MONOCLONAL ANTIBODY PRODUCTION FOR CANCER IMMUNOTHERAPY

By

SARA ELIZABETH SLOSKY

_____

A Thesis Submitted to The Honors College

In Partial Fulfillment of the Bachelors degree
With Honors in

Chemical Engineering

THE UNIVERSITY OF ARIZONA

MAY 2018

Approved by:

Dr. Kim Ogden
Department of Chemical and Environmental Engineering

University of Arizona

Monoclonal Antibody Production for Cancer Immunotherapy

Team 12

Karen Leon

Samantha Louzek

Jacob Rischar

Sara Slosky

CHEE 443

Dr. Ogden

4/27/2018

Abstract

The goal of the proposed plant is to produce 8760 g of bevacizumab at a concentration of 25 mg/mL which yields 349 L of product per year. Bevacizumab is a monoclonal antibody that is used to treat six different types of cancer. It acts by binding to the growth factor which essentially starves the cancerous tumor. To mass produce the antibody, recombinant Chinese Hamster Ovary cells that produce the desired humanized antibody are grown in a cascade of bioreactors. The cell solution then goes through a series of purification steps to isolate the desired antibody. A centrifuge is used to remove cell mass and chromatography columns are used to rid the solution of the undesired proteins. Filtration removes the buffer salts and then the final product is freeze dried. While the startup cost of the proposed plant is high, the pharmaceutical market is very lucrative and the facility is expected to make a profit within the first year. After seven years the investment has an estimated net present value of over $120 million. Due to the high economic return, the proposed plant design should move on to further development and implementation.

Overview of Contributions

The group working on the proposed plant design consisted of four members: Samantah Louzek, Karen Leon, Jacob Rischar, and Sara Slosky. Samantha wrote the ion exchange chromatography column design and optimization. She also contributed to the overall goal, current market info, and project premises and assumptions.

Karen worked on writing up the ultrafiltration unit, the bottler, and freeze dryer, and centrifuge for the design and optimization section. She also worked on the safety section and environmental regulations section.

Jacob wrote up the bioreactor cascade design and optimization. He worked on the BFD, economic analysis and LCA. He also created the stream tables, performed the utilities calculations, and created the utilities table.

Sara wrote the Protein A Chromatography and heat exchanger design and optimization sections. She also wrote the summary and the rationale for process choice. She contributed to the environmental section by working on the environmental waste and chemical spills.

Project Summary

The goal of the proposed plant is to produce 8760 g of bevacizumab at a concentration of 25 mg/mL which yields 349 L of product per year. Bevacizumab is one of the highest selling monoclonal antibodies and it is used to treat glioblastoma as well as colorectal, lung, kidney, cervical, and ovarian cancers. The antibody inhibits angiogenesis in tumors by binding to the growth factor that stimulates the formation of new vasculature, resulting in the starvation of cells from nutrients and prevents proliferation of the cancer cells in the tumor (Syrop).

The production process consists of producing antibodies using Chinese Hamster Ovary cells followed by a downstream purification process. The cells are grown in a series of bioreactors with increasing volume to allow for optimal cell growth and biomass concentration. The cell solution spends seven days in each bioreactor at 37 °C and the cell growth follows a Monod-like kinetic model.

After the bioreactor cascade, the solution goes through a heat exchanger where the temperature is reduced to 5°C to preserve the antibody during downstream processing. The product solution then goes through a series of purification steps. First, a centrifuge is used to rid the solution of solid cell biomass. Then the solution goes to a series of three different chromatography columns to increase antibody purity. Protein A chromatography is the first column and retains the antibodies from the solution along with a fraction of other cell proteins. The cell media and remaining cell mass is washed out during this step. The antibodies are eluted from the Protein A column and then go to ion exchange columns where the "other" proteins are removed from the solution as well as any remaining contaminants.

Final processing of the antibody begins with an ultrafiltration system where small peptides and salts are removed from the solution. The product is then freeze dried to remove the

water from the solution. Excipients such as trehalose are added to increase therapeutic impact, dilute antibody concentration, and extend shelf life. The product is then packaged as a liquid at a concentration of 25 mg/mL and is ready for shipment.

The antibody production and purification plant is capable of producing 195 g of antibody per batch. With each batch taking a week to run, the proposed plant can produce 45 batches a year to achieve the goal of 8760 g a year. The pharmaceutical business is very lucrative and the plant turns a profit after only the first year. After seven years the net present value of the production facility is $124 million. The environmental impact of plant is within regulated limits. The CO_2 emissions are well below regulated limits. It is important that the fluids used in the proposed plant do not enter drains but beyond that the plant does not pose environmental or health risks to the community.

There are a few important uncertainties in the plant design that could impact the final economic evaluation and plant feasibility. Approximations were used to find bulk volume cost of buffer and resin based off of a pilot plant proposal. There is also the assumption that the plant will have a water purification unit on site that will produce water for injection. This cost is included in the raw material cost but may not accurately represent the operating cost of the purification process. The yield for the bioreactor cascade is much lower than average values used in production which underestimates the economic value of the plant (Reinhart).

Even with the uncertainties of the plant design, the net present value of the plant makes it a promising investment. The design has potential to be further improved by modeling the cell growth with an updated model or setting up another bioreactor cascade in parallel. The analysis in this paper provides the groundwork for a monoclonal antibody production facility that can be expanded upon to create a profitable investment.

Table of Contents

Introduction

Overall Goal

Antibodies, or immunoglobins, are proteins produced by the human immune system by plasma cells. The purpose of these proteins is to neutralize pathogens that may have entered the body. Every antibody has an antigen binding site that recognizes a unique molecule of the pathogen, which allows the antibody and the pathogen to precisely bind. In doing this, the pathogen becomes a target for the body's immune system. If successful, the antibody will prevent the pathogen from spreading and decrease the host's probability of becoming ill (Mandal 1). Due to the extreme variability of the antigen binding site of immunoglobins, the number of pathogens or viruses that the body can be protected from is innumerable.

Bevacizumab is a recombinant humanized monoclonal antibody that targets the vascular endothelial growth factor or VEGF. By binding with the VEGF, it inhibits the receptors from binding and ultimately prevents tumor blood vessels from growing or maintaining themselves ("NCI Drug Dictionary"). With this capability, bevacizumab can be used to treat several cancer types including lung, colon, rectal, and breast cancers ("Avastin (bevacizumab) Uses").

The following proposal explores the capability of producing mass quantities of bevacizumab in-vitro, taking into account the economic feasibility and environmental responsibility. The determined goal is to successfully produce over 8 kg of pure product that can be sold for use in humans. A bioreactor cascade is used to grow the desired antibody with Chinese Hamster Ovary (CHO) cells and the bioreactor products are sent through a multi-step purification and filtration process to ensure product purity and abide by all necessary safety regulations as outlined by the FDA.

Current Market Info

Bevacizumab was approved for sale and use in humans in 2008. By 2013, global sales were up to $1.7 billion (Ecker 1). As a possible treatment for a variety of cancers, its value only continues to grow along with demand. In the current market place, a single dose of intravenous solution at a concentration of 25 mg/mL, costs $205 ("Avastin (bevacizumab) Uses"). Treatments are usually given every 2 to 3 weeks, depending on the cancer type, and can require up to 15 mg of bevacizumab per dose. The treatments continue until a significant reduction in tumor occurs. This can take anywhere from 5 months to a year or longer ("Important Safety Information"). Based on an average of 6 months of treatment, with doses taken every 2 week, it can cost upwards of $1,500 for the given therapy. In addition to this, the number of projected cancer cases in the world are projected to increase by 50% between 2012 and 2030 ("Cancer Statistics"). This saddening statistic portrays the increasing demand for cancer treatments, such as bevacizumab.

The proposed production and purification process will produce 8,760 g of bevacizumab or 349 L of a final product with a concentration of 25 mg/mL, per year. At the current market value this will provide an approximate total revenue of $71.5 million in the first year alone.

Project Premises and Assumptions

Table 1 lists major project premises and assumptions made throughout the process of designing the plant. References and/or reasoning that support assumptions is provided along with the specific assumptions made.

Table 1: Assumptions and Premises

Assumption/ Premises	**Reference/ Reasoning**
Bulk pricing accurate as defined in "Biopharmaceutical Process Optimization with Simulation and Scheduling Tools".	(Petrides, Demetri, et al. 167)
All solutions in process have similar properties to water	Throughout the process, water makes up more than 97% of the solution (“BalanCD® CHO Growth A.” 1)
Diaphragm pumps are sufficient for use throughout entire process	The majority of pumps need to be diaphragm pumps due to the sensitivity of the antibody. For convenience of purchasing all pumps are diaphragm pumps (“Quattroflow Fluid Systems”)
Stainless steel is optimal for use in all pieces of equipment	Stainless steel is ideal for minimizing contamination (Fleming 2)
Water purification plant located on site	The amount of water required in this process makes it unreasonable to purchase from a supplier.
Oxygen into bioreactors is fed at 100% excess and is sufficient to maintain desired dissolved oxygen concentration	(Dr. Ogden, personal communication)
Heat loss to the system and agitation in bioreactors negligible	(Dr. Ogden, personal communication)
CO2 emissions due to transportation negligible	Hybrid vehicles used along with short transportation distances
Data relating to Avastin used for reference	Avastin® is the trade name for bevacizumab

Process Description, Rationale and Optimization

Figure 1 is a quantitative block flow diagram of the process that shows the overall mass balance and each major process step for the proposed plant. Figures 2-4 are the process flow diagrams for the process. Table 2 shows process parameters for each piece of equipment listed on the PFD, and Tables 3-6 detail the contents of each stream listed on the PFD, and Table 7 describes the utilities used in the process.

