mol}{L}) = 5970 mol \cdot 60 \frac{g}{mol} \\ &= 358 kg \\ m_{aa,product\ out} &= V_{product\ out} \cdot \% 0.1M_{aa} = 2800 L \cdot 0.847 \cdot 0.1 \frac{mol}{L} \cdot 60 \frac{g}{mol} = 14.2 kg \\ m_{aa,waste\ out} &= m_{aa,in} - m_{aa,product\ out} = 358 kg - 14.2 kg = 343.8 kg \end{aligned}$$

Calculation 43: The inlet and outlet masses for sodium acetate. Volumes were calculated in MATLAB (*Appendix C*).

$$\begin{aligned} m_{sa,in} &= (V_{pH5} \cdot \% 0.1M_{sa}) + (V_{pH4} \cdot \% 0.1M_{sa}) \\ &= (56200 L \cdot 0.643 \cdot 0.1 \frac{mol}{L}) + (46800 L \cdot 0.153 \cdot 0.1 \frac{mol}{L}) = 5330 mol \cdot 82 \frac{g}{mol} \\ &= 437 kg \\ m_{aa,product\ out} &= V_{product\ out} \cdot \% 0.1M_{aa} = 2800 L \cdot 0.153 \cdot 0.1 \frac{mol}{L} \cdot 82 \frac{g}{mol} = 3.5 kg \\ m_{aa,waste\ out} &= m_{aa,in} - m_{aa,product\ out} = 437 kg - 3.5 kg = 433.5 kg \end{aligned}$$

Calculation 44: The inlet and outlet masses for urea. Volumes were calculated in MATLAB (*Appendix C*).

$$m_{urea,in} = m_{urea,waste\ out} = V_{urea\ solution} \cdot M_{urea} = 28120 L \cdot 8 \frac{mol}{L} \cdot 60 \frac{g}{mol} = 13500 kg$$

Calculation 45: The inlet and outlet masses for WFI. Volumes were calculated in MATLAB (*Appendix C*).

$$\begin{aligned} m_{WFI,in} &= (V_{pH5} + V_{pH4} + V_{urea\ solution} + V_{production}) \cdot \rho_{WFI} \\ &= (56200L + 46800L + 28120L + 35000L) * 1 \frac{kg}{L} = 166120 kg \end{aligned}$$

$$\begin{aligned} m_{WFI,product\ out} &= V_{product} * \rho_{WFI} = 2357L * 1 \frac{kg}{L} = 2357 kg \\ m_{WFI,waste\ out} &= m_{WFI,in} - m_{WFI,product\ out} = 166120 kg - 2357 kg = 163763 kg \end{aligned}$$

Energy Balances and Utilities::

Calculation 46: Pressure Drop along Protein A Column.

$$\Delta P = \frac{150(1 - \xi)^3 \cdot \mu \cdot v_o \cdot L}{\xi^3 \cdot d_p^2}$$

$$\Delta P = \frac{150(1 - 0.8)^3 \cdot 0.001 Pa s \cdot 0.000833 \frac{m}{s} \cdot 0.89m}{0.8^3 \cdot 0.000085^2} = 235 Pa$$

Calculation 47: Pump P-115 Electrical Requirements

$$\begin{aligned}\Delta P &= \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (0.89m - 0m) = 8705 Pa \\ Energy(E) &= \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches} \\ E &= \frac{8705 Pa + 235 Pa}{0.75} \cdot 35000 L \cdot \frac{1m^3}{1000L} \cdot 25 = 10451 \frac{kJ}{yr}\end{aligned}$$

Calculation 48: Pump P-116 Electrical Requirements

$$\begin{aligned}\Delta P &= \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (10.29m - 0m) = 100640 Pa \\ Energy(E) &= \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches} \\ E &= \frac{100640 Pa + 235 Pa}{0.75} \cdot 56230 L \cdot \frac{1m^3}{1000L} \cdot 25 = 1.89 \cdot 10^5 \frac{kJ}{yr}\end{aligned}$$

Calculation 49: Pump P-117 Electrical Requirements

$$\begin{aligned}\Delta P &= \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (10.29m - 0m) = 100640 Pa \\ Energy(E) &= \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches} \\ E &= \frac{100640 Pa + 235 Pa}{0.75} \cdot 46857 L \cdot \frac{1m^3}{1000L} \cdot 25 = 1.57 \cdot 10^5 \frac{kJ}{yr}\end{aligned}$$

Calculation 50: Pump P-118 Electrical Requirements

$$\begin{aligned}\Delta P &= \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (10.29m - 0m) = 100640 Pa \\ Energy(E) &= \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches} \\ E &= \frac{100640 Pa + 235 Pa}{0.75} \cdot 12812 L \cdot \frac{1m^3}{1000L} \cdot 25 = 42978 \frac{kJ}{yr}\end{aligned}$$

Viral Inactivation

Design Equations:

Calculation 51: Operational time for viral inactivation

$$t_{operation} = \frac{V}{\dot{V}} = \frac{2800L}{60 \frac{L}{hr}} = 47 \text{ hr} \sim 50 \text{ hr}$$

Energy Balances and Utilities::

Calculation 52: Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot N_{batches} \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot \left(2.3 \frac{rev}{s}\right)^3 \cdot (0.5 m^3)^5 \cdot 25 \text{ batches} \cdot 3600s = 14291 \frac{kJ}{yr}$$

Calculation 53: Pump P-119 Electricity Requirement

Pressure drop across filters is assumed to be 15000 Pa.

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (2.25m - 0m) = 22006 Pa$$

$$Energy(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot N_{batches}$$

$$E = \frac{22006 Pa + 15000 Pa}{0.75} \cdot 2800 L \cdot \frac{1m^3}{1000L} \cdot 25 = 3454 \frac{kJ}{yr}$$

Polishing

Design Equation:

The protein A unit operation was sized in MATLAB consult *Appendix C*.

Material Balances:

The protein A unit operation was sized in MATLAB consult *Appendix C*.

Energy Balances and Utilities:

Calculation 54: Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot N_{batches} \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot \left(2.3 \frac{rev}{s}\right)^3 \cdot (0.5 m^3)^5 \cdot 25 \text{ batches} \cdot 3600s = 14291 \frac{kJ}{yr}$$

Calculation 55: Pressure Drop along Polishing Column

$$\Delta P = \frac{150(1 - \xi)^3 \cdot \mu \cdot v_o \cdot L}{\xi^3 \cdot d_p^3}$$

$$\Delta P = \frac{150(1 - 0.85)^3 \cdot 0.001 Pa \cdot s \cdot 0.0017 \frac{m}{s} \cdot 2.03m}{0.8^3 \cdot 0.000120^3} = 198 Pa$$

Calculation 56: Pump P-120 Electricity Requirement

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (2.25m - 0m) = 22006 Pa$$

$$Energy(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} * N_{batches}$$

$$E = \frac{22006 Pa}{0.75} \cdot 2800 L \cdot \frac{1m^3}{1000L} \cdot 25 = 2054 \frac{kJ}{year}$$

Calculation 57: Pump P-121 Electricity Requirement

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (10.29 m - 0m) = 100640 Pa$$

$$Energy(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} * N_{batches}$$

$$E = \frac{100640 Pa}{0.75} \cdot 2800 L \cdot \frac{1m^3}{1000L} \cdot 25 = 9393 \frac{kJ}{year}$$

Storage

Design Equations:

Calculation 58: The number of drums per year

Drum size: 200L

Diameter = 0.5 m

Height = 0.8 m

$$N_{drums} = 25 \text{ batches} * 8000 \frac{L}{batch} * \frac{1}{200} \frac{\text{drum}}{L} = 1000 \text{ drums}$$

Calculation 59: The storage room size

Stack the drums 4 high

$$h = 0.8 m * 4 = 3.2 m + 0.8 \text{ (m of spacing)} = 4m$$

The storage room is a square

$$N_{drums \text{ per level}} = \frac{1000 \text{ drums}}{4 \text{ drums per level}} = 250 \text{ drums per level}$$

$$N_{drums \text{ per side}} = \sqrt{250 \text{ drums}} = 15.8 \text{ drums per side}$$

$$\text{length of side} = 16 \text{ drums} * 0.5 m = 8m + 2 \text{ (m for spacing)} = 10 m$$

Storage room size

$$height = 4m, \quad width = 10 m, \quad length = 10m$$

Calculation 60: Total mass of storage room occupants

35/65 water/glycerol mixture

$$\text{Total mass of water} = 1000 \text{ drums} \cdot 200 \frac{L}{\text{drum}} \cdot 35\% \text{ water} \cdot 1000 \frac{kg}{m^3} = 70000kg$$

$$\text{Total mass of glycerol} = 1000 \text{ drums} \cdot 200 \frac{L}{\text{drum}} \cdot 65\% \text{ glycerol} \cdot 1260 \frac{kg}{m^3} = 163800kg$$

$$\text{Total mass of air in room} = (400 m^3 \text{ storage room} - 200 m^3 \text{ drums}) \cdot 1.25 \frac{kg}{m^3} = 250kg$$

Calculation 61: Energy required per year to bring product from 20°C to -20°C

$$\text{Total energy per year} = \Delta H_{\text{water}} + \Delta H_{\text{glycerol}} + \Delta H_{\text{air}} = 3.69 \cdot 10^7 kJ - \text{HYSYS}$$

Calculation 62: Energy requirement for refrigeration cycle

Assumptions:

- 3 days to cool one batch (40 drums)
- The barrels remain at -20°C
- Perfect insulation
- The entire room is cooled every 3 days
-

$$\dot{Q}_{\text{refrigeration cycle}} = \frac{\text{Total energy per year}}{3 \text{ day} * 24 \frac{\text{hr}}{\text{day}} * 3600 \frac{\text{s}}{\text{hr}}} = 142.5 \text{ kW} \text{ operating for 75 days of the year}$$