Block Flow Diagram

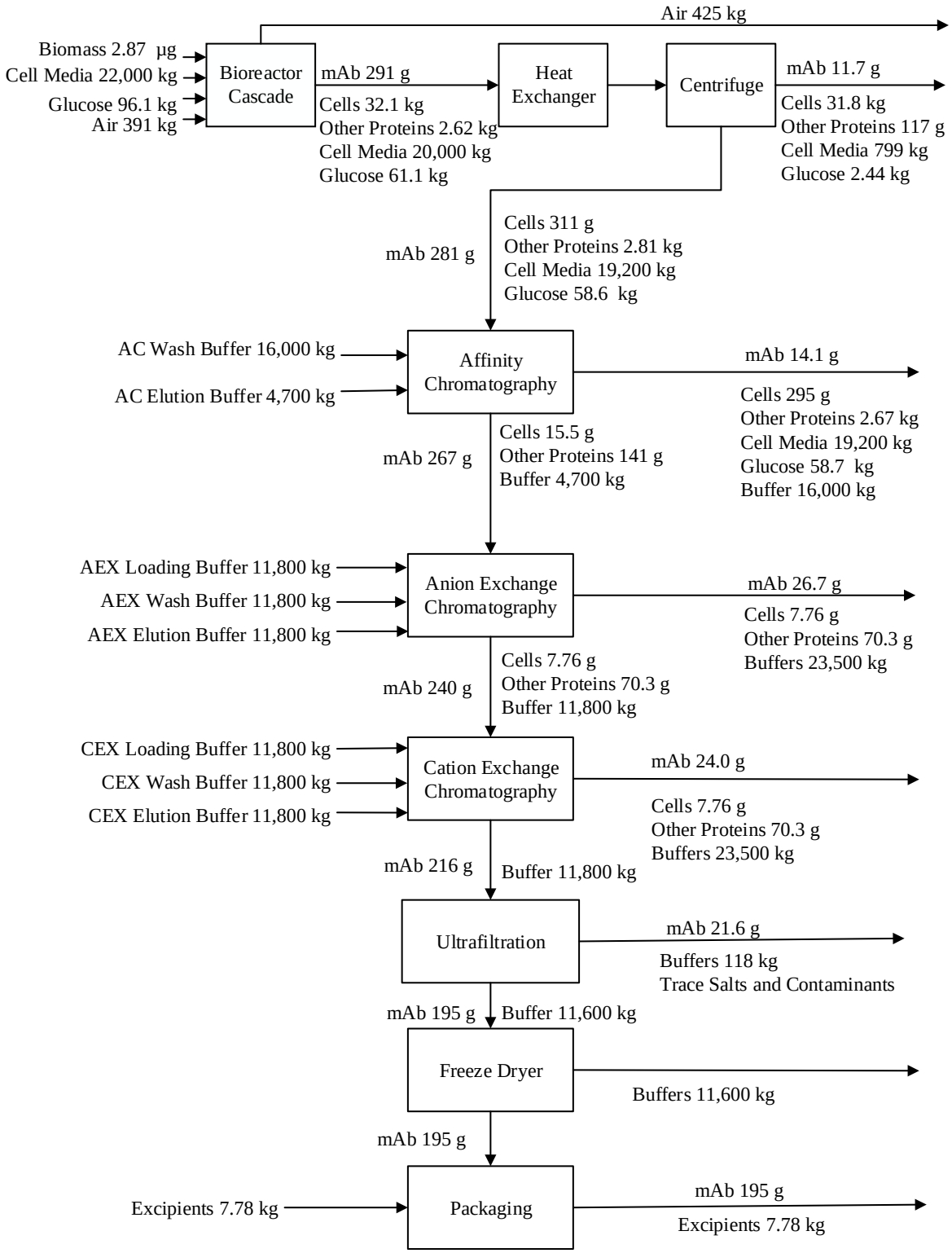

Figure 1: Block Flow Diagram

Process Flow Diagram

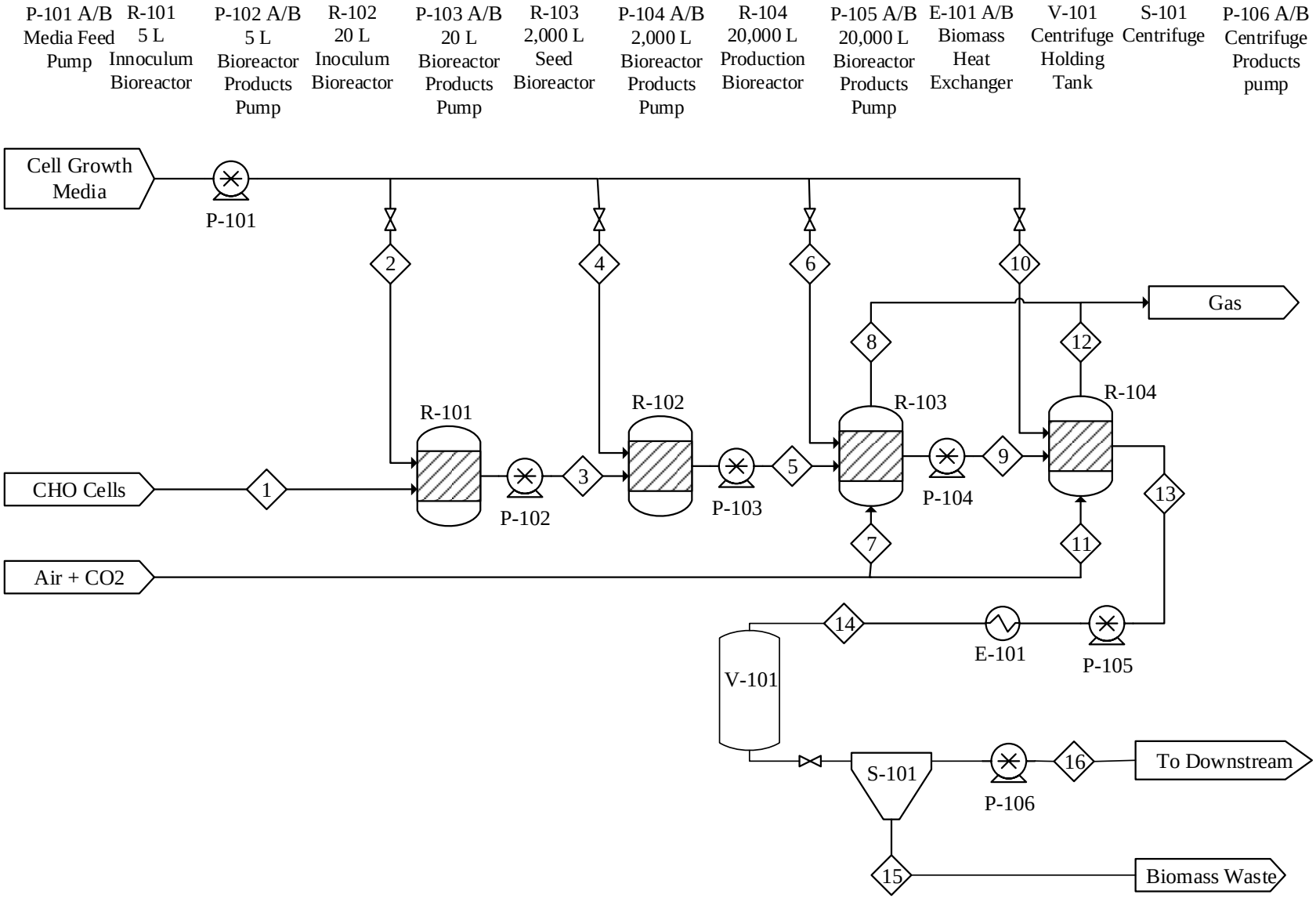

Figure 2: PFD of Upstream Process

V-201 C-201 Holding Tank | P-201 A/B Holding Tank Exit Pump | P-202 A/B AC Elution Buffer Pump | P-203 A/B AC Wash Buffer Pump | C-201 Protein A Chromatography Column | P-204 A/B C-201 Column Waste Pump | V-202 C-202 Holding Tank | P-205 A/B C-201 Holding Tank Exit Pump | P-207 A/B AEX Wash Buffer Pump | P-208 A/B AEX Elution Buffer Pump | C-202 AEX Chromatography Column | P-209 A/B AEX Waste Pump | V-203 C-202 Exit Holding Tank | P-206 A/B AEX Loading Buffer Pump

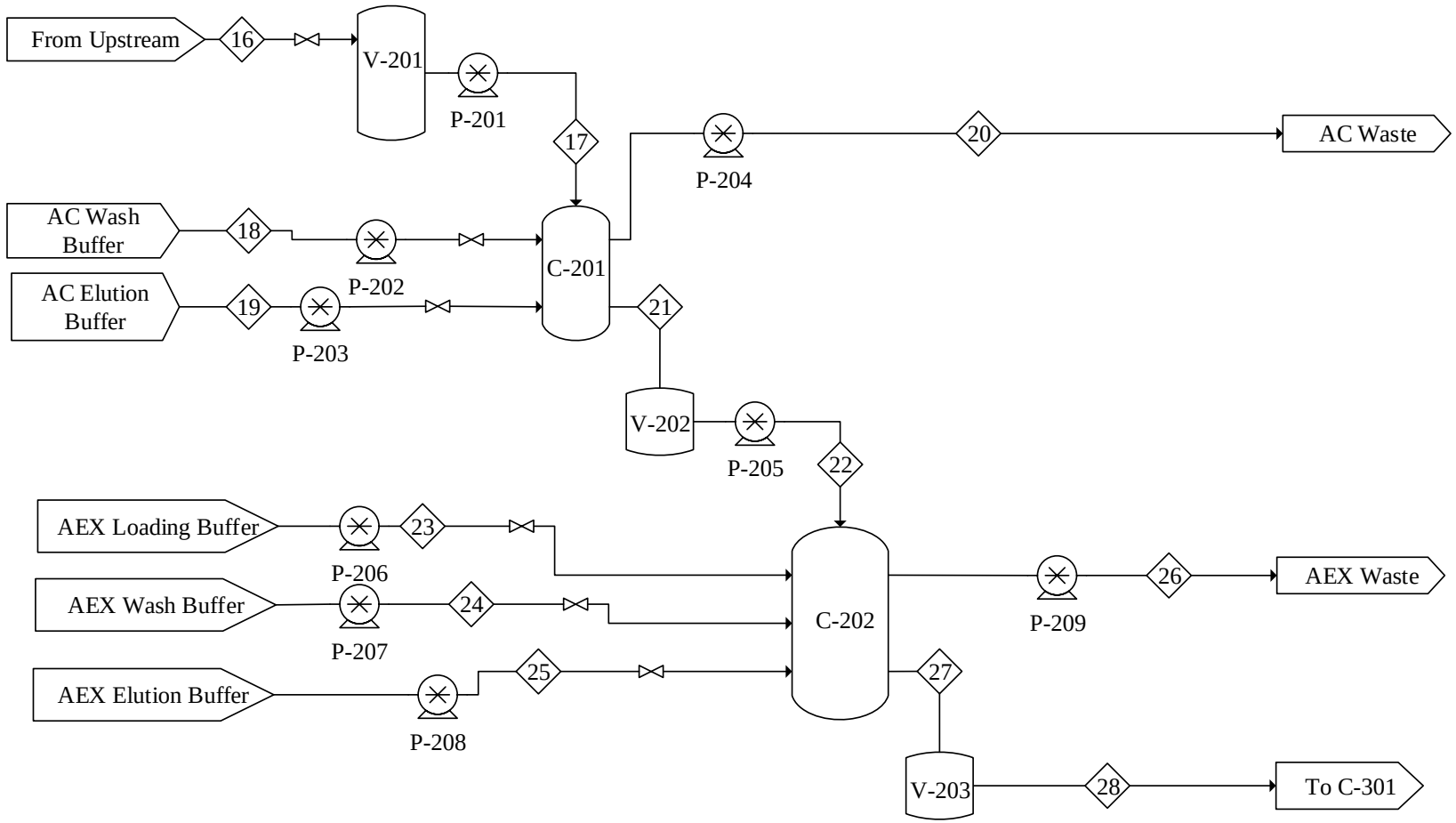

Figure 3: PFD of Separation Process

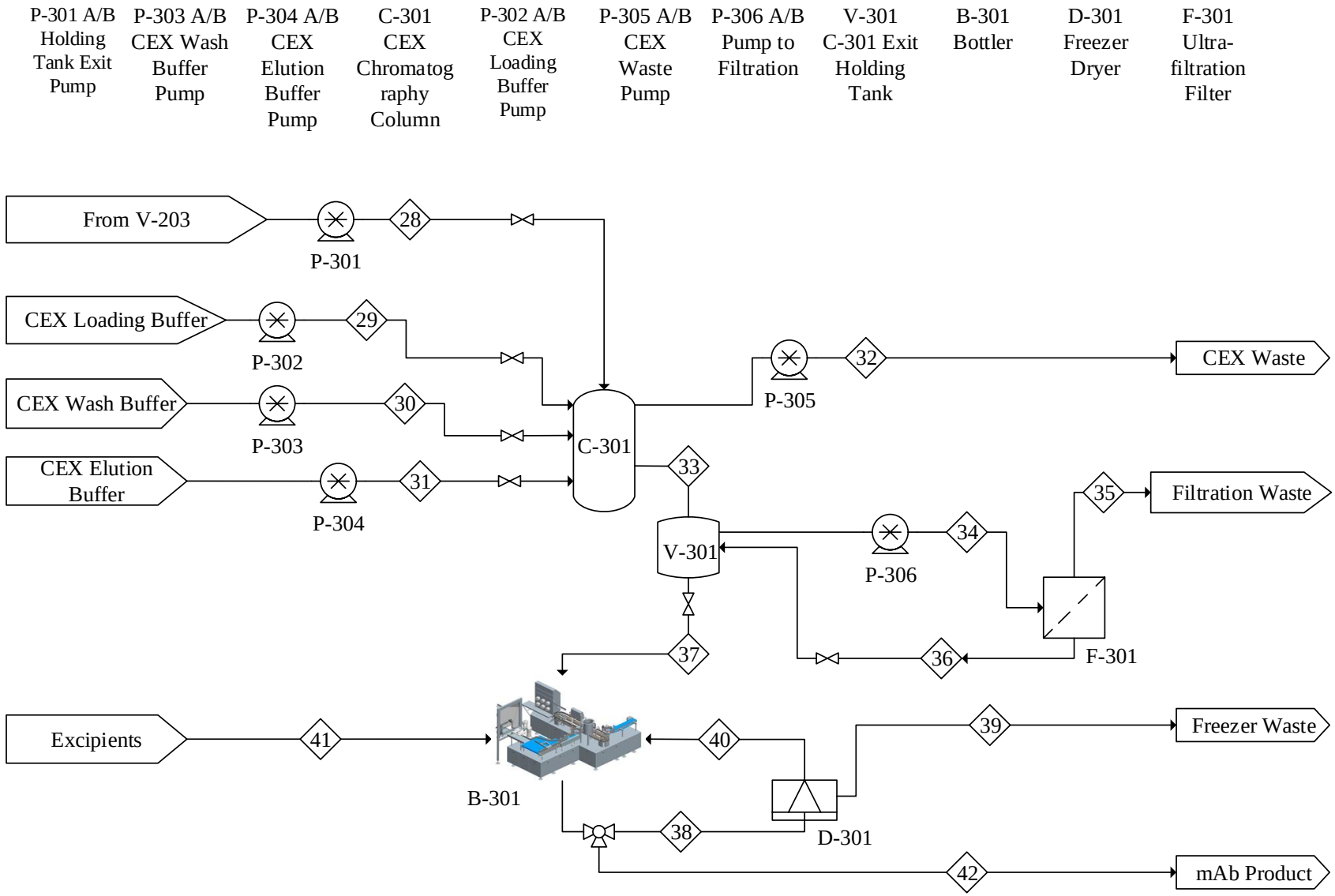

Figure 4: PFD of Final Purification Process

Equipment Tables

Table 2: Equipment Summary for Monoclonal Antibody Production PFD

Heat Exchangers	**E-101**					
Type	Shell and Tube					
Area (m^2)	64					
Duty (BTU/hr)	312,046					
Tube	**Inlet**	**Outlet**				
Temp (^oC)	-10	30				
Pressure (bar)	1.3	1.1				
Phase	Vapor	Vapor				
Shell	**Inlet**	**Outlet**				
Temp (^oC)	37	4.94				
Pressure (bar)	1	0.977				
Phase	Liquid	Liquid				
Reactors	**R-101**	**R-102**	**R-103**	**R-104**		
Residence time (Days)	7	7	7	7		
Temp (^oC)	37	37	37	37		
Pressure (atm)	1	1	1	1		
Orientation	Vertical	Vertical	Vertical	Vertical		
pH	7.0	7.0	7.0	7.0		
Organism	CHO cells	CHO cells	CHO cells	CHO cells		
Media	BalanCD® CHO Growth A Medium					
Size						
Height (m)	0.3	0.5	2	3		
Diameter (m)	0.2	0.3	1.5	4.5		
Volume capacity (L)	5	20	2000	20000		
Pumps	**P-101A/B**	**P-102A/B**	**P-103A/B**	**P-104A/B**	**P-105A/B**	**P-106A/B**
Flow (LPH)	5,000	5	20	2000	5000	2,500
Fluid Density (g/mL)	1	1	1	1	1	1
Power (kW)	3	0.05	0.37	2.2	4	3
Pumps	**P-201A/B**	**P-202A/B**	**P-203A/B**	**P-204A/B**	**P-205A/B**	**P-206A/B**
Flow (LPH)	5,500	5,500	5,500	5,500	5,000	5,000
Fluid Density (g/mL)	1	1	1	1	1	1
Power (kW)	4	4	4	4	4	4
Pumps	**P-207A/B**	**P-208A/B**	**P-209A/B**	**P-301A/B**	**P-302A/B**	**P-303A/B**
Flow (LPH)	5,000	1,200	5,000	5,000	5,000	5,000
Fluid Density (g/mL)	1	1	1	1	1	1
Power (kW)	4	2.2	4	4	4	4
Pumps	**P-304A/B**	**P-305A/B**	**P-306A/B**			
Flow (LPH)	1,200	5,000	1			
Fluid Density (g/mL)	1	1	1			
Power (kW)	2.2	4	0.05			

Chromatography Column	**C-201**	**C-202**	**C-301**		
Temp (^{o}C)	5	5	5		
Pressure (atm)	1	1	1		
pH	6-8	8-8.5	8-8.5		
Orientation	Vertical	Vertical	Vertical		
Size					
Height/Length (m)	3	3	3		
Diameter (m)	1	1	1		
Internals	Packed Bed	Packed Bed	Packed Bed		
Centrifuge	**S-101**				
Throughput capacity (m^3/h)	20				
Solids Handling (L/h)	600				
Power (kW)	20				
Holding Tanks	**V-101**	**V-201**	**V-202**	**V-203**	**V-301**
Temperature (^{o}C)	5	5	5	5	5
Pressure (atm)	1	1	1	1	1
Volume (m^3)	25	36.8	36.8	18.8	18.8
Height (m)	4.5	7.5	7.5	6	6
Diameter (m)	3	2.5	2.5	2	2
MOC	SS	SS	SS	SS	SS
Ultra-Filtration	**F-301**				
Area of membrane (m^2)	0.133				
Pore size (kDa)	50				
Pressure (psi)	10				
Freeze Dryer	**D-301**				
Temperature (^{o}C)	-55				
Condenser Capacity (kg/day)	300				
Throughput Capacity (Vials/batch)	65				
Length (m)	6				
Width (m)	3.4				
Height (m)	4.2				

Stream Tables

Table 3: Stream Table for Streams 1-12

Stream number	1	2	3	4	5	6	7	8	9	10	11	12
Temperature (°C)	37	37	37	37	37	37	37	37	37	37	37	37
Pressure (bar)	1	1	1	1	1	1	1	1	1	1	1	1
Vapor Fraction	0	0	0	0	0	0	1	1	0	0	1	1
Mass Flow (kg/batch unless specified)	2.87 µg	5.02	5.02	15.1	20.1	2010	1.08	1.18	2010	20100	358	425
Component Mass Flow (kg/batch unless specified)												
Glucose	0	24 g	24 g	72 g	95.7 g	9.5	0	0	9.49	86.5	0	0
Media	0	5	5	15	20	2,000	0	0	2,000	20,000	0	0
Biomass	2.87 µg	0	953 µg	0	317 mg	0	0	0	105 g	0	0	0
Cells	2.87 µg	0	874 µg	0	291 mg	0	0	0	96.6 g	0	0	0
Antibodies	0	0	7.9 µg	0	2.63 mg	0	0	0	875 mg	0	0	0
Other Proteins	0	0	71.1 µg	0	23.7 mg	0	0	0	7.88 g	0	0	0
Air	0	0	0	0	0	0	1.08	1.18	0	0	390	425
O_2	0	0	0	0	0	0	224 g	112 g	0	0	74.4	37.2
N_2	0	0	0	0	0	0	842 g	842 g	0	0	280	280
CO_2	0	0	0	0	0	0	10.7 g	165 g	0	0	35.4	86.6
H_2O	0	0	0	0	0	0	0	63 g	0	0	0	20.9

Table 4: Stream Table for Streams 13-24

Stream number	13	14	15	16	17	18	19	20	21	22	23	24
Temperature (°C)	37	5	5	5	5	5	5	5	5	5	5	5
Pressure (bar)	1	1	1	1	1	1	1	1	1	1	1	1
Vapor Fraction	0	0	0	0	0	0	0	0	0	0	0	0
Mass Flow (kg/batch unless specified)	20,100	20,100	833	19,200	19,200	16,000	4,700	35,200	4,700	4,700	11,800	11,800
Component Mass Flow (kg/batch unless specified)												
Glucose	61.1	61.1	2.44	58.6	58.6	0	0	58.6	0	0	0	0
Media	20000	20000	799	19200	19200	0	0	19200	0	0	0	0
Biomass	35	35	31.9	3.4	3.4	0	0	2.98	423 g	423 g	0	0
Cells	32.1	32.1	31.8	311 g	311 g	0	0	295 g	15.5 g	15.5 g	0	0
Antibodies	291 g	291 g	11.7 g	281 g	281 g	0	0	14.1 g	267 g	267 g	0	0
Other Proteins	2.62	2.62	117 g	2.81	2.81	0	0	2.67	141 g	141 g	0	0
Air	0	0	0	0	0	0	0	0	0	0	0	0
Protein A Wash Buffer	0	0	0	0	0	16000	0	16000	0	0	0	0
Protein A Elution Buffer	0	0	0	0	0	0	4700	0	4700	4700	0	0
AEX Loading buffer	0	0	0	0	0	0	0	0	0	0	11800	0
AEX Washing Buffer	0	0	0	0	0	0	0	0	0	0	0	11800
AEX Elution Buffer	0	0	0	0	0	0	0	0	0	0	0	0
CEX Loading buffer	0	0	0	0	0	0	0	0	0	0	0	0
CEX Washing Buffer	0	0	0	0	0	0	0	0	0	0	0	0
CEX Elution Buffer	0	0	0	0	0	0	0	0	0	0	0	0
Excipients	0	0	0	0	0	0	0	0	0	0	0	0