Calculation 63: Cold heat exchanger area for refrigeration cycle

$$A = \frac{\dot{Q}}{U \cdot \Delta T_{Lm}} = \frac{142.5 \frac{kJ}{s} \cdot \frac{1 \text{ kcal}}{4.18 \text{ kJ}} \cdot 3600 \frac{s}{hr}}{200 \frac{\text{kcal}}{\text{hr} \text{ m}^2 \text{ }^\circ\text{C}} \cdot \left(\frac{(-40 - -20) - (-40 - 25)}{LN \left(\frac{-40 - -20}{-40 - 25} \right)} \right)} = 13.8 \text{ m}^2$$

Calculation 64: Hot heat exchanger area for refrigeration cycle

$$Q_h = (h_4 - h_3)\dot{n}_{cool} = \left(261.9 \frac{kJ}{kg} - 386.3 \frac{kJ}{kg} \right) \cdot 1.87 \frac{kg}{s} = -232.6 \frac{kJ}{s}$$

$$T_{air,out} = \frac{Q}{C_p \cdot \dot{m}} + T_{air,in} = \frac{232.6 \frac{kJ}{s}}{4.184 \frac{kJ}{kg \text{ } ^\circ\text{C}} \cdot \frac{5.5kg}{s}} + 25^\circ\text{C} = 35.1^\circ\text{C}$$

$$A = \frac{\dot{Q}}{U \cdot \Delta T_{Lm}} = \frac{-232.6 \frac{kJ}{s} \cdot \frac{1 \text{ kcal}}{4.18 \text{ kJ}} \cdot 3600 \frac{s}{hr}}{200 \frac{\text{kcal}}{hr \text{ m}^2 \text{ }^\circ\text{C}} \cdot \left(\frac{(63 - 35) - (25 - 20)}{LN\left(\frac{63 - 35}{25 - 20}\right)} \right)} = 64.8 \text{ m}^2$$

Energy Balances and Utilities:*Calculation 65:* Storage Tank Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot (2.3 \frac{rev}{s})^3 \cdot (0.64 \text{ m}^3)^5 \cdot 365 \frac{days}{year} \cdot \frac{86400s}{day} = 9.99 \cdot 10^6 \frac{kJ}{yr}$$

Calculation 66: Glycerol Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot (2.3 \frac{rev}{s})^3 \cdot (1.85 \text{ m}^3)^5 \cdot 365 \frac{days}{year} \cdot \frac{86400s}{day} = 4.69 \cdot 10^8 \frac{kJ}{yr}$$

Calculation 67: Pump P-122 Electricity Requirement

$$\Delta P = \rho * g * \Delta h = 998 \frac{kg}{m^3} * 9.8 \frac{m}{s^2} * (0.85 \text{ m} - 0\text{m}) = 8323 \text{ Pa}$$

$$Energy(E) = \frac{\Delta P * \dot{Q}}{\eta} * \frac{V}{\dot{Q}} = \frac{\Delta P * V}{\eta} * (N_{batches} * N_{drums})$$

$$E = \frac{8323 \text{ Pa}}{0.75} * 8000 \text{ L} * \frac{1\text{m}^3}{1000\text{L}} * 25 * 40 = 2222 \frac{kJ}{yr}$$

Calculation 68: Pump P-130 Electricity Requirement

$$\Delta P = \rho * g * \Delta h = 998 \frac{kg}{m^3} * 9.8 \frac{m}{s^2} * (4.24 \text{ m} - 0\text{m}) = 41500 \text{ Pa}$$

$$Energy(E) = \frac{\Delta P * \dot{Q}}{\eta} * \frac{V}{\dot{Q}} = \frac{\Delta P * V}{\eta} * (N_{batches})$$

$$E = \frac{41500 \text{ Pa}}{0.75} * 5240\text{L} * \frac{1\text{m}^3}{1000\text{L}} * 25 * 40 = 7250 \frac{kJ}{yr}$$

Calculation 69: Rankine Refrigeration Cycle*Table 51:* A summary of the Refrigeration cycle used for this process. The position refers to Figure 37.

Position	Temperature [°C]	Pressure[mPa]	Enthalpy[kJ/kg]	Entropy[J/g/K]
1	-40	0.706	261.9	1.292
2	-40	0.706	338.2	1.619
3	63	4.69	386.3	1.619
4	25	4.69	261.9	1.206

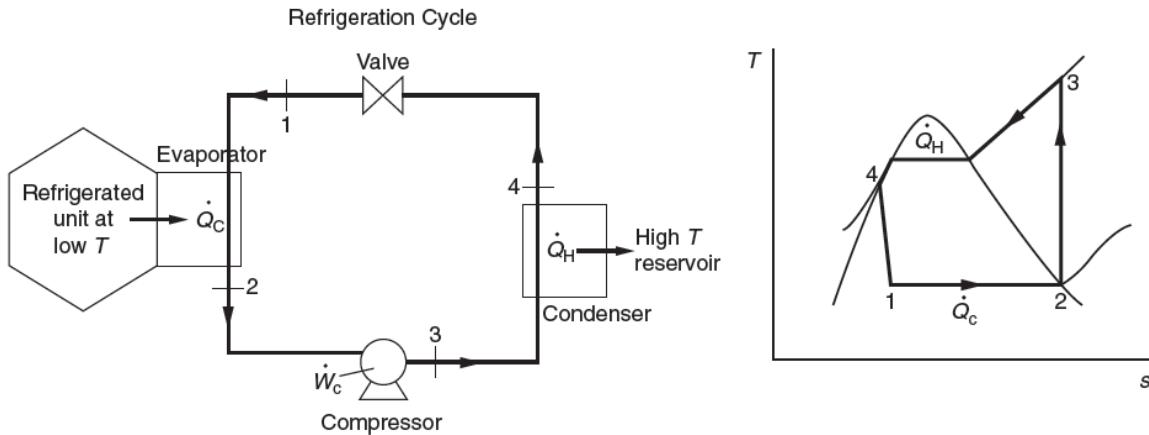


Figure 37: Ideal Rankine Refrigeration cycle. The refrigerated unit is modeled as the storage room where heat is transferred through HEX-102. The compressor will increase the pressure and temperature of the refrigerant(R-23) where it will give off heat through HEX-103. The valve located between position four and one will reduce the pressure, cooling the liquid below the storage room temperature set point.⁴⁰

Calculation 70: Required coolant flow rate

$$n_{cool} = \frac{\dot{Q}_c}{q_c} = \frac{142.5 \text{ kW}}{338.2 \frac{\text{kJ}}{\text{kg}} - 261.94 \frac{\text{kJ}}{\text{kg}}} = 1.87 \frac{\text{kg}}{\text{s}}$$

Calculation 71: Required compressor duty

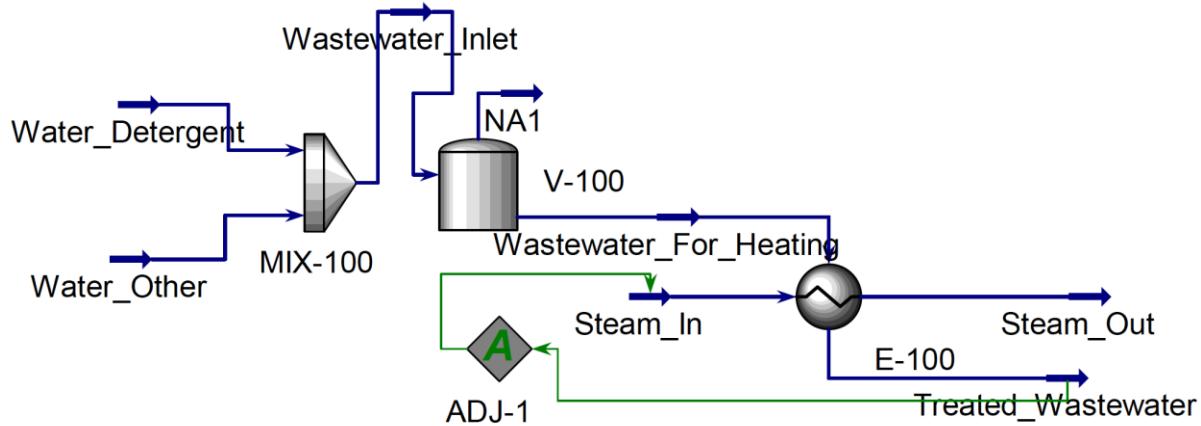
$$w_c = (h_3 - h_2) \cdot n_{cool} = \left(386.3 \frac{\text{kJ}}{\text{kg}} - 338.2 \frac{\text{kJ}}{\text{kg}} \right) \cdot 1.87 \frac{\text{kg}}{\text{s}} = 89.85 \frac{\text{kJ}}{\text{s}}$$

Calculation 72: Required valve duty

$$w_v = (h_4 - h_1) \cdot n_{cool} = \left(261 \frac{\text{kJ}}{\text{kg}} - 261 \frac{\text{kJ}}{\text{kg}} \right) \cdot 1.87 \frac{\text{kg}}{\text{s}} = 0$$

Kill Tanks

⁴⁰ Koretsky, Milo D. *Engineering and Chemical Thermodynamics*. Hoboken, NJ: Wiley, 2004. Print.