Table 5: Stream Table for Streams 25-36

Stream number	25	26	27	28	29	30	31	32	33	34	35	36
Temperature (°C)	5	5	5	5	5	5	5	5	5	5	5	5
Pressure (bar)	1	1	1	1	1	1	1	1	1	1	1	1
Vapor Fraction	0	0	0	0	0	0	0	0	0	0	0	0
Mass Flow (kg/batch unless specified)	11,800	23,500	11,800	11,800	11,800	11,800	11,800	23,500	11,800	11,800	118	11,600
Component Mass Flow (kg/bactch unless specified)												
Glucose	0	0	0	0	0	0	0	0	0	0	0	0
Media	0	0	0	0	0	0	0	0	0	0	0	0
Biomass	0	105 g	318 g	318 g	0	0	0	102 g	216 g	216 g	21.6 g	195 g
Cells	0	7.76 g	7.76 g	7.76 g	0	0	0	7.76 g	0	0	0	0
Antibodies	0	26.7 g	240 g	240 g	0	0	0	24.0 g	216 g	216 g	21.6 g	195 g
Other Proteins	0	70.3 g	70.3 g	70.3 g	0	0	0	70.3 g	0	0	0	0
Air	0	0	0	0	0	0	0	0	0	0	0	0
Protein A Wash Buffer	0	0	0	0	0	0	0	0	0	0	0	0
Protein A Elution Buffer	0	0	0	0	0	0	0	0	0	0	0	0
AEX Loading buffer	0	11,800	0	0	0	0	0	0	0	0	0	0
AEX Washing Buffer	0	11,800	0	0	0	0	0	0	0	0	0	0
AEX Elution Buffer	11,800	0	11,800	11,800	0	0	0	0	0	0	0	0
CEX Loading buffer	0	0	0	0	11,800	0	0	11,800	0	0	0	0
CEX Washing Buffer	0	0	0	0	0	11,800	0	11,800	0	0	0	0
CEX Elution Buffer	0	0	0	0	0	0	11,800	0	11,800	11,800	118	11,600
Excipients	0	0	0	0	0	0	0	0	0	0	0	0

Table 6: Stream Table for Streams 37-42

Stream number	37	38	39	40	41	42
Temperature (°C)	5	5	5	5	5	5
Pressure (bar)	1	1	1	1	1	1
Vapor Fraction	0	0	0	0	0	0
Mass Flow (kg/batch unless specified)	11,600	11,600	11,600	195 g	7.78	8.17
Component Mass Flow (kg/batch unless specified)						
Glucose	0	0	0	0	0	0
Media	0	0	0	0	0	0
Biomass	195 g	195 g	0	195 g	0	195 g
Cells	0	0	0	0	0	0
Antibodies	195 g	195 g	0	195 g	0	195 g
Other Proteins	0	0	0	0	0	0
Air	0	0	0	0	0	0
Protein A Wash Buffer	0	0	0	0	0	0
Protein A Elution Buffer	0	0	0	0	0	0
AEX Loading buffer	0	0	0	0	0	0
AEX Washing Buffer	0	0	0	0	0	0
AEX Elution Buffer	0	0	0	0	0	0
CEX Loading buffer	0	0	0	0	0	0
CEX Washing Buffer	0	0	0	0	0	0
CEX Elution Buffer	11,600	11,600	11,600	0	0	0
Excipients	0	0	0	0	7.78	7.78

Utility Table

Table 7: Utility Table

Utility	Usage Per Batch	Annual Usage
Cooling Water	2480 kg	112,000 kg
Electricity	1,910 MJ	86,000 MJ

Written Description of Process

The antibody production process is split into two main parts: upstream growth and downstream processing. In the upstream processing steps, the antibodies are produced in cell cultures in a series of bioreactors, and in the downstream processing steps, the antibody product is separated, purified, and packaged.

The upstream production process consists of a cascade of four bioreactors. The cell growth media is prepared by mixing the media powder with water for injection (WFI) in a tank (not shown on PFD). The media in this tank is preheated to 37 ˚C using an electric heater. The media feed pump (P-101) pumps the media into the first bioreactor, which has a working volume of 5 L. Approximately 2 micrograms of recombinant CHO cells are added to the reactor. The cells culture for 7 days in the 5 L reactor, then the contents of R-101 are pumped by P-102 into the second bioreactor, R-102, which has a working volume of 20 L. Media is fed to R-102 through the same media feed pump (P-101). R-102 cultures for 7 days, then the process is repeated: P-103 pumps the contents of R-102 into R-103, and P-101 adds media to R-103. R-103 has a working volume of 2,000 L. After 7 days in R-103, the process is repeated a final time to fill the 20,000L bioreactor (R-104). P-104 pumps the contents of R-103 into R-104, and the

media is fed through P-101. For the two larger bioreactors (R-103, R-104), an air feed is added to the reactors. Air is bubbled through the reactors to provide oxygen and CO_2 to maintain an environment in which the cells can grow. After 7 days, the contents are pumped out of R-104 through P-105 and fed into a shell and tube heat exchanger to lower the temperature to 5^{o}C. The fluid stream is split to go to two different heat exchangers in parallel where it is cooled from 37 °C to 5°C in four hours.

Following the heat exchanger, the product solution is fed to a disk-stack centrifuge, S-101, to remove large cell debris from the product solution. The cell debris will settle at the bottom and the product solution will be pumped to the next purification step. This entire process takes about 2 hours to complete. Removing the cell debris facilitates the purification of the product in the downstream process.

The product solution is then sent to a series of chromatography columns where the antibody is captured and purified. The first column (C-101) uses affinity chromatography to retain as much of the proteins in the product solution as possible. The column is packed with a Protein A resin that has a very high affinity for human and humanized antibodies. Four column volumes (CVs) of an equilibration buffer that is 30 mM sodium phosphate is used to prime the column before batches. The product solution is then run through the column followed by a washing step where 7 CVs of the same buffer are used to rinse out all unbound material from the column. Two CVs of a hydrochloric acid buffer at a pH of around 2 are used to elute the bound proteins from the column. The whole process starting from product solution entering the column through the elution step takes around 7 hours. The eluted solution is stored in a holding tank (V-202) before going to the next steps of the purification process. The ion exchange columns will

purify the antibody solution by removing the other biologic proteins and any leeched Protein A molecules.

For this process an anion anda cation exchange column is used for further purification. These columns work by utilizing the isoelectric point of the desired protein. In the anion exchange column, 5 CVs of tricine buffer are run through the resin-packed column (C-202). This step changes the overall net charge of the antibodies to positive using a pH of 8.5 which is above the isoelectric point of bevacizumab (pH 8.3). When the product solution from the Protein A column is run through, the desired antibodies attach to the resin. The column is then washed with 5 CVs of the same buffer that was used to charge the column to remove unbound compounds. Then, tricine is again used to elute the antibodies from the resin. This time, however, the tricine is at a pH of 8.3 in order to release the antibody from the resin. From there it is sent to a storage vessel (V-203),before ultimately being delivered to the cation exchange column. This process is identical to the anion exchange column except a pH lower than the isoelectric point of the antibody is used to create a negatively charged resin for capturing the protein. After the antibodies are eluted from the cation exchange column they are fed into a storage vessel (V-301) where they are held until they are sent to the ultrafiltration process.

The final step in the purification process is ultrafiltration. In this step small peptides and salts are removed from the product solution. The product solution is continuously pumped through the membrane, F-301, until the target purity of product has been achieved. After the ultrafiltration step, the product is placed in small 4 mL vials by the bottling unit (B-301). Sugars such as trehalose are added to the vial to be sent to the freeze dryer. Sugars are added to the product solution to preserve the stability of the antibody. After the water has been removed from the vials in the freeze dryer, phosphate buffered saline is added to the final product to dilute the

product to 25 mg/mL. This final product is shipped off to healthcare providers that will use the antibodies to treat affected patients

Rationale for Process Choice

Monoclonal antibody production is a quickly advancing industry and while the foundational process is well established, there are variations from one facility to another. The proposed plant utilizes batch processing which is the most common processing mode in the biopharmaceutical industry (Petrides et al. 158). For the designed process it allows one batch per week and then an additional day between batches for cleaning and sanitizing the equipment. The bioreactor cascade with corresponding volumes is based off of the method proposed by Petrides et al. The model for cell growth is modeled by a Monod-like kinetic model found by Lopez-Meza et al. This specific model was chosen because it provides a simple model for ease of calculating product yield and manipulating conditions to maximize cell growth.

A heat exchanger is used after the bioreactors to decrease the temperature from ideal cell growing conditions (37 °C) to 5 °C to prevent the antibody from denaturing during processing. From there it is necessary to remove the solid cell debris, cell media, and undesired proteins from the monoclonal antibody. A centrifuge is used to remove the cell debris. The process was originally designed with a settling tank prior to the centrifuge but in order to decrease processing time and reduce product waste, the settling tank was removed and the solution goes directly to the centrifuge.

From the centrifuge, the product solution goes through a series of three chromatography columns. The first affinity chromatography column uses Protein A to adsorb the antibody which is an industry standard (Low et al. 50). To further purify the product ion chromatography or hydrophobic interaction chromatography are typically used. As suggested by Low et al. ion

exchange chromatography is used in this process because it is optimal for commercial practices compared to other types of chromatography. Ultrafiltration is used to remove the remaining salts from the chromatography column buffers. The final freeze drying step removes the water to complete the antibody purification process. The powder is mixed with excipients to dilute the product, increase therapeutic effect, and extend shelf life. Trehalose was selected as the main excipient to be added because it has been shown to provide stability for pharmaceutical components with minimal protein degradation (Colaco). The product is sold in a liquid form so it is ready for use.

This process rationale provides adequate plant capacity to meet the goal of 8760 g of bevacizumab per year. Equipment calculations to further justify this process are in Appendix A. This process provides groundwork for an efficient antibody production facility. The process could be improved in the future by using an improved kinetic growth model. One way to improve the yield would be to design a semi-batch reactor cascade instead of the designed batch system (Lopez-Meza). Additionally, the concentrations achieved in the paper "Benchmarking of commercially available CHO cell culture media for antibody production" are almost a full order of magnitude greater than the model by Lopez-Meza (Reinhart et al.). The bioreactor cascade is the limiting step in the process, requiring a full week in each reactor to achieve desired growth. The time limitations are calculated in Appendix B. To increase production capacity a second bioreactor cascade could be used in parallel to reduce the time per batch by 50%, doubling the plant output.

Equipment Description, Rationale, and Optimization

Reactors

R-101 – R-104: Bioreactor Cascade

The bioreactor cascade consists of four jacketed stainless steel stirred-tank bioreactors. Batch bioreactors are used to minimize contamination of the cell culture. Additionally, batch reactors are the industry standard for producing monoclonal antibodies (Petrides et al. 158). The operating conditions for the bioreactors are summarized in Table 8.

Table 8: Summary of Bioreactor Operating Conditions

	R-101	R-102	R-103	R-104	Source:
Working Volume	5 L	20 L	2,000 L	20,000 L	Petrides et al., 158
Diameter	0.2 m	0.3 m	1.5 m	3 m	Appendix A.1
Height	0.3 m	0.5 m	2 m	4.5 m	Appendix A.1
Time	7 days	7 days	7 days	7 days	Appendix A.1
Mixer speed	80 rpm	80 rpm	80 rpm	80 rpm	Sellick et al.
Dissolved [O_2]	30%	30%	30%	30%	Sellick et al.
pH	7.0	7.0	7.0	7.0	Sellick et al.
Temperature	37 ˚C	37 ˚C	37 ˚C	37 ˚C	Lopez-Meza et al., 158 Sellick et al.
Media Type	BalanCD® CHO Growth A Medium				Reinhart et al.

R-101 is a 5 L bioreactor that is 30 cm in diameter and 20 cm in height. R-102 is a 20 L bioreactor that is 30 cm in diameter and 50 cm in height. R-103 is a 2,000 L bioreactor that is 1.5 m in diameter and 2 m in height. R-104 is a 20,000 L bioreactor that is 3 m in diameter and 4.5 m in height. The working volumes chosen for the bioreactors are based on industry standards used in the antibody production process (Petriedes et al., 158). The calculations used to determine the dimensions of each bioreactor are detailed in Appendix A.1 - Bioreactors (A.1.33-A.1.37).

A Monod-like kinetic model was used to model the component concentrations of each component in the reactor cascade to determine the residence time for each reactor. This model is taken from the paper titled "Using simple models to describe the kinetics of growth, glucose consumption, and monoclonal antibody formation in naïve and infliximab producer CHO cells" by Lopez-Meza, et al. The paper investigates the production of infliximab, but, it is reasonable to assume that the monoclonal antibody kinetics are roughly the same between bevacizumab and infliximab. The solution to the proposed model and the results from running the model are found in Appendix A.1 - Bioreactors in equations A.1.1 - A.1.17. Figure 5 is a plot of the mAb concentration versus time in the bioreactor cascade. Since the antibody production is directly proportional to the amount of CHO cells in the culture, the mAb production curve exhibits exponential behavior.

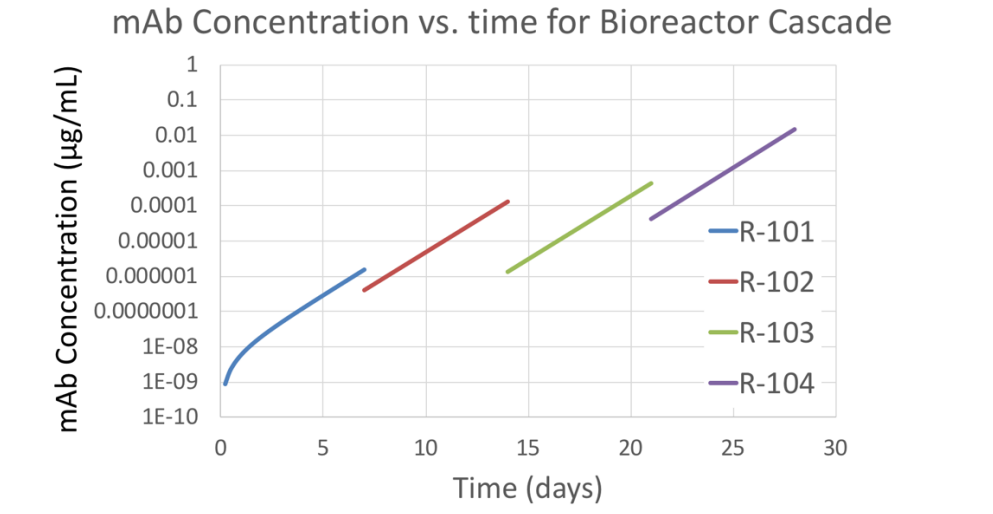

Figure 5: mAb Concentration in Bioreactor Cascade

An energy balance is performed to determine the amount of cooling water required to maintain the bioreactor temperature at a constant 37 ˚C. This calculation is found in Appendix A – Bioreactors in equations A.1.18 - A.1.32.

The operating conditions for each bioreactor are identical. In 2015, Sellicik et al. investigated the yield of CHO cell cultures and determined the optimal bioreactor operating conditions and detailed their findings in the article “Optimizing CHO Cell Culture Conditions”. The bioreactor cascade in the proposed plant uses the optimal conditions discussed in the paper. The speed of the agitators in the bioreactors is 80 revolutions per minute (rpm), the dissolved oxygen concentration is kept above 30%, and the pH in the reactors is maintained at 7.0.

The temperature of the bioreactors is controlled at 37 ˚C. The feed to each bioreactor is pre-heated to 37 ˚C, so that the temperature of the contents in the bioreactor is initially at the set point. All of the bioreactors utilize cooling jackets to cool the contents of the bioreactor as heat is released through exothermic metabolic processes.

The media type used in the bioreactors is the BalanCD® CHO Growth A Medium, from Irvine Scientific. Reinhart et al. studied the effect of different media types used in CHO cell cultures and found that BalanCD® CHO Growth A Medium can be used to produce high cell and product concentrations.

Chromatography Columns

C-201: Protein A Chromatography Column

The protein A chromatography column is the first column used in the antibody purifcation process. The purpose of the column is to capture only the proteins in the solution and then elute them for further antibody purification. This removes all of the compounds that are not proteins from the product solution. To maximize protein capture a resin needs to be selected that

can handle large linear velocities and has high protein binding capacitiy. POROS™ MabCapture™ resin is chosen because it is designed for a large-scale manufacturing process ("POROS™ MabCapture™ A Select Affinity Chromatography Resin"). Using the binding capacity of 37 mg/mL the total volume of resin needed is approximately 80 L. The tank is designed to be 1 m in diameter and 3 m high based off of heuristic relationship of 3:1 for the height to diameter ratio (eqns. A.2.1-5). This tank size allows for easier purchase and there is space to expand the process if more bioreactors are implemented in the future. The tank is constructed of stainless steel to ensure a sterile process and allow for ease of cleaning.

The resin allows for a high linear velocity of 700 cm/hr and at these conditions the resin has only a 5% breakthrough capacity ("POROS™ MabCapture™" 2017). The residence time is 26 minutes and the solution will take three and a half hours to flow through the column. In between batches the column should be sanitized with sodium hydroxide. To prevent contamination the resin should be replaced annually.

C-202: Anion Exchange Chromatography Column

Anion exchange chromatography columns are widely used in industry to separate a large range of molecules from amino acids and nucleotides as well as large proteins. In this process all of the proteins captured in the protein A chromatography column are sent through the anion exchange column in order to capture only the desired antibodies. Exchange chromatography is a sterile process, which makes it a viable method for purification in the proposed process.

For anion exchange chromatography to be successful on a large scale, a resin needs to be selected, similar to the Protein A resin, that allows for fast linear velocities and has a high binding capacity to capture all of the monoclonal antibodies, and reduce loss in the system. Nuvia Q was selected because it allows for a linear velocity of 600 cm/hr and has a binding

capacity of 170 mg of antibody/mL of resin. This resin also has a low breakthrough capcity of 10% ("Nuvia™ Q Resin."). Based on the binding capacity and the amount of antibody that needs to be captured the column was sized to 1 m in diameter and 3 m in height. This is much larger than necessary to allow for further upscaling in the future. Similar to the Protein A column the column should be sanitized with sodium hydroxide between batches and the resin replaced annually to prevent potential contamination.

C-301: Cation Exchange Chromatography Column

A cation exchange chromatography column is used in addition to an anion exchange column to ensure the highest purity possible. The process is identical to the anionic process however a different resin was selected to allow for maximum capture. Nuvia S is utilized allowing a maximum linear velocity of 600 cm/hr and a binding capacity of 110 mg of antibody/mL of resin with a breakthrough capacity of 10% ("Nuvia™ S Resin"). The column was sized using the same process as the anion column as well. This calculation resulted in a column of the same size with a height of 3 m and a diameter of 1 m.

Vessels

V-101: Centrifuge holding tank

The centrifuge holding tank is located between the bioreactor and the centrifuge. This tank is designed to hold a total volume of 20 m^3. The working volume of this holding tank is set to 80% to reduce the chances of the vessel overfilling. The dimensions of the vessel are determined by using the relationship of the vessel height being equal to three times the column diameter. Using that relationship the dimensions of the holding vessel are found to be 3 m in diameter and 4.5 m in height.

V-201: Protein A Chromatography Holding Tank

The Protein A chromatography holding tank is located in between the centrifuge and the protein A chromatography column. It was designed to be able to hold the total volume coming out of the centrifuge per batch which is 19.23 m^3. Using the heuristic relationship of the vessel height being equal to three times the column diameter with an 80% working volume, the diameter was found to be 2.2 m with a height of 6.5 m (eqns. A.3.5-6). In order to mak the vessel easier to purchase, the diameter was adjusted up to the nearest half meter with the diameter being 2.5 m and a height of 7.5 m. The vessel is constructed of stainless steel for ease of cleaning.

V-202: AEX Holding Tank

This holding tank is located before the AEX column and after the protein A Column (C-201). The required storage volume necessary is 2.5 L. Assuming an 80% working volume and adjusting for purchasing purposes , a diameter of 2 m and height of 6 m was calculated providing a total tank volume of 18.8 m^3 Stainless steel was selected to reduce the risk of contamination and allow for easy routine cleaning/maintenance.

V-203 and V-301: AEX/CEX Exit Holding Tanks

The AEX/CEX exit holding tanks are located at the exit of the AEX and CEX columns respectively. These tanks each need to be able to hold the total volume of elution buffer that is run through the columns before it. That is, it must be able to hold 11,775 L of solution. To size this, the heuristics ration of 3:1 was used and that the required capacity must be 80% of the working volume. Based on these specifications, the tank dimensions of 2.5 m in diameter and 7.5 m in height were determined. The calculations for this can be found in the equipment calculations. Additionally, the tanks must be made up of stainless steel as it is ideal for the sterile conditions needed in operations.