Material Streams									
		NA1	Steam_In	Steam_Out	Water_Other	Water_Detergent	Wastewater_Inlet	Wastewater_For_Heating	Treated_Wastewater
Vapour Fraction		1.0000	1.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Temperature	C	32.17	100.0	99.00	25.00	60.00	32.17	32.17	80.00
Pressure	kPa	101.3	101.3	101.3	101.3	101.3	101.3	101.3	101.3
Molar Flow	kgmole/h	0.0000	8.093	8.093	72.16	18.62	90.78	90.78	90.78
Mass Flow	kg/h	0.0000	145.8	145.8	1300	335.5	1635	1635	1635
Liquid Volume Flow	m ³ /h	0.0000	0.1461	0.1461	1.303	0.3361	1.639	1.639	1.639
Heat Flow	kJ/h	-0.0000	-1.931e+006	-2.260e+006	-2.056e+007	-5.256e+006	-2.581e+007	-2.581e+007	-2.548e+007

Design Equations:

Calculation 73: Overall Heat Transfer Area:

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

$$323057 \frac{kJ}{hr} = 418 \frac{kJ}{hr m^2 K} \cdot A \cdot \frac{(373K - 305.73K) - (373 K - 353 K)}{\ln \left(\frac{373 K - 305.73 K}{373 K - 353 K} \right)}$$

$$A = 19.8 m^2$$

Energy Balances and Utilities:

Calculation 74: Yearly Heating Required

$$Q = \dot{m}C_p dT$$

$$\dot{m} = \frac{V_{waste\ water\ per\ batch} \cdot N_{batches} \cdot \rho_{waste\ water}}{t_{operation}} = \frac{429800L \cdot 25 \cdot 1 \frac{kg}{L}}{365 \frac{day}{yr} \cdot 24 \frac{hrs}{day} \cdot 0.75} = 1635 \frac{kg}{hr}$$

$$Q = \left(1635 \frac{kg}{hr} \right) \cdot \left(4.18 \frac{kJ}{kgK} \right) \cdot (353 K - 305.73 K) = 323057 \frac{kJ}{hr}$$

$$E = \dot{Q} \cdot 24 \frac{hr}{day} \cdot 365 \frac{day}{yr} \cdot 0.75 = 2.12 \cdot 10^9 \frac{kJ}{yr}$$

Calculation 75: City Water Required

$$Q = \dot{m}\Delta H_{vap}$$

$$323057 \frac{kJ}{hr} = \dot{m}_{steam} (2256.5 \frac{kJ}{kg})$$

$$\dot{m}_{steam} = \dot{m}_{city\ water} = 143.2 \frac{kg}{hr} = 9.4 \cdot 10^5 \frac{kg}{yr}$$

CIP

Material Balance:

Calculation 76: CIP Flow Rate used to calculate the amount of cleaning solution and WFI used for the process.

V = Volume [L]

h = height [m]

d = diameter [m]

c = circumference [m]

\dot{V} = flow rate [gpm]

V = 5000 L

d = 1.5 m

h = 2.8 m

$$c = d \cdot \pi = 1.5 \text{ m} \cdot \pi = 4.7 \text{ m} \cdot 3.3 \frac{ft}{m} = 15.6 \text{ ft}$$

$$\dot{V} = c \cdot \frac{3 \text{ gpm}}{ft} = 47 \text{ gpm}$$

Energy Balance and Utilities:

Calculation 77: Yearly Heating Requirements

$$E = \dot{m}_{detergent} \cdot C_p_{water} \cdot \Delta T = \left(2.2 \cdot 10^6 \frac{kg}{yr}\right) \cdot 4.18 \frac{kJ}{kg} \cdot (60^\circ\text{C} - 25^\circ\text{C}) = 3.22 \cdot 10^8 \frac{kJ}{yr}$$

Calculation 78: Non-Contact Steam to Heat Detergent

$$\dot{m}_{steam\ max} = \frac{\dot{Q}_{max}}{\Delta H_{vap}} = \frac{3335640 \frac{kJ}{hr}}{2256.5 \frac{kJ}{kg}} = 1478 \frac{kg}{hr}$$

$$\dot{m}_{steam\ year} = \frac{E}{\Delta H_{vap}} \cdot 24 \frac{hr}{day} \cdot 365 \frac{day}{yr} \cdot 0.75 \text{ efficiency} = 142569 \frac{kg}{yr}$$

Calculation 79: Pump P-123 Electricity Requirements

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (4.85m - 0m) = 47434 Pa$$

$$Energy(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot 0.75 \text{ of year}$$

$$Energy(E) = \frac{47434 \text{ Pas} \cdot 0.00309 \frac{\text{m}^3}{\text{s}}}{0.75} \cdot 0.75 \left(365 \text{ days} \cdot \frac{86400 \text{ s}}{1 \text{ day}} \right) = 4.62 \cdot 10^6 \frac{\text{kJ}}{\text{yr}}$$

Calculation 80: Pump P-124 Electricity Requirements
Pressure drop over HEX-101 is 75 kPa

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{\text{kg}}{\text{m}^3} \cdot 9.8 \frac{\text{m}}{\text{s}^2} \cdot (4.85 \text{ m} - 0 \text{ m}) + 75 \text{ kPa} = 122434 \text{ Pa}$$

$$Energy(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot 0.75 \text{ of year}$$

$$Energy(E) = \frac{122434 \text{ Pa} \cdot 0.00309 \frac{\text{m}^3}{\text{s}}}{0.75} \cdot 0.75 \left(365 \text{ days} \cdot \frac{86400 \text{ s}}{1 \text{ day}} \right) = 1.19 \cdot 10^7 \frac{\text{kJ}}{\text{yr}}$$

Calculation 81: Air Compressor (K-100)

$$E = W_S * t = \frac{R \cdot T_{in} \cdot k}{\eta \cdot (k - 1)} \left[\left(\frac{P_{out}}{P_{in}} \right)^{\frac{(k-1)}{k}} - 1 \right] \cdot t$$

$$E = \frac{8.31 \frac{\text{J}}{\text{mol} \cdot \text{K}} * (22 + 273) \cdot k \cdot 1.4}{0.75 \cdot (1.4 - 1)} \left[\left(\frac{3 \text{ bar}}{1 \text{ bar}} \right)^{\frac{(1.4-1)}{1.4}} - 1 \right] \cdot \left(0.75 \cdot 365 \frac{\text{days}}{\text{year}} \cdot 86400 \frac{\text{s}}{\text{day}} \right)$$

$$= 9.97 \cdot 10^7 \frac{\text{kJ}}{\text{yr}}$$

Calculation 82: Detergent Heat Exchanger Area

Assume saturated steam is used as the heating fluid and overall heat transfer coefficient is 418 (kJ/hr/m²/°C). The max flow rate for CIP is 100 gpm, so the heat exchanger is sized off this flow rate.

$$\dot{Q} = UA(\Delta T_{lm})$$

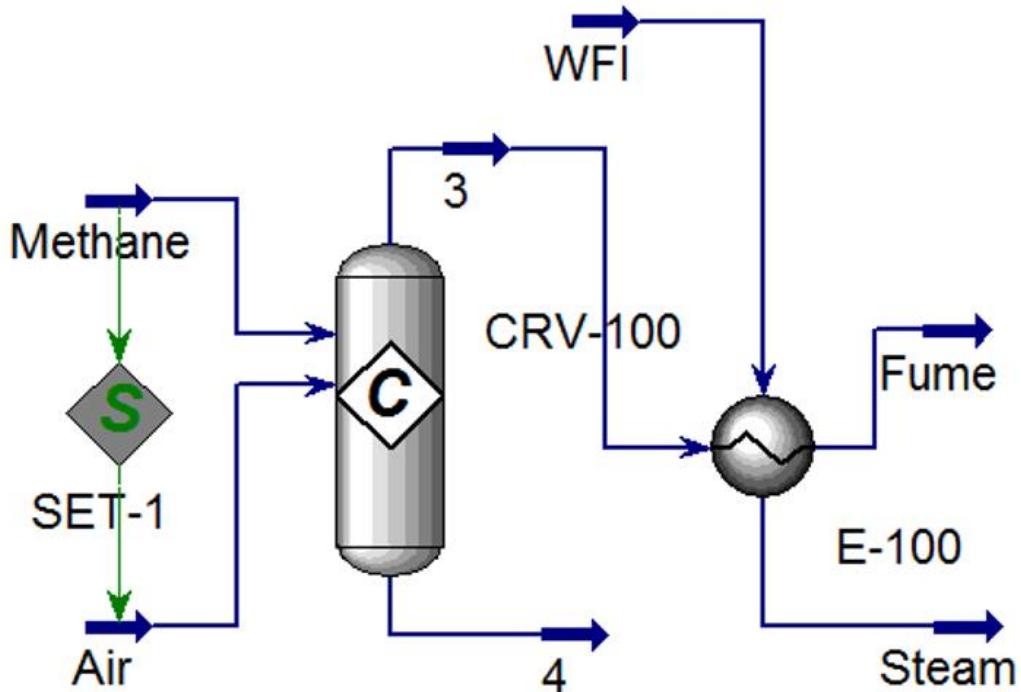
$$\Delta T_{lm} = \frac{\Delta T_2 - \Delta T_1}{\ln \frac{\Delta T_2}{\Delta T_1}} = \frac{(100 - 60) - (100 - 25)}{\ln \frac{(100 - 60)}{(100 - 25)}} = 56^\circ\text{C}$$

$$\dot{Q}_{max} = \dot{V}_{max} \cdot \rho_{water} \cdot Cp_{water} \cdot \Delta T = 100 \frac{\text{gal}}{\text{min}} \cdot 3.8 \frac{\text{L}}{\text{gal}} \cdot 1 \frac{\text{kg}}{\text{L}} \cdot 4.18 \frac{\text{kJ}}{\text{kg}^\circ\text{C}} \cdot (60^\circ\text{C} - 25^\circ\text{C}) \cdot 60 \frac{\text{min}}{\text{hr}} = 3335640 \frac{\text{kJ}}{\text{hr}}$$

$$A = \frac{3335640 \frac{kJ}{hr}}{418 \frac{kJ}{hr m^2 ^\circ C} \cdot 56^\circ C} = 142.5 m^2$$