Pumps

P-101: Media Feed Pump

The media feed pump is used to pump cell growth media into the bioreactors. This pump is unique because it is used to fill four bioreactors with volumes ranging from 5 L to 20,000 L, therefore, this pump must be able to operate at a wide range of flowrates. The QF5050S pump from "Quattroflow Fluid Systems" has an operable range from 50 LPH to 5,000 LPH (7). This operating range is appropriate to use the QF5050s pump as P-101. The calculations used to arrive at this conclusion are found in Apendix A.4 - Pumps (Eqns A.4.1-A.4.2). A diaphram pump is used for this pump because it is gentle and doesn't disrupt the components in the media solution.

P-102 – P-104: Bioreactor Products Pumps

The bioreactor products pumps are used to pump the products from one batch reactor to the next batch reactor. The size of each pump is based on the volume of products in the preceeding bioreactor that will be pumped to the following bioreactor. It takes 1 hour to pump the products out of each of the bioreactors. This time is important because there is only one day for cleaning the bioreactors in between batches- pumping the bioreactor products quickly allows more time to clean the bioreactor after the products have been removed. The following models are used as pumps in the plant: P-102: QF150S, P-103: QF1200S, P-104:QF4400S ("Quattroflow Fluid Systems" 7). The calculations that support these choices as appropriate pumps for the process are detailed in Appendix A.4 - Pumps (Eqns A.4.3-A.4.5). Diaphram pumps are used for P-102 – P-104 because they are gentle and do not introduce a lot of shear forces into the fluid being pumped. Shear forces are detrimental to the bioreactor product solution because it can lead to bursting the CHO cells and/or breaking apart the antibody product.

P-105: 20,000L Bioreactor Products Pump

The 20,000 L bioreactor products pump is used to pump the products of R-104 through the heat exchanger (E-101) to the holding tank before centrifugation (V-101). The design flowrate of the bioreactor product through the heat exchanger is 5,000 LPH. Therefore, a pump that can accommodate a flowrate of 5,000 LPH is chosen for P-105. The QF20K is equipped to handle a flowrate of 5,000 LPH, so it is used for P-105. The calculations that support this conclusion are found in Appendix A.4 - Pumps (Eqn A.4.6). Again, a diaphram pump is chosen to reduce the shear force exerted on the bioreactor products.

P-106: Centrifuge Products Pump

The centrifuge pump is used to move the antibodies from the centrifuge to the holding tank prior to Protein A chromotogrpahy (V-201). The centrifuge has a bowl volume of 30 L, which means only 30 L of product solution need to be moved to the holding tank at a time. There is 20,000 L of the porduct solution that needs to goes through the centrifuge. That 20,000 L product solution is split into batches of 30 L and each batch takes approximately 10 seconds to run. Therefore the pump must be equipped to handle at least 10,000 LPH. The pump chosen for this application was a quaternary diaphragm pump QF20K with a capacity of 20,000 LPH ("Quattroflow Fluid Systems" 7). The pump will be running at half of its max capacity to increase the efficiency of the pump.

P-201 – P-204: Pumps for Protein A Chromatography Column

The pumps for the Protein A chromatography column are all designed based off of the maximum allowable flowrate in the column. Since the column is capable of handling a linear velocity of 7 m/hr, that corresponds to a volumetric flow rate of 5.5 m^3/hr with the given diameter of 1 m (eqns. A.4.8-9). Diaphragm pumps are used to in this process and the

appropriate sized pump is QF20K which has the capacity of 1-20 m^3 per hour ("Quattroflow Fluid Systems" 7).

P-205 – P-207, P-209: AEX Column Buffer and Waste Pumps

The four pumps used in the ion exchange chromatography process must be capable of pumping in buffer solutions at the maximum allowable flowrate for the AEX column. Using the desired linear velocity in the column, which is 600 cm/hr, and the cross-sectional area of the column the necessary volumetric flowrate through the pump is 4.71 m3/hr or approximately 5,000 LPH. Because diaphragm pumps are used throughout the process the appropriate sized pump here is a QF20K ("Quattroflow Fluid Systems" 7).

P-208: AEX Column Elution Buffer Pump

The elution buffer pump differs from the other buffer pumps because the recommended design specifications for the selected resin in the AEX column states that the antibody must be eluted at a velocity no greater than 150 cm/hr. This leads to the determination that the required volumetric flowrate neede from the pump is no greater than 1,200 LPH. The diaphragm pump qualified for this flowrate is the QF4400S ("Quattroflow Fluid Systems" 7).

P-301 – P-303, P-305: CEX Column Buffer and Waste Pumps

Similar to the AEX column buffer and waste pumps, all of the CEX buffer and waste pumps must be able to produce a flowrate of 5,000 LPH since the allowable maximum velocity for the resin in the CEX column is also 600 cm/hr and both columns have the same dimensions. Therefore the same pump, a QF20K is utilized here ("Quattroflow Fluid Systems" 7).

P-304: CEX Column Elution Buffer Pump

The maximum required velocity for the elution buffer through the CEX column is only 150 cm/hr, similar to the AEX column. Due to both columns having equivalent dimensions the

required flowrate through the pump is approximately 1,200 LPH. Once again, the QF4400S diaphragm pump is the most cost effective and efficient pump available ("Quattroflow Fluid Systems" 7).

P-306: Pump to filtration

The pump to the filtration membrane is a small pump because only about 1 liter of solution is being moved through the filter. The chosen pump is QF150S Quaternary diaphragm pump. The max flowrate of this pump is 180 LPH which is more than enough capacity to move the solution through the ultrafiltration membrane.

Additional Equipment

E-101: Heat Exchanger

The heat exchanger was designed using ASPEN by specifying input flowrates, temperatures, pressure and outlet temperature of the product stream. The heat exchanger is shell and tube with counter-current flow. It is constructed of stainless steel to allow for ease of cleaning. Tetrafluoroethane is a refrigerant that is used as the coolant fluid since the outlet temperature of the product solution must be reduced to 5 °C. To increase cooling efficiency two heat exchangers are used in parallel with flow rates of the product stream being 2500 kg/hr. The resultant heat exchanger has an area of 64 m^2 with tube lengths of approximately 5 m. Two heat exchangers are used in parallel to increase the efficiency and reduce the process time to only four hours.

S-101: Centrifuge

A stainless steel disk stack centrifuge is used in the first purification step in the process. Disk stack centrifuges are ideal for shear sensitive products because of its hermetic design with a

bottom feed. The centrifuge also avoids the pick up of oxygen. The throughput capacity of the centrifuge is a maximum of 20 m^3/hr. The stainless steel material makes it easier for sterilizing and cleaning the centrifuge. The centrifuge can hold up to 30 L per batch with a sludge space volume of 10 L. The amount of sludge created is minimal because the cells that are being removed are failry small. In the process a product solution of 20 m^3 must pass through the bowl. Thus, it will take approximately 2 hrs to run the solution through the centrifuge.

F-301: Ultrafiltration

Tangentital flow filtration is the most effective ways of clarifying, concentrating, and purifying antibodies. Normal flow filtration is not used because pressure is applied directly towards the mebrane which makes it more succeptible to foul the small pores of the membrane. The transmembrane pressure of the system is the driving force of the solution through the membrane. A rule of thumb for ultrafiltration is to set a transmembrane presure of 9-12 psig. The molecular weight cut off of the membrane is 50 kDa. This means that 90% of the salts and polypepetides from the upstream process is retained in the membrane. Any compounds with a molecular weight higher than 50 kDa will stay in the retenate stream. The membrane area is determined using the flux and process time. About one liter of solution is fed through the ultrafriltration memebrane thus, the membrane area is fairly small.

B-301: Bottler

The bottler is a stainless steel semi-autoamitc bottler used to place antibodies into 4 mL vials. Within the bottling machine trehalose will be added to ensure stability of the product. This bottler can bottle up to 10 vials per hour.

D-301: Freeze dryer

The freeze dryer chosen for this process is a SMART LYO ™ freeze dryer. This a pharmecetutical freeze dryer system that is composed of a condensor unit, venting system, and vaccumm. The benefit of using this freeze dryer is that it has the option of clean in place (CIP)/ sterilization in place (SIP). The material of contrustruction is stainless steel which facilitates the cleaning process.

Safety Issues

The monoclonal antibody production site is a pharmaceutical site where cleanliness is crucial to the effectiveness of the manufacturing process. The entire site will be a clean room to avoid any contamination of the product. The personnel working on site will be required to wear coveralls made of Tyvek which is a synthetic material made from flash spun high-density polyethylene fibers (Samson). Coveralls made of Tyvek have a very low particle shed count. In addition to the coveralls, personnel will also wear standard personal protective equipment (PPE) such as gloves and safety glasses as required by The Occupational Safety and Health Administration (OSHA). PPE is necessary to protect personnel from toxic substances on site such as hydrochloric acid and to protect the manufacturing process from human contamination.

There are safety risks that need to be considered with the large equipment used on site. Large vessels are used throughout the entire site; the largest vessel is the final bioreactor which has a working volume of 20 m^3. The level of these vessels needs to be closely monitored to avoid any major spills. Disturbances to the process that can cause increased tank level include controller malfunctions or inlet/outlet valve malfunction.

The temperature of the antibody must be kept cool at 5 °C in the purification stages to reduce the risk of degradation of the product. Refrigeration of the purification facility will allow the process to be kept at 5 °C. The production of antibodies is an exothermic reaction thus, in the production stages the antibodies will be kept at 37 °C by cooling the bioreactors with a cooling jacket. Any malfunction in the refrigeration equipment will affect the integrity and stability of the product. More information on safety risks related to the major equipment on site can be found in the hazard and operability analysis found in Appendix D – HAZOP Forms.

There are various chemicals that are stored in large vessels throughout the plant. The most hazardous chemical stored in a large vessel is hydrochloric acid. Hydrochloric acid must be stored in a metallic or coated fiberboard drum using a strong polyethylene inner package. Hydrochloric acid should not be stored near any oxidizing agents, organic materials, metals, or alkalis ("*Hydrochloric acid*"). Other chemicals that are stored in large vessels include tricine and cell media. These two chemicals are not as hazardous as hydrochloric acid, but safety precautions still need to be taken into consideration. All storage vessels for buffers to the chromatography columns and cell media must be kept in a cool, well ventilated area.

Regulations

The monoclonal antibody manufacturing site must follow federal regulations set forth by the Food and Drug Administration (FDA). There is a need for quality control and assurance departments to ensure that the site is meeting the requirements of good manufacturing practices. In the manufacturing site, Chinese Hamsters are injected with an antigen in the lab to obtain the ovary cells that will produce the specific antibody to combat that antigen. The lab must be in accordance with the NIH guide for Care and Use of Laboratory Animals. There will be frequent serological testing of the Chinese Hamster established to test for any type of virus contamination or infectious disease.

Commercial production of antibodies requires considerable efforts to purify the antibody. The FDA requires that sites have a purification scheme that has the ability to remove adventitious agents and other contaminants. The site has implemented various purification equipment such as Protein A and Ion Exchange Chromatography columns and ultrafiltration to ensure removal of contaminants. The FDA also requires the proposed plant to have limits on the number of times a purification component can be reused. Monitoring systems will be

implemented throughout every piece of equipment in the plant to ensure acceptable performance of equipment.

The stability of the antibody must meet the needs imposed by the clinical protocol. To meet those needs, a stability testing program is to be implemented to test for the integrity, potency, sterility, identity, and pH of final product at regular intervals. A small amount of final product lot will go through various test to ensure they meet the standards.

Environmental Impact Statement

Gaseous, aqueous, and solid waste

Due to the biologic process of antibody production, the majority of waste created from the plant is biohazardous and requires appropriate measures to be taken. The gaseous waste out of the bioreactor consists of 1,600 kg/year of carbon dioxide which is released to the environment along with unreacted oxygen and water vapor. The aqueous waste includes the salts from the buffers and dissolved biomass. There are 4.7 m^3 per year of hydrochloric acid buffer disposed of along with 26 m^3 of sodium phosphate and 71 m^3 of tricine. There are 2,000 kg per year of both solid and aqueous biomass. The biomass and chemical waste must be further treated before being disposed of based on biohazard standards.

Utilities

The two major utilities used in the proposed plant are cooling water and electricity. Since the cooling water is not consumed in the process, it can be recycled after it has been cooled in a cooling tower. Therefore, the cooling water has a minimal impact on the environment. No water is consumed, and the only step in the process that has an environmental impact is the cooling tower. Since such a small amount of cooling water is used per batch, the cooling tower is located offsite; for this reason, the cooling tower environmental impact is not considered as part of the environmental impact of the proposed plant.

The other utility used in the proposed plant is electricity. Electricity is consumed in the process through the pumps, centrifuge (S-101), bottler (B-301), and the media feed pre-heater (not shown on PFD). 86,000 MJ of electricity are consumed annually by the process equipment. The calculations used to find the electricity requirements for the process are documented in Appendix B.1 – Equipment Calculations and Appendix B.2 – Economic Analysis Spreadsheet.

The electricity consumption translates to a $CO_{2,}e$ of 17.8 metric tons (US, EPA). Additionally, a large amount of electricity is consumed to maintain the temperature of the downstream process at 5 ˚C. This amount of electricity was not calculated for the scope of this project; however, since the proposed plant is located in Tucson, Arizona, the electricity costs for cooling are significant and will need to be quantified to fully investigate the environmental impact of the utilities used in the production process.

Potential Chemical Spills

Large volumes of buffer are used throughout the process which requires the preparation for the possibility of chemical spills. The salts used in buffers are delivered as solids before being mixed with water for injection to make the aqueous buffers. If there is a spill of these solids then they should be disposed of in a regular waste and then the area should be cleaned with water. In the scenario of a large spill, a shovel should be used to dispose of the salts in a proper waste container and then the area should still be cleaned up with water. To clean up minor to moderate liquid spills, a commercially available kit including an inert adsorbent can be used. Hydrochloric acid is used in the process and requires special care when cleaning up a spill. For a small spill the hydrochloric acid buffer should be diluted and then cleaned up with an inert adsorbent. If there is a large spill then the solution should be adsorbed with dry earth and a water spray should be used to divert toxic vapors (*Hydrochloric Acid*). The chemical waste will be stored on site and then sent to a treatment facility.

The plant can be equipped with floor drainage that leads to a hazardous waste collection container. It is vital that the buffers do not enter drains although the cell media does not pose any environmental harm. To prevent the buffers from entering drains it is important that all waste is directed toward appropriate chemical disposal.

Regulations

The U.S. Environmental Protection Agency (EPA) requires that new and existing industrial facilities obtain Clean Air Act permits for non-GHGs and GHGs. Per the EPA fact sheet, if an industrial facility emits less than 50,000 tons of GHGs per year on a carbon dioxide equivalent (CO_{2e}) basis, an operating permit will not be required ("Clean Air Act Permitting for Greenhouse Gas Emissions – Final Rules "). The proposed production facility emits approximately 17.8 metric tons of $CO_{2,e}$ annually by the process equipment. Additional $CO_{2,e}$ is emitted by the refrigeration units for the facility but not enough to reach the 50,000 tons mark. A Clean Air Act permit for GHGs will not be required for the proposed production facility. Although permits will not be required, CO_2 will be closely monitored on each piece of process equipment.

Life Cycle Assessment

The Life Cycle Assessment (LCA) impact of the proposed plant consists of raw material usage (resource depletion) and CO_2e (global warming potential). The land use, nitrification potential and ozone depletion impacts of the proposed plant are not considered in this LCA, but would need to be quantified to fully analyze the LCA impact of the proposed plant. The raw materials consumed are listed in the Table 9.

Table 9: Raw Material Usage

Raw Material	**Annual amount consumed**
Water For Injection	968,000 kg
Media Powder	2,350 kg
CHO Cells	129.15 µg
CO_2	1600 kg
Various Buffers	4,570,000 kg
Excipients	350 L

The raw materials that have the largest impact on the environment are the WFI and the buffers. Since the buffers consist mainly of WFI, the total pure water consumed annually for the process is the sum of the WFI and the buffers; the total pure water consumed by the plant annually is 5.54 million kg. The other raw materials consumed in the process are in low quantities and they are not precious compounds, so the environmental impact of the rest of the raw materials is minimal.

There are two components of the proposed process that contribute to global warming potential (CO_2e): the electricity consumed by process equipment, and the CO_2 emitted from the cell cultures in the bioreactor cascade. As explained in the utilities section above, the process equipment consumes 86,000 MJ of electricity, which translates to a CO_2e of 17.8 metric tons (17,800 kg). The bioreactor cascade produces 251 kg of CO_2 per batch, or 11,295 kg of CO_2 annually. The calculations used to arrive at this value are found in Appendix B.3 – Mass Balance Spreadsheet. Combining the CO_2 emissions from the bioreactor cascade with the CO_2e from the process equipment yields a total global warming potential of 29,100 kg of CO_2e. This is well below the CO_2e threshold set forth by the EPA for environmental regulation permits.

Economic Analysis Including Economic Hazards

One of the main predictors of plant feasability is the economic outlook. The equipment cost is summarizaed in Table 10 and these costs are one-time startup costs. The reoccurring annual costs are found in Table 11 which includes the annual raw material and usage costs. The annual production costs are in Table 12. Table 13 shows the components that contribute to the total capital investment for the proposed plant. The net present value of the plant is shown in Table 14. Table 10 displays the bare module cost of each piece of equipment used in this process. Most equipment costs were calculated using the formulas and diagrams provided in Seider et al., (2009); however, the bare module costs of the jacketed bioreactors (R-101:104), the bottler (B-301) and the freeze dyer (D-301) used in the proposed plant are not included in the book. The bioreactors were calculated using figures in Ulrich and Vasudevan, 2004. The costs of the bottler (B-301) and the freeze dryer (D-301) were sourced from the manufacturer of those pieces of equipment (Liberti and SMART LYO^{TM} Pharma Freeze Dryer). The calculations used to arrive at the bare module equipment costs are detailed in Appendix B.2 – Economic Calculations in the sheet titled “Equipment Costs”.

Table 10: Equipment Cost Breakdown

Equipment	Bare Module Cost

Reactors		
R-101	$	40,500
R-102	$	45,000
R-103	$	63,100
R-104	$	243,000
Total	$	392,000

Vessels		
C-201	$	37,100
C-202	$	37,100
C-301	$	37,100
V-101	$	124,000
V-201	$	152,000
V-202	$	108,000
V-203	$	108,000
V-301	$	108,000
Total	$	710,000

Additional Equipment		
E-101	$	362,000
B-301	$	225,000
S-101	$	216,000
D-301	$	546,000
F-301	$	97,400
Total	$	1,450,000

Equipment	Bare Module Cost

Pumps		
P-101	$	33,700
P-102	$	16,000
P-103	$	17,400
P-104	$	29,400
P-105	$	38,600
P-106	$	33,700
P-201	$	38,600
P-202	$	38,600
P-203	$	38,600
P-204	$	38,600
P-205	$	38,600
P-206	$	38,600
P-207	$	38,600
P-208	$	29,400
P-209	$	38,600
P-301	$	38,600
P-302	$	38,600
P-303	$	38,600
P-304	$	29,400
P-305	$	38,600
P-306	$	16,000
Total	$	707,000

Total Equipment Cost:	$	3,250,000

Table 11 displays the cost breakdown of the raw materials used in the process. The calculations used to arrive at these costs are detailed in Appendix B.2 – Economic Calculations in the sheet titled "Raw Material and Product Costs." The main contributors to the raw material cost for the proposed plant are Water For Injection (WFI), powdered media solution, and Protein A resin. Water For Injection is very expensive ($3 per liter), and a large amount of water for injection is needed to prepare the media solution (per the instructions in the BalanCD Growth A Directions For Use) and to prepare the buffer solutions used to elute the chromatography columns. Additionally, Protein A is very expensive; an alternative compound to replace Protein A for affinity chromatography is discussed later in this section.

Table 11 : Annual Raw Material Usage and Costs

Component	Annual Consumption (kg)	Annual Cost
WFI	968,000	$ 2,900,000
Media Powder	23,500	$ 900,000
CHO Cells	129	$ 45,100
CO2	1,590	$ 49,400
AC Equilibration Buffer	1,170,000	$ 176,000
AC Elution Buffer	212,000	$ 31,700
IEX Equilibration Buffer	531,000	$ 101,000
IEX Wash Buffer	531,000	$ 117,000
IEX Elution Buffer	531,000	$ 186,000
AEX Equilibration Buffer	531,000	$ 101,000
AEX Wash Buffer	531,000	$ 117,000
AEX Elution Buffer	531,000	$ 186,000
Excipients	349	$ 26,200
Protein A Resin	80	$ 480,000
IEX Resin	20	$ 24,000
AEX Resin	20	$ 24,000
	Total Raw Material Cost:	$ 5,470,000

The bare module costs from Table 10 and the raw material costs in Table 11 are used in Table 12 along with other factors to determine the annual production cost for the proposed plant. The annual cost of utilities is calculated in Appendix B.2 – Economic Calculations in the sheet titled "Raw Material and Product Costs". The annual costs for operating overhead, maintenance, operations, property taxes and general expenses were calculated using equations found in Chapter 17 of Seider et al., 2009. These calculations are documented in Appendix B.2 - Economic Calculations in the sheet titled "Annual Costs." The annual cost of feedstocks, utilities, operating overhead, maintenance, operations, and property taxes and insurance combine to yield an annual cost of manufacture of $4.46 million. When general expenses are added in, the total production cost is $12.7 million.