SIP Boiler 101

Design Equations: HYSYS simulation for Boiler 101



Material Streams								
		Methane	Air	3	4	WFI	Steam	Fume
Vapour Fraction		1.0000	1.0000	1.0000	0.0000	0.0000	1.0000	1.0000
Temperature	C	22.00	22.00	1406	1406	22.00	150.8	90.00
Pressure	kPa	600.0	300.0	300.0	300.0	101.0	200.0	101.0
Molar Flow	kgmole/h	178.4	396.3	574.7	0.0000	699.4	699.4	574.7
Mass Flow	kg/h	2862	1.145e+004	1.431e+004	0.0000	1.260e+004	1.260e+004	1.431e+004
Liquid Volume Flow	m3/h	9.559	13.19	21.86	0.0000	12.63	12.63	21.86
Heat Flow	kW	-3723	-12.35	-3735	0.0000	-5.553e+004	-4.617e+004	-1.309e+004

Compositions							
	Methane	Air	3	4	WFI	Steam	Fume
Comp Mole Frac (Methane)	1.0000	0.0000	0.2346	0.2346	0.0000	0.0000	0.2346
Comp Mole Frac (H ₂ O)	0.0000	0.0000	0.1517	0.1517	1.0000	1.0000	0.1517
Comp Mole Frac (Oxygen)	0.0000	0.2200	-0.0000	0.0000	0.0000	0.0000	-0.0000
Comp Mole Frac (CO ₂)	0.0000	0.0000	0.0759	0.0759	0.0000	0.0000	0.0759
Comp Mole Frac (Nitrogen)	0.0000	0.7800	0.5379	0.5379	0.0000	0.0000	0.5379

Energy Balances and Utilities:

Calculation 83: Yearly Energy Requirements

$$E = \dot{m}_{steam}(\hat{h}_{steam(121^{\circ}\text{C}, Sat.)} - \hat{h}_{water(25^{\circ}\text{C}, 101kPa)}) = 289000 \frac{\text{kg}}{\text{yr}} \left(2707.4 \frac{\text{kJ}}{\text{kg}} - 104.82 \frac{\text{kJ}}{\text{kg}} \right)$$

$$= 7.52 * 10^8 \frac{\text{kJ}}{\text{yr}}$$

Calculation 84: Maximum Energy Requirement

$$\dot{E}_{max} = \dot{m}_{steam,max}(\hat{h}_{steam(121^{\circ}\text{C}, Sat.)} - \hat{h}_{water(25^{\circ}\text{C}, 101kPa)}) = 3.5 \frac{\text{kg}}{\text{s}} \left(2707.4 \frac{\text{kJ}}{\text{kg}} - 104.82 \frac{\text{kJ}}{\text{kg}} \right)$$

$$= 9109 \text{ kW}$$

Calculation 85: Yearly WFI Requirement

$$\dot{m}_{WFI} = \dot{m}_{steam\ per\ batch} + N_{batches} \cdot \dot{m}_{blow\ down} = \dot{m}_{steam} + 25 \cdot 0.05 \dot{m}_{steam\ per\ batch} =$$

$$11560 \frac{\text{kg}}{\text{yr}} + 25 \cdot 0.05 \cdot \frac{11560 \text{kg}}{\text{yr}} = 26010 \frac{\text{kg}}{\text{yr}}$$

Calculation 86: Pump P-128 Electricity Requirement

$$\Delta P = \rho \cdot g \cdot \Delta h = 75 \text{ kPa} \text{ (Assumed heat exchanger)}$$

$$Energy(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot 0.75 \text{ of year}$$

$$Energy(E) = \frac{75000 \text{ Pa} * 0.0035 \frac{\text{m}^3}{\text{s}}}{0.75} \cdot 0.75 \left(365 \text{ days} \cdot \frac{86400 \text{ s}}{1 \text{ day}} \right) = 8.28 * \frac{\text{kJ}}{\text{year}}$$

Calculation 87: Pump P-129 Electricity Requirement

$$\Delta P = \rho \cdot g \cdot \Delta h = 998 \frac{kg}{m^3} \cdot 9.8 \frac{m}{s^2} \cdot (6.13m - 0m) = 62249 Pa$$

$$Energy(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot \frac{V}{\dot{Q}} = \frac{\Delta P \cdot V}{\eta} \cdot (N_{batches})$$

$$E = \frac{62249 Pa}{0.75} \cdot 397000L \cdot \frac{1m^3}{1000L} \cdot 25 = 8.24 \cdot 10^5 \frac{kJ}{year}$$

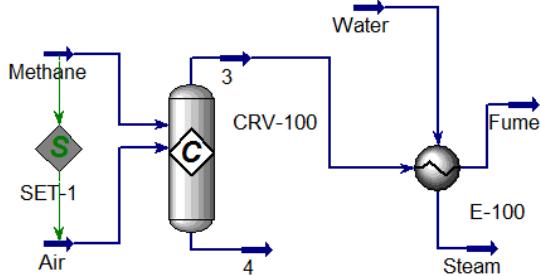
Calculation 88: Boiler Size (Boiler 101)

$$A = \frac{\dot{Q}}{U \cdot \Delta T_{Lm}}$$

$$A = \frac{9109 \frac{kJ}{s} \cdot \frac{1 kcal}{4.18 kJ} \cdot 3600 \frac{s}{hr}}{200 \frac{kcal}{hr m^{2 \circ C}} \cdot \left(\frac{(151 - 90) - (1406 - 22)}{LN\left(\frac{151 - 90}{1406 - 22}\right)} \right)} = 92.47 m^2$$

Heat Boiler 100

Design Equations: HYSYS simulation of Boiler 100



Material Streams						
	Methane	Air	3	4	Water	Steam
Vapour Fraction	1.0000	1.0000	1.0000	0.0000	0.0000	1.0000
Temperature	C	22.00	22.00	1406	1406	22.00
Pressure	kPa	600.0	300.0	300.0	300.0	101.0
Molar Flow	kgmole/h	2.061	4.577	6.638	0.0000	8.326
Mass Flow	kg/h	33.06	132.2	165.3	0.0000	150.0
Liquid Volume Flow	m ³ /h	0.1104	0.1524	0.2525	0.0000	0.1503
Heat Flow	kW	-43.00	-0.1426	-43.14	0.0000	-661.1
						-553.6
						-150.6

Compositions						
	Methane	Air	3	4	Water	Fume
Comp Mole Frac (Methane)	1.0000	0.0000	0.2346	0.2346	0.0000	0.0000
Comp Mole Frac (H ₂ O)	0.0000	0.0000	0.1517	0.1517	1.0000	1.0000
Comp Mole Frac (Oxygen)	0.0000	0.2200	0.0000	0.0000	0.0000	0.0000
Comp Mole Frac (CO ₂)	0.0000	0.0000	0.0759	0.0759	0.0000	0.0000
Comp Mole Frac (Nitrogen)	0.0000	0.7800	0.5379	0.5379	0.0000	0.5379

Energy Balances and Utilities:

Calculation 89: Maximum Energy Requirement

$$\dot{E}_{max} = \dot{m}_{steam,max} (\hat{h}_{steam(100^\circ C, Sat.)} - \hat{h}_{water(25^\circ C, 101kPa)}) = 0.44 \frac{kg}{s} \left(2695 \frac{kJ}{kg} - 104.82 \frac{kJ}{kg} \right) = 1135 kW$$

Calculation 90: Boiler Size (Boiler 100)

$$A = \frac{\dot{Q}}{U \cdot \Delta T_{Lm}}$$

$$A = \frac{1135 \frac{kJ}{s} * \frac{1 kcal}{4.18 kJ} * 3600 \frac{s}{hr}}{200 \frac{kcal}{hr m^2 \circ C} \cdot \left(\frac{(100 - 90) - (1406 - 22)}{LN\left(\frac{100 - 90}{1406 - 22}\right)} \right)} = 17.56 m^2$$

Calculation 91: Pump P-127 Electricity Requirement

$$\Delta P = \rho \cdot g \cdot \Delta h = 75 kPa \text{ (Assumed heat exchanger)}$$

$$Energy(E) = \frac{\Delta P \cdot \dot{Q}}{\eta} \cdot 0.75 \text{ of year}$$

$$Energy(E) = \frac{75000Pa \cdot 0.0000416 \frac{m^3}{s}}{0.75} \cdot 0.75 \left(365 \text{days} \cdot \frac{86400s}{1 \text{day}} \right) = 9.85 \cdot 10^7 \frac{kJ}{yr}$$

Media Preparation

Energy Balances and Utilities:

Calculation 92: M-100 Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot \left(2.3 \frac{rev}{s} \right)^3 \cdot (1.05 m^3)^5 \cdot 365 \frac{days}{year} \cdot \frac{86400s}{day} \cdot 0.75 = 1.15 \cdot 10^8 \frac{kJ}{yr}$$

Buffer Preparation

Energy Balances and Utilities:

Calculation 93: M-102 Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot \left(2.3 \frac{rev}{s} \right)^3 \cdot (1.25 m^3)^5 \cdot 365 \frac{days}{year} \cdot \frac{86400s}{day} \cdot 0.75 = 2.49 \cdot 10^8 \frac{kJ}{yr}$$

Calculation 94: M-103 Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot \left(2.3 \frac{rev}{s} \right)^3 \cdot (1.14 m^3)^5 \cdot 365 \frac{days}{year} \cdot \frac{86400s}{day} \cdot 0.75 = 1.76 \cdot 10^8 \frac{kJ}{yr}$$

Calculation 95: M-104 Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot \left(2.3 \frac{rev}{s} \right)^3 \cdot (0.98 m^3)^5 \cdot 365 \frac{days}{year} \cdot \frac{86400s}{day} \cdot 0.75 = 8.18 \cdot 10^7 \frac{kJ}{yr}$$

Calculation 96: M-105 Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot \left(2.3 \frac{rev}{s} \right)^3 \cdot (0.428 m^3)^5 \cdot 365 \frac{days}{year} \cdot \frac{86400s}{day} \cdot 0.75 = 1.30 \cdot 10^6 \frac{kJ}{yr}$$

Calculation 97: M-107 Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot \left(2.3 \frac{rev}{s}\right)^3 \cdot (1.41 m^3)^5 \cdot 365 \frac{days}{yr} \cdot \frac{86400s}{day} \cdot 0.75 = 3.40 \cdot 10^8 \frac{kJ}{yr}$$

Calculation 98: M-108 Agitator Electricity Requirement

$$E = N_p \cdot \rho \cdot n^3 \cdot D_a^5 \cdot t_{operation}$$

$$E = 0.3 \cdot 998 \frac{kg}{m^3} \cdot \left(2.3 \frac{rev}{s}\right)^3 \cdot (1.12 m^3)^5 \cdot 365 \frac{days}{yr} \cdot \frac{86400s}{day} \cdot 0.75 = 1.61 \cdot 10^8 \frac{kJ}{yr}$$

Costing

Calculation 98: Example U-tube Shell and Tube Heat Exchanger (Costing Curve)

$$C = a + b(S)^n$$

From Appendix D: S=19.8 m²; a=28000; b=54; n=1.2

$$C = 28000 + 54 * (19.8 m^2)^{1.2} = \$29942.6$$

Calculation 99: Example Cone Roof Tank (Used to model holding tanks) (Costing Curve)

$$C = a + b(S)^n$$

From Appendix D: S=250000 m³; a=5800; b=1600; n=0.7

$$C = 5800 + 1600 * (250000 m^3)^{0.7} = \$9.615 \cdot 10^6$$

Calculation 100: Example Centrifugal Pump (Costing Curve)

$$C = a + b(S)^n$$

From Appendix D: S=6.31 L/s; a=8000; b=240; n=0.9

$$C = 8000 + 240 * \left(6.31 \frac{L}{s}\right)^{0.9} = \$9259.61$$

Calculation 101: Equipment Costing Using Scaling

$$C = a + b(S)^n$$

$$C = C_{SuperPro} * \left(\frac{\text{size}}{\text{size}_{SuperPro}}\right)^{0.6}$$

Calculation 102: Net Present Value (NPV) Analysis

$$\text{Depreciation} = (\text{Plant Cost} - \text{Salvage Value}) * \text{MACRS}$$

$$Tax = (Profit - Depreciation) * (tax \%)$$

$$Cash Flow = Profit - Tax$$

$$Present Value (PV) = \frac{Cash Flow}{(1 + interest)^{year}}$$

$$NPV = \sum_{n=1}^{n=t} \frac{Cash Flow}{(1 + i')^n}$$

21 Appendix C: MATLAB Simulation

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21.2 General

This section lists all general values needed to start the process. The values found here are either from the AIChE report, calculated, or from an external source.

```
% Housekeeping
close all; clc; format short; clear all
tic %starts timer

final_needed = 1000; %kg/year
final_needed = final_needed/365/24; %kg/hr
cell_production_rate = 25; % pg/(cell·day)
```

```

cell_production_rate = cell_production_rate*(10^(-15))/(24); % Kg/(cell·hr)
cell_conc_cutoff = 7*10^9; % [cell/L] maximum concentration cells can reach
cell_weight = 2*10^(-9); % Kg/cell
productivity = 25*10^(-12)/24; %g/cell/hour

plot_switch = 0; % turns plots on [1] and off [0]

```

21.3 Seed Train

The seed train calculations incorporate ODE45 to model the batch growth of cells in each tank. Once the cells reach the maximum concentration, the code moves onto the tank. The concentration of cells decreases and the concentration of substrate increases, promoting cell growth.

```

%Define growth characteristics, determined from runs in bioreactor
Ks = 0.286; % g/L
mu_max = 0.0193; % 1/hr    max growth rate
kd = 0.000;      % endogenous (cell death) maintanence metabolism

%batch growth parameters
t_input_batch = 10000; % (hr) length of time for batch
initial_cell = 1*10^6; % intial number of cells
P_init = 0; % g/L

% Substrate Polynomial - taken from source, used to optimize yield
Sub_x = flip([4.8 3.6 2.4 1.2]); % substrate conc.
Sub_y = flip([1.08E+10 7.25E+9 3.58E+9 2.69E+9]); % productivity conc.
Trend_Sub = polyfit(Sub_x,Sub_y,2); % Builds 2nd order polynomial

vol = [5/1000 15/1000 35/1000 50/1000 1 5 20 80 300 1000 2100];
Sub_conc = ones(1,length(vol))*5.5; % starting sub. conc. in each tank

% Allocated space
Seed_volume = zeros(length(vol),1);
cell_plot = zeros(124,1);
time_plot = zeros(1,124);
ppp = zeros(length(vol),1);
time_seed = zeros(length(vol),1);
final_num_cell = zeros(124,1);
final_num_substrate = zeros(124,1);
substrate_balance = zeros(1,length(vol));
product_balance = zeros(1,length(vol));
cell_balance = zeros(1,length(vol));

% For loop to run through each tank volume
for tank = 1 : length(vol)

    %If statement to kickstart the substrate and cell concentrations
    if tank == 1
        Seed_volume(tank) = Vol(tank);
        X_init = initial_cell/Seed_volume(tank); % [cell/L] init. cell c
        Y_xs = polyval(Trend_Sub,Sub_conc(tank)); % [cell/mg] yield

```

```

s_init = Sub_conc(tank); % Initial substrate concentration
else
    Seed_volume(tank) = (vol(tank) + Seed_volume(tank-1));
    X_init = final_num_cell(tank-1)/Seed_volume(tank); % finds new cell
    S_init = (final_num_substrate(tank-1)+Sub_conc(tank)...
        *vol(tank))/Seed_volume(tank); % Finds new substrate conc
    Y_xs = polyval(Trend_Sub,Sub_conc(tank)); % [cell/mg] yield
    P_init = (product_plot(end)*Seed_volume(tank-1))...
        /Seed_volume(tank); % finds new product concentration

end
ppp(tank) = S_init;

%%Define the time span for batch growth
t_span_batch = [0,t_input_batch]; % (hr)

components_initial_batch = [X_init; % X initial (g/L)
    S_init; % S initial (g/L)
    P_init]; % P initial (g/L)

% ODE - Sent to function found below
[t_batch,components_batch] = ode45(@(t_batch,components_batch)...
    batch(t_batch,components_batch,Ks,mu_max,Y_xs,Kd,vol(tank)...
    ,productivity),t_span_batch,components_initial_batch);

% Delete Function - This is implemented to find where the cells reach
% their maximum concentration and then the next tank overwrites the
% next portion
for i = 1:length(components_batch(:,2))
    if components_batch(i,1) < cell_conc_cutoff || components_batch(i,1) < 0.96
        buffer(i,1) = components_batch(i,1); % temporary variable
        buffer(i,2) = components_batch(i,2); % temporary variable
        buffer(i,3) = components_batch(i,3); % temporary variable
        time_buff(i) = t_batch(i);
    end
end

clear components_batch t_batch % prepares for next loop
components_batch = buffer; % sets new variable
t_batch = time_buff; % sets new variable
time_seed(tank) = t_batch(end)/24; % reports time for each tank

% Stores in array for plotting
if tank == 1
    time_plot = t_batch; % xaxis - time
    cell_plot = components_batch(:,1); % cells conc.
    substrate_plot = components_batch(:,2); % substrate conc.
    product_plot = components_batch(:,3); % product conc.
    final_num_cell(tank) = cell_plot(end)*Seed_volume(tank); % cells
    num_cell = components_batch(:,1)*Seed_volume(tank); % cells
    final_num_substrate(tank) = substrate_plot(end)*...
        Seed_volume(tank); % substrate
end

```

```

else
    time_plot = horzcat(time_plot,(t_batch+...
        time_plot(end))); % builds time conc data
    cell_plot = vertcat(cell_plot,...%
        components_batch(:,1)); % builds cell conc data
    substrate_plot = vertcat(substrate_plot,...%
        components_batch(:,2)); % builds substrate conc data
    product_plot = vertcat(product_plot,...%
        components_batch(:,3)); % builds product conc data
    final_num_cell(tank) = cell_plot(end)...
        *Seed_volume(tank); % builds cell data
    num_cell = vertcat(num_cell,components_batch(:,1)*...
        Seed_volume(tank)); % builds cell data
    final_num_substrate(tank) = substrate_plot(end)*...
        Seed_volume(tank); % builds substrate data
end

substrate_balance(tank) = substrate_plot(end).*Seed_volume(tank);
product_balance(tank) = product_plot(end).*Seed_volume(tank);
cell_balance(tank) = cell_plot(end).*Seed_volume(tank);

clear buffer time_buff % prepares for next loop

end
Tank_Number = 1:length(vol);
blake = table(Tank_Number',substrate_balance',product_balance',cell_balance');
blake.Properties.VariableNames = {'Tank_Number' 'Substrate_Out' 'Product_Out',...
    'Cells_Out'}; % Changes table nammes
fprintf('=====\\n')
fprintf('Seed Train:\\n\\n')
fprintf('Days for final cell conc: %.2f\\n\\n', time_plot(end)/24)
disp(blake)
fprintf('=====\\n')
=====
```

Seed Train:

Days for final cell conc: 38.26

Tank_Number	Substrate_Out	Product_Out	Cells_Out
1	0.025396	0.001672	3.039e+07
2	0.10177	0.0065432	1.1597e+08
3	0.27809	0.019408	3.4191e+08
4	0.53105	0.036951	6.4988e+08
5	5.543	0.425	7.4669e+09
6	30.874	2.1497	3.7765e+10
7	133.12	8.3139	1.4607e+11
8	530.69	42.059	7.3869e+11
9	2077.2	124.32	2.184e+12

10	7128.9	480.85	8.4469e+12
11	17850	1140.3	2.0029e+13

21.4 Production reactors

Production reactors are implemented into obtain the final concentration of product as well as the final amount of product. The cell broth from the final seed train is split into seven production reactors, where each reactor has 1000 L of media to start off. Once the cells enter the reactors, a fed-batch process begins until the final volume reaches 5000L

```

number_reactors = 7; % number or production reactors
Reactor_vol = 500; % Initial reactor volumes
C_init = num_cell(end)/number_reactors/Seed_volume(end); % init. Conc.
Product_final_conc = [1 2]; % Final product
P_init = product_plot(end);
X_init = cell_plot(end);

%fed batch growth parameters
t_input_fed = 180; % (hr) length of time for fed bath
S_fed = 2.9; % (g/L) substrate concentration
F = 25; % (L/hr) feed flowrate

% Define the time span for fed batch growth
t_span_fed = [0,t_input_fed]; % (hr)

components_initial_fed = [Reactor_vol; % v (L)
    X_init; % X (g/L)
    S_fed; % S (g/L)
    P_init; % P (g/L)
    C_init]; % C (cells)

% ODE for fed batch
[t_fed,components_fed] = ode45(@(t_fed,components_fed)...
    fed_batch(t_fed,components_fed,ks,mu_max,Y_xs,S_fed,F, ...
    productivity,Kd),t_span_fed,components_initial_fed);

% Stores array for plot access
fed_time_plot = t_fed;
fed_volume_plot = components_fed(:,1);
fed_cell_plot = components_fed(:,2);
fed_substrate_plot = components_fed(:,3);
fed_product_plot = components_fed(:,4);
fed_num_cell = components_fed(:,5);

% The amount of product created totally.
amount_product_weight = fed_product_plot(end)*fed_volume_plot(end)*...
    number_reactors/1000; %with all three reactors, [Kg]

%Final Feed calcs

```

```

yearly_runtime = (365-45)*0.8; % predicted time of year [in days]
days = 10; % dyas per cycle
yearly_runs = floor(yearly_runtime/days); % amount of times is ran
yearly_amount = yearly_runs *...
    amount_product_weight; %amount of product outputted from reactors
fprintf('=====
fprintf('Production reactors:\n\n')
fprintf('Final Concentration of Product: %.1f [g/L]\n',...
    fed_product_plot(end))
fprintf('Total days (Fed and Batch): %.1f\n',...
    (fed_time_plot(end)+time_plot(end))/24)
fprintf('Total amount of product per year at 0.75 yearly run time of reactors: %.1f
[kg]\n\n',yearly_amount)
fprintf('=====

```

Production reactors:

Final Concentration of Product: 1.4 [g/L]
Total days (Fed and Batch): 45.8
Total amount of product per year at 0.75 yearly run time of reactors: 1198.9 [Kg]

21.5 Protein A

The Protein A exchange column is optimized to find the lowest yearly operating cost with the resin being replaced every year. The values are based off of the Protein A unit operation found in SuperPro. Time of operation was also found by using the same as the

```

% Liquid outputted by all the production reactors
Liq_Volume = number_reactors*fed_volume_plot(end)/1000; % [m^3]

% Range of column volumes to iterate through
Column_Vol = linspace(0.1,2,100); % [m^3]

Qd = 15*1000; % resin capacity [g/m^3] - source
un = 5.5556e-04;% [m/s] - superpro
C_o = 1.5*1000; % [g/m^3]
uL = 8.3333e-04; % [m/s] - superpro
Column_A = 0.5; % [m^2] - superpro
Column_D = (4*Column_A/pi)^0.5; % [m]

% SuperPro values
wash = 11597/4; % [L/cycle]
elution = 9664/4; % [L/cycle]
regen = 5800/4; % [L/cycle]
amount_superpro = 14436.9714/1000; %[m^3]

wash_price = 1; % [$/Kg]
elute_price = 0.745; % [$/Kg] ICIS

```

```

regen_price = 0.345; % [$/Kg]

eluant_in_product = 0.8; % [per cycle per unit] - based off of resin volume

% for loop to find column length, number of cycles, productivity, time,
% outproduct, waste, all buffers through column, price of buffers, and
% resin price.

% Allocated space
Length = zeros(length(Column_Vol),1);
Cycles = zeros(length(Column_Vol),1);
Productivity = zeros(length(Column_Vol),1);
Time = zeros(length(Column_Vol),1);
ratio = zeros(length(Column_Vol),1);
elutant_needed = zeros(length(Column_Vol),1);
wash_needed = zeros(length(Column_Vol),1);
regen_needed = zeros(length(Column_Vol),1);
price_wash = zeros(length(Column_Vol),1);
price_elut = zeros(length(Column_Vol),1);
price_regen = zeros(length(Column_Vol),1);
Price1 = zeros(length(Column_Vol),1);
Price = zeros(length(Column_Vol),1);
price_resin = zeros(length(Column_Vol),1);
elutant_out = zeros(length(Column_Vol),1);

for i = 1:length(Column_Vol)

    Length(i,:) = Column_Vol(i)/Column_A; % [m]
    Cycles(i,:) = (C_o*Liq_Volume/(Column_Vol(i)*Qd)); % [cycles]
    Productivity(i,:) = 1/(Length(i)*(1/(C_o*uL) + ...
        (cycles(i)/(Qd*un)))); % [kg_prod/v_co1/s];
    Time(i,:) = (Liq_Volume*C_o)/(Column_Vol(i))/Productivity(i)/3600;%[hr]
    elutant_out(i,:) = Column_Vol(i)*eluant_in_product*(Cycles(i,:))*1000;%[L]
    ratio(i,:) = Liq_Volume /amount_superpro;

    elutant_needed(i,:) = elution*ratio(i,:)*ceil(Cycles(i,:)); %[L]
    wash_needed(i,:) = wash*ratio(i,:)*ceil(Cycles(i,:)); %[L]
    regen_needed(i,:) = regen*ratio(i,:)*ceil(Cycles(i,:)); %[L]

    price_resin(i,:) = Column_Vol(i)*8000*1000; % [$]
    price_wash(i,:) = wash*ratio(i,:)*wash_price; % [$/cycle]
    price_elut(i,:) = elution*ratio(i,:)*elute_price; % [$/cycle]
    price_regen(i,:) = regen*ratio(i,:)*regen_price; %[L]

    Price1(i,:) = yearly_runs *ceil(Cycles(i,:))*(price_wash(i) + ...
        price_elut(i) + price_regen(i,:)); % $
    Price(i,:) = price_resin(i,:) + Price1(i,:); % $
end

volume_A = Column_Vol';% Stores vector in column vector

```

```
[optimize,s] = min(Price); % Finds

jol = table(Volume_A(s),Length(s),ceil(cycles(s)),Time(s),...
    Price(s), elutant_out(s)); % Stores into a table
jol.Properties.VariableNames = {'Volume' 'Length' 'Cycles' ...
    'Time' 'Price' 'Eluted'}; % Changes table names
fprintf('=====\\n')
fprintf('Protein A:\\n\\n')
disp(jol)
fprintf('=====\\n')
protein_A_efficiency = 0.9; %Protein Efficiency -relates to conc and tot

conc = protein_A_efficiency*fed_product_plot(end)*fed_volume_plot(end)...
    *number_reactors/elutant_out(s); % [g/L] prod conc
amount = conc*elutant_out(s)*yearly_runs/1000; % [Kg] yearly amount
```

=====

Protein A:

Volume	Length	Cycles	Time	Price	Eluted
0.44545	0.89091	8	50.833	6.0847e+06	2800

=====

21.6 Polishing

The polishing unit operation is based off of vendor values of the Unosphere resin. The polishing unit will run for four cycles to remove unwanted components through anion exchange. A tris buffer will be used to strip the column and prepare the apparatus for the next cycle.

```
total_amount_resin = amount_product_weight*protein_A_efficiency*...
    1000/1000; % [m^3], amount of resin needed

polish_cycle = 4; % number of polish cycles
polish_batch_size = elutant_out(s)/polish_cycle; % volume product ran
resin_efficiency = 0.9; % the efficiency of resin in column
true_amount_resin = total_amount_resin/...
    polish_cycle/resin_efficiency; % The real amount of resin needed [m^3]

polish_velocity = 615; % [cm/hr] linear velocity of fluid
polish_velocity = polish_velocity/100/3600; % [m/s]
polish_time_hr = 1.58; % [hr] Time to run a single cycle
polish_time = polish_time_hr*3600; % [s]
polish_area = polish_batch_size/1000/...
    polish_time/polish_velocity; % [m^2] area of polish column
polish_D = (4*polish_area/pi)^0.5; % [m] diameter of polish column
polish_length = true_amount_resin/polish_area; % [m] length of polish column
polish_time_total = (polish_time_hr*polish_cycle...
    *2+1)/24; % [day] total amount of time needed for process
```

```
% Needed components to regenerate column
polish_wash = polish_area*polish_velocity*3600*1000*polish_cycle; % [L]
polish_strip = polish_area*polish_velocity*polish_time...
    *1000*polish_cycle; % [L]
polish_efficiency = 0.95;

fprintf('=====\\n')
fprintf('Polishing:\\n\\n')
fprintf('Amount resin: %.2f [L]\\n',true_amount_resin*1000)
fprintf('Polish time: %.2f [hours]\\n',polish_time_total*24)
fprintf('=====\\n')
conc = conc*polsih_efficiency; % [g/L] conc after polish
amount = amount*polsih_efficiency; % [Kg] amount after polish
```

=====
Polishing:

Amount resin: 119.89 [L]
Polish time: 13.64 [hours]

21.7 Media Prep

This section sums all needed amounts of media needed to produce mAbs, assuming the media is CD OptiCHO

```
fprintf('=====\\n')
fprintf('Media Prep:\\n\\n')
media_volume = number_reactors*(fed_volume_plot(end))...
    + Seed_volume(end); % amount of media needed
safe_media_volume = media_volume*1.05; % safety factor
fprintf('Tank volume to hold all media (seed and production): %.0f [L]\\n',safe_media_volume )
fprintf('=====\\n')
```

=====
Media Prep:

Tank volume to hold all media (seed and production): 40431 [L]

21.8 Buffer Prep

This portion calculates all needed buffers to carry out the process

```
fprintf('=====\\n')
fprintf('Buffer Prep:\\n\\n')

% Amount of buffer needed
ph4_amount = elutant_needed(s);
ph5_amount = wash_needed(s) ;
ph8_urea_amount = regen_needed(s);
ph8_tris_amount = polish_strip;
```

```

V_3 = 1.1*(10^6)/25; % L/batch of pH 3 buffer needed for CIP
V_tris = 2500/25; % L/batch of pH 7.5 0.02- 0.05 Tris buffer needed
V_detergent = 2.204*(10^6)/25; %L/batch of 5 wt% detergent needed for CIP
V_AA = (ph4_amount*.847)+(ph5_amount*.357)...
    +(V_3*0.9823); % L/yr of 0.1M acetic acid
V_SA = (ph4_amount+V_3+ph5_amount)-V_AA; % L/yr of 0.1M sodium acetate
V_AA_95 = V_AA*.1/16.5; % L/yr of 95wt% acetic acid
m_urea = ph8_urea_amount * 8 * 60.06; % g urea/ yr needed 8 M urea solution
m_SA = V_SA * .1 * 82; % g sodium acetate/yr
m_tris = (ph8_tris_amount + V_tris)*0.05*121.14; % g tris/yr
m_detergent = V_detergent * 53; % g/yr detergent

fprintf('pH 4 Tank Size: %.0f [L]\n',ph4_amount)
fprintf('pH 5 Tank Size: %.0f [L]\n',ph5_amount)
fprintf('pH 8 (urea) Tank Size: %.0f [L]\n',ph8_urea_amount)
fprintf('pH 8 (tris) Tank Size: %.0f [L]\n',ph8_tris_amount)
fprintf('=====\\n')
=====\\n

```

Buffer Prep:

```

pH 4 Tank Size: 46857 [L]
pH 5 Tank Size: 56230 [L]
pH 8 (urea) Tank Size: 28122 [L]
pH 8 (tris) Tank Size: 2800 [L]
=====\\n

```

21.9 Kill tank

The code found in this section sums all buffers, elutants, and wastes found in the process and divides the amount into two, equally-sized kill tanks.

```

fprintf('=====\\n')
fprintf('Kill Tank:\\n\\n')

% Amount of stuff going into kill tanks
pH3_AA = V_3;
pH4_AA = ph4_amount;
pH5_AA = ph5_amount;
pH8_urea = ph8_urea_amount;
Tris_NaCl = ph8_tris_amount;
Tris = V_tris;
Detergent = V_detergent;
CIP = 3287000/25;

Media = fed_volume_plot(end)*number_reactors;

% All of the wastes put into a table showing amounts and titles of each
kyle = table(pH3_AA,pH4_AA,pH5_AA,pH8_urea,Tris_NaCl,Tris,Detergent,Media,CIP);
disp(kyle) % Displays waste
waste = CIP + Tris + Tris_NaCl + pH8_urea + pH5_AA + pH4_AA + ...

```

```
pH3_AA + Detergent + Media - elutant_out(s); % Total amount waste
```

```
fprintf('Size of Kill Tank 1: %.0f [L]\n',waste/2)
fprintf('Size of Kill Tank 2: %.0f [L]\n',waste/2)
fprintf('=====\\n')
```

=====
Kill Tank:

pH3_AA	pH4_AA	pH5_AA	pH8_urea	Tris_NaCl	Tris	Detergent	Media	CIP
_____	_____	_____	_____	_____	_____	_____	_____	_____
44000 1.3148e+05	46857	56230	28122	2800	100	88160	35000	

```
Size of Kill Tank 1: 214975 [L]
Size of Kill Tank 2: 214975 [L]
=====
```

21.10 Storage

The mAb final product is mixed with glycerol to reduce the freezing temperature well below -20°C. The volume percent of glycerol needed in must be at least 50%. The final, mixed product will be stored into plastic, 200 L barrels.

```
volume = amount*1000/(conc)/yearly_runs; % [L], volume of product
conc2 = 5.1; % Concentration of needed glycerol
V_glycerol = conc*volume/conc2 - volume; % Amount glycerol needed
Vf = ceil(volume + V_glycerol); % Final volume
Drum_Size = 200; % [L], size of an individual drum
Drum_count = floor(Vf/Drum_Size); % amount of drums
Drum_year = Drum_count*yearly_runs; % amount of drums for total year
```

```
fprintf('=====\\n')
fprintf('Storage:\\n\\n')
fprintf('Amount glycerol needed: %.2f [L]\\n',V_glycerol)
fprintf('Final volume: %.2f [L]\\n',Vf)
fprintf('Final concentration: %.2f [g/L]\\n',conc2)
fprintf('Final amount of yearly product: %.2f [kg]\\n',amount)
fprintf('Drums a cycle: %.0f [drums]\\n',Drum_count)
fprintf('Drums a year: %.0f [drums]\\n',Drum_year)
fprintf('=====\\n\\n')
```

toc

=====
Storage:

```
Amount glycerol needed: 5240.00 [L]
Final volume: 8040.00 [L]
```

```

Final concentration: 5.10 [g/L]
Final amount of yearly product: 1025.10 [Kg]
Drums a cycle: 40 [drums]
Drums a year: 1000 [drums]
=====

```

Elapsed time is 13.359849 seconds.

21.11 Plots

```

if plot_switch == 1

    % Seed Train

    % Number of cells: Seed Train, Figure 1
    figure('Position',[800,50,800,700])
    plot(time_plot/24,num_cell*cell_weight,'-k','LineWidth',2)
%     title('Number of cells: Seed Train')
    ylabel('N_c_e_l_l_s [ ]')
    xlabel('Time [Days]')
    set(gcf,'color','w');
    box on;
    set(gca,'FontSize',20)

    % Product Concentration: Seed Train, Figure 2
    figure('Position',[800,50,800,700])
    plot(time_plot/24,product_plot,'-k','LineWidth',2)
%     title('Product Concentration: Seed Train')
    xlabel('Time [Days]')
    ylabel('c_p_r_o_d_u_c_t [g/L]')
    set(gcf,'color','w');
    box on;
    set(gca,'FontSize',20)

    % cell Concentration: Seed Train, Figure 3
    figure('Position',[800,50,800,700])
    plot(time_plot/24,cell_plot*cell_weight,'-k','LineWidth',2)
    ylabel('C_c_e_l_l_s [g/L]')
    xlabel('Time [Days]')
%     title('Cell Concentration: Seed Train')
    set(gcf,'color','w');
    box on;
    set(gca,'FontSize',20)

    % Substrate Concentration: Seed Train, Figure 4
    figure('Position',[800,50,800,700])
    plot(time_plot/24,substrate_plot,'-k','LineWidth',2)
%     title('Substrate Concentration: Seed Train')
    xlabel('Time [Days]')
    ylabel('C_s_u_b_s_t_r_a_t_e [g/L]')
    set(gcf,'color','w');
    box on;

```

```
set(gca,'FontSize',20)

% Production Reactors

%Product Concentration: Production Reactor, Figure 5
figure('Position',[800,50,800,700])
plot(fed_time_plot/24,fed_product_plot,'k','LineWidth',2)
hold on
x1=get(gca,'xlim');
plot(x1,[Product_final_conc(2) Product_final_conc(2)],':k','LineWidth',3)
hold on
plot(x1,[Product_final_conc(1) Product_final_conc(1)],':k','LineWidth',3)
%    title('Product Concentration: Production Reactor')
xlabel('Time [Days]')
ylabel('C_p_r_o_d_u_c_t [g/L]')
ylim([0 2.5])
legend('C_p_r_o_d_','C_l_i_m_,_p_r_o_d_')
set(gcf,'color','w');
box on
set(gca,'FontSize',20)

% Cell Concentration: Production Reactor, Figure 6
figure('Position',[800,50,800,700])
plot(fed_time_plot/24,fed_cell_plot*cell_weight,'k','LineWidth',2)
x1=get(gca,'xlim');
hold on
plot(x1,[15.06415,15.06415],':k','LineWidth',2)
ylabel('C_c_e_l_l_s [g/L]')
%    title('Cell Concentration: Production Reactor')
legend('C_c_e_l_l_s','C_m_a_x_,_c_e_l_l_s')
xlabel('Time [Days]')
ylim([7,17])
set(gcf,'color','w');
box on;
set(gca,'FontSize',20)

% Substrate Concentration: Production Reactor, Figure 7
figure('Position',[800,50,800,700])
plot(fed_time_plot/24,fed_substrate_plot,'k','LineWidth',2)
hold all
plot(x1,[Product_final_conc(2) Product_final_conc(2)],':k','LineWidth',3)
%    title('Substrate Concentration: Production Reactor')
xlabel('Time [Days]')
ylabel('C_s_u_b_s_t_r_a_t_e [g/L]')
ylim([1.75 3])
legend('C_s_u_b_','C_m_i_n_,_s_u_b_')
set(gcf,'color','w');
set(gca,'FontSize',20)
```

```
% Reactor Volume, Figure 8
figure('Position',[800,50,800,700])
plot(fed_time_plot/24,fed_volume_plot,'k','LineWidth',2)
ylabel('V_r_e_a_c_t_o_r [L]')
xlabel('Time [Days]')
% title('Reactor volume')
set(gcf,'color','w');
box on
set(gca,'FontSize',20)

% Protein A Plot, Figure 9
figure('Position',[800,50,800,700])
hold on
plot(column_Vol,Price,'k','LineWidth',2)
yyaxis left
xlabel('Volume_r_e_s_i_n [L]')
% title('Protein A Optimization')
ylabel('Cost [$]')
yyaxis right
plot(column_Vol,Time,'--k','LineWidth',2);
ylabel('Time [ hr ]')
set(gcf,'color','w');
box on
y3 = get(gca,'ylim');
plot([volume_A(s) volume_A(s)],y3,:k','LineWidth',2)
annotation('textarrow',[volume_A(s)-0.05 volume_A(s)-0.14],[mean(y3) ...
    mean(y3)]/y3(2),'String',{{'Min_v_o_l= ' num2str(volume_A(s))}},'FontSize',17)
set(gca,'FontSize',20,'YColor','k')
legend('Cost','Time')

end
```

21.12 Functions for batch growth

```
function d_dt_batch = batch(~,components_batch,Ks,mu_max,Y_xs,Kd,~,productivity)

% unpack components for readable variable names
X = components_batch(1);
S = components_batch(2);

%%Mass Balance for X, S.
mu = mu_max*S/(Ks + S);

%dx/dt
d_dt_batch(1) = (mu * X) - (Kd * X);

% ds/dt
d_dt_batch(2) = -(mu * X) / (Y_xs);

% dp/dt
```

```
d_dt_batch(3) = x * productivity;

d_dt_batch = d_dt_batch';
end
```

21.13 Function for fed batch

```
function d_dt_fed = fed_batch(~,components_fed,Ks,mu_max,Y_xs,S_fed,F,productivity,Kd)

% unpack components for readable variable names
V = components_fed(1);
X = components_fed(2);
S = components_fed(3);

% calculate mu
mu = mu_max*S/(Ks + S);

% dv/dt
d_dt_fed(1) = F;

% dx/dt
if X < 7*10^9
    d_dt_fed(2) = (mu - (F/V) - Kd)*X ;
else
    d_dt_fed(2) = 0;
end

% ds/dt
d_dt_fed(3) = (F/V)*(S_fed - S) - (1/Y_xs)*mu*X;

% dp/dt
d_dt_fed(4) = x * productivity ;

%dc/dt
d_dt_fed(5) = x * V;

% transpose to column
d_dt_fed = d_dt_fed';
end
```

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22 APPENDIX D (Costing)

Table 7.2 Purchased Equipment Cost for Common Plant Equipment

Equipment	Units for Size, S	S _{lower}	S _{upper}	a	b	n	Note
<i>Agitators & mixers</i>							
Propeller	driver power, kW	5.0	75	17,000	1,130	1.05	
Spiral ribbon mixer	driver power, kW	5.0	35	30,800	125	2.0	
Static mixer	liters/s	1.0	50	570	1,170	0.4	
<i>Boilers</i>							
Packaged, 15 to 40 bar	kg/h steam	5,000	200,000	124,000	10.0	1.0	
Field erected, 10 to 70 bar	kg/h steam	20,000	800,000	130,000	53	0.9	
<i>Centrifuges</i>							
High speed disk	diameter, m	0.26	0.49	57,000	480,000	0.7	
Atmospheric suspended basket	power, kW	2.0	20	65,000	750	1.5	
<i>Compressors</i>							
Blower	m ³ /h	200	5,000	4,450	57	0.8	
Centrifugal	driver power, kW	75	30,000	580,000	20,000	0.6	
Reciprocating	driver power, kW	93	16,800	260,000	2,700	0.75	
<i>Conveyors</i>							
Belt, 0.5 m wide	length, m	10	500	41,000	730	1.0	
Belt, 1.0 m wide	length, m	10	500	46,000	1,320	1.0	
Bucket elevator, 0.5m bucket	height, m	10	30	17,000	2,600	1.0	
<i>Crushers</i>							
Reversible hammer mill	t/h	30	400	68,400	730	1.0	
Pulverizers	kg/h	200	4,000	16,000	670	0.5	
Jaw crusher	t/h	100	600	-8,000	62,000	0.5	
Gyratory crusher	t/h	200	3,000	5,000	5,100	0.7	
Ball mill	t/h	0.7	60	-23,000	242,000	0.4	
<i>Crystallizers</i>							
Scraped surface crystallizer	length, m	7	280	10,000	13,200	0.8	
<i>Distillation columns</i>							
See pressure vessels, packing and trays							

Table 7.2 Purchased Equipment Cost for Common Plant Equipment—Cont'd

Equipment	Units for Size, <i>S</i>	<i>S</i> _{lower}	<i>S</i> _{upper}	<i>a</i>	<i>b</i>	<i>n</i>	Note
<i>Dryers</i>							
Direct contact Rotary	m ²	11	180	15,000	10,500	0.9	1
Atmospheric tray batch	area, m ²	3.0	20	10,000	7,900	0.5	
Spray dryer	evap rate kg/h	400	4,000	410,000	2,200	0.7	
<i>Evaporators</i>							
Vertical tube	area, m ²	11	640	330	36,000	0.55	
Agitated falling film	area, m ²	0.5	12	88,000	65,500	0.75	2
<i>Exchangers</i>							
U-tube shell and tube	area, m ²	10	1,000	28,000	54	1.2	
Floating head shell and tube	area, m ²	10	1,000	32,000	70	1.2	
Double pipe	area, m ²	1.0	80	1,900	2,500	1.0	
Thermosiphon reboiler	area, m ²	10	500	30,400	122	1.1	
U-tube Kettle reboiler	area, m ²	10	500	29,000	400	0.9	
Plate and frame	area, m ²	1.0	500	1,600	210	0.95	2
<i>Filters</i>							
Plate and frame	capacity, m ³	0.4	1.4	128,000	89,000	0.5	
Vacuum drum	area, m ²	10	180	-73,000	93,000	0.3	
<i>Furnaces</i>							
Cylindrical	duty, MW	0.2	60	80,000	109,000	0.8	
Box	duty, MW	30	120	43,000	111,000	0.8	
<i>Packings</i>							
304 ss Raschig rings	m ³			0	8,000	1.0	
Ceramic intalox saddles	m ³			0	2,000	1.0	
304 ss Pall rings	m ³			0	8,500	1.0	
PVC structured packing	m ³			0	5,500	1.0	
304 ss structured packing	m ³			0	7,600	1.0	3
<i>Pressure vessels</i>							
Vertical, cs	shell mass, kg	160	250,000	11,600	34	0.85	4
Horizontal, cs	shell mass, kg	160	50,000	10,200	31	0.85	4

Table 7.2 Purchased Equipment Cost for Common Plant Equipment—Cont'd

Equipment	Units for Size, S	<i>S_{lower}</i>	<i>S_{upper}</i>	<i>a</i>	<i>b</i>	<i>n</i>	Note
Vertical, 304 ss	shell mass, kg	120	250,000	17,400	79	0.85	4
Horizontal, 304 ss	shell mass, kg	120	50,000	12,800	73	0.85	4
<i>Pumps and drivers</i>							
Single stage centrifugal	flow, liters/s	0.2	126	8,000	240	0.9	
Explosion proof motor	power, kW	1.0	2,500	-1,100	2,100	0.6	
Condensing steam turbine	power, kW	100	20,000	-14,000	1,900	0.75	
<i>Reactors</i>							
Jacketed, agitated	volume, m ³	0.5	100	61,500	32,500	0.8	2
Jacketed, agitated, glass lined	volume, m ³	0.5	25	12,800	88,200	0.4	
<i>Tanks</i>							
floating roof	capacity, m ³	100	10,000	113,000	3,250	0.65	
cone roof	capacity, m ³	10	4,000	5,800	1,600	0.7	
<i>Trays</i>							
Sieve trays	diameter, m	0.5	5.0	130	440	1.8	5
Valve trays	diameter, m	0.5	5.0	210	400	1.9	
Bubble cap trays	diameter, m	0.5	5.0	340	640	1.9	
<i>Utilities</i>							
Cooling tower & pumps	flow, liters/s	100	10,000	170,000	1,500	0.9	6
Packaged mechanical refrigerator							
evaporator	duty, kW	50	1,500	24,000	3,500	0.9	
Water ion exchange plant	flow m ³ /h	1	50	14,000	6,200	0.75	
<i>Notes:</i>							
1. Direct heated.							
2. Type 304 stainless steel.							
3. With surface area 350 m ² /m ³ .							
4. Not including heads, ports, brackets, internals, etc. (see <i>Chapter 14</i> for how to calculate wall thickness).							
5. Cost per tray, based on a stack of 30 trays.							
6. Field assembly.							
7. All costs are U.S. Gulf Coast basis, Jan. 2010 (CEPCI index = 532.9, NF refinery inflation index = 2281.6).							

Table 52: Pump pricing

Pumps	Cost
P-106	9123.38034
P-107	9123.38034
P-108	9123.38034
P-109	9123.38034

P-110	9123.38034
P-111	9123.38034
P-112	9123.38034
P-113	9452.12584
P-114	9260.99598
P-115	9447.43724
P-116	9447.43724
P-117	9447.43724
P-118	9447.43724
P-120	9092.74904
P-121	9092.74904
P-123	10479.4024
P-124	10479.4024
P-125	9178.27311
P-126	9178.27311
P-131	9178.27311
P-127	9178.27311