Table 12: Annual Production Cost Breakdown

Feedstocks	**$**	**5,470,000**
Utilities	**$**	**1,680**
Cooling Water	$	3
Electricity	$	1,670
Refrigerant (-10 ˚C)	$	1
Operating Overhead	$	440,000
General Plant Overhead	$	78,000
Mechanical Dept. Services	$	26,400
Employee Relations Dept.	$	64,800
Business Services	$	81,300
Maintenance	**$**	**764,000**
Wages & Benefits	$	332,000
Salaries & Benefits	$	83,000
Materials and Services	$	332,000
Maintenance overhead	$	16,600
Operations	**$**	**3,070,000**
Direct Wages & Benefits	$	2,020,000
Direct Salaries & Benefits	$	302,000
Operating Supplies & Services	$	360,000
Control Laboratory	$	390,000
Property Taxes & Insurance	**$**	**190,000**
COST OF MANUFACTURE	**$**	**4,460,000**

General Expenses	**$**	**8,270,000**
Selling Expense	$	2,150,000
Direct Research	$	3,440,000
Allocated Research	$	358,000
Administrative Expense	$	1,430,000
Management Incentives	$	895,000

TOTAL PRODUCTION COST	**$**	**12,700,000**

The components of the total capital investment are shown in Table 13. The table includes the description of each cost that contributes to the total capital investment. The bare module equipment cost from Table 10 is used and the cost of spare pumps and storage tanks are also accounted for to get the total bare module investment. The costs of site preparation, service facilities, and allocated costs are added to the total bare module investment to yield the direct permanent investment. The costs of contingencies and the Contractor's fee are added to the direct permanent investment to yield the total depreciable cost. Adding the costs of land, royalties and plant startup yield the total permanent investment. Finally, the working capital is added to the total permanent investment to yield the total capital investment. The calculations that yield these values are found in Appendix B.2 - Economic Calculations in the sheet titled "Computing C_TCI."

Table 13: Components of the Total Capital Investment (C_{TCI})

$C_{Equipment}$	Bare module cost of all equipment used in the plant	$	3,250,000
C_{spare}	Bare module cost of spares for pumps	$	707,000
$C_{storage}$	Bare module cost of storage tanks for raw materials	$	1,240,000
C_{TBM}	**Total Bare Module Investment ($C_{Equipment} + C_{spare} + C_{storage}$)**	**$**	**5,210,000**
C_{site}	Cost of Site preparation	$	781,000
C_{serv}	Cost of service facility	$	1,040,000
C_{alloc}	Allocated costs for utilities and related facilities	$	1,2300,000
C_{DPI}	**Direct Permanent Investment Cost ($C_{TBM} + C_{site} + C_{serv} + C_{alloc}$)**	**$**	**8,330,000**
C_{cont}	Contingencies and Contractor's Fee	$	1,450,000
C_{TDC}	Total Depreciable Capital Cost ($C_{DPI} + C_{cont}$)	**$**	**9,830,000**
C_{land}	Cost of land	$	197,000
C_{royal}	Cost of royalties	$	197,000
$C_{startup}$	Cost of plant startup	$	983,000
C_{TPI}	**Total Permanent Investment Cost ($C_{TDC} + C_{land} + C_{royal} + C_{startup}$)**	**$**	**11,200,000**
C_{wc}	Working Capital	$	2,240,000
C_{TCI}	**Total Capital Investment Cost ($C_{TPI} + C_{wc}$)**	**$**	**13,400,000**

The Net Present Value table is shown below in Table 14. The total depreciable capital investment and working capital cost is taken from Table 13 above, and the total production cost from Table 12 is used as the annual cost of the process. The sales of the proposed plant are based on the sale price of Avastin®. The calculation for the annual sales of the plant is found in Appendix B.2 - Economic Calculations in the sheet titled "Raw Material and Product Costs." A MACRS 5-year depreciation model is used to compute the depreciation of the total depreciable capital investment over the 7 year period that the NPV is based on. At an annual compound growth rate of 20% (Dr. Kim Ogden, 2018), the net present value of the proposed plant is $124 million. The investor's rate of return (IRR) is 313%. The calculations that lead to the NPV table are detailed in Appendix B.2 - Economics Calculations in the sheet titled "NPV."

Table 14: Net Present Value Table (Values in Millions of Dollars)

Year	Total Depreciable Capital Investment	Working Capital	Depreciation	Costs Excluding Depreciation	Sales	Net Earnings	Cash Flow	Cum PV 12.5%
0	$ (9.83)	$ (2.24)	$ -	$ -	$ -	$ -	$ (12.10)	$ (12.10)
1	$ -	$ -	$ 1.97	$ 12.80	$ 71.60	$ 35.80	$ 37.80	$ 19.40
2	$ -	$ -	$ 3.15	$ 12.80	$ 71.60	$ 35.10	$ 38.20	$ 45.90
3	$ -	$ -	$ 1.89	$ 12.80	$ 71.60	$ 35.80	$ 37.70	$ 67.80
4	$ -	$ -	$ 1.13	$ 12.80	$ 71.60	$ 36.30	$ 37.50	$ 85.80
5	$ -	$ -	$ 1.13	$ 12.80	$ 71.60	$ 36.30	$ 37.50	$ 101.00
6	$ -	$ -	$ 0.57	$ 12.80	$ 71.60	$ 36.70	$ 37.20	$ 113.00
7	$ -	$ 2.24	$ -	$ 12.80	$ 71.60	$ 37.00	$ 39.30	$ 124.00

While the economic outlook is very promising, there are hazards with this investment. The pharmaceutical market is fast paced and competitive. There is constant technological advancement and there is a risk of the plant or the technology becoming obsolete. The process is very sensitive to contamination and sterilization is vital for the process. If a contaminant gets into the process, it could cause production to shut down until the contaminant is completely eliminated. Further, the plant is located in Tucson, Arizona which has a very warm and dry climate. This process requires very pure water and since Arizona is in a long-term drought, this water could become difficult to acquire in the coming years. The summers in Tucson can reach temperatures over 100 °F and so the refrigeration units in the plant are very important. If these units ever break down then reagents for dozens of batches could be compromised.

To cut costs and optimize the process, a few steps can be taken. One of the largest raw materials costs is the cost of Protein A resin. An alternative to Protein A has been developed (del Carmen Candia-Plata et al., 2009) which could greatly decrease the cost of the affinity chromatography resin. Additionally, the yield of the bioreactors can be improved. The final concentration of mAbs achieved in the bioreactor cascade in the proposed plant is 15.6 µg/mL (Appendix A.1). MAb concentrations up to 200 µg/mL have been achieved (Reinhart et al., 2015). By improving the yield of the bioreactor cascade, more product can be produced, which translates to more revenue due to sales. Improving the bioreactor yield has additional impacts to the proposed plant; more mAb products would affect the size and design parameters of downstream processs equipment, and there is a possibility of affecting the market for the mAb product.

Conclusions and Recommendations

The designed production facility meets the objective of producing 8760 g of bevacizumab a year. More than 140,000 people could receive treatments produced from the proposed plant (Genentech, Foss, Walpole). The design is economically viable with a net present value of $124 million in only seven years. The yields from the bioreactors are lower than industry averages, though, which provides room for improvement. Yield could be improved by implementing a fed-batch process through the bioreactors or by adding additional bioreactor cascades in parallel. The environmental impact of the proposed plant is within the regulated limits but it is important to minimize waste in pharmaceutical production because the process involves large volumes of water.

References

“Alfa Laval Culturefuge 400B High Capacity Continuously Solids-Discharging Centrifuge for Biotech Industries.”

"Avastin (bevacizumab) Uses, Dosage, Side Effects." *Drugs.com*. Web. Apr 25, 2018 <https://www.drugs.com/avastin.html>.

“BalanCD® CHO Growth A.” Irvine Scientific, www.irvinesci.com/uploads/technical-documentations/40960_BalanCD_CHO_Growth_A_Rev1_WEB.pdf.

“BioNumber Details Page.” Diameter of Cell - Chinese Hamster Ovary (CHO) - BNID 101901, bionumbers.hms.harvard.edu/bionumber.aspx?id=101901&ver=6.

"Cancer Statistics." *National Cancer Institute.* Web. Apr 25, 2018 <https://www.cancer.gov/about cancer/understanding/statistics>.

“Clean Air Act Permitting for Greenhouse Gas Emissions – Final Rules .” www.epa.gov/sites/production/files/2015-12/documents/20101223factsheet.pdf.

Colaco, C. A. L. S., et al. "Chemistry of protein stabilization by trehalose." 1994. 222-240.

del Carmen Candia-Plata, Maria, et al. "Isolation of human serum immunoglobulins with a new salt-promoted adsorbent." Journal of Chromatography A 1118.2 (2006): 211-217.

Ecker, Dawn M., Susan Dana Jones, and Howard L. Levine. "The Therapeutic Monoclonal Antibody Market." *mAbs* 7.1 (2015): 9-14. *MEDLINE.* Web.

Fleming, James R., et al. "Material of construction for pharmaceutical and biotechnology processing: moving into the 21st century." Pharmaceutical engineering (2001): 1-6.

Foss, Alexander JE, et al. "Comparing different dosing regimens of bevacizumab in the treatment of neovascular macular degeneration: study protocol for a randomised controlled trial." *Trials* 16.1 (2015): 85.

Genentech, Inc. "Highlights of Prescribing Information". South San Francisco, CA. 2016.

Han, Yi, et al. "Cultivation of recombinant Chinese hamster ovary cells grown as suspended aggregates in stirred vessels." Journal of bioscience and bioengineering 102.5 (2006): 430-435.

Hydrochloric acid; Sciencelab.com: Houston, TX October 9, 2005. http://www.sciencelab.com/msds.php?msdsId=9924285 (accessed April 23, 2018)

Important Safety Information.” *Avastin® (Bevacizumab) Efficacy, Safety, Dosing & Prescribing Information*, www.avastin-hcp.com/.

Katsu-Kimura, Yumiko, et al. "Substantial energy expenditure for locomotion in ciliates verified by means of simultaneous measurement of oxygen consumption rate and swimming speed." Journal of Experimental Biology 212.12 (2009): 1819-1824.

Keijzer, Timo, and Halotec Instruments BV. "Advances in the Design of Bioreactor Systems."

Liberti, Ross. “Bottle Filler Request.” Bottle Filler Request, 13 Apr. 2018.

López-Meza, Julián, et al. "Using simple models to describe the kinetics of growth, glucose consumption, and monoclonal antibody formation in naive and infliximab producer CHO cells." Cytotechnology 68.4 (2016): 1287-1300.

Mandal, Ananya. "What is an Antibody?" *News-Medical.net.* 2011-01-18T03:45:00.0000000-05:00 Web. Apr 25, 2018 <https://www.news-medical.net/health/What-is-an-Antibody.aspx>.

Milo, Ron, and Ron Philips. “» What Is the Density of Cells?” Cell Biology by the Numbers What Is the Density of Cells Comments, book.bionumbers.org/what-is-the-density-of-cells/.

"NCI Drug Dictionary." National Cancer Institute. Web. Apr 25, 2018 <https://www.cancer.gov/publications/dictionaries/cancer-drug>.

“Nuvia™ Q Resin.” *Nuvia™ Q Resin | Process Separations | Bio-Rad*, www.bio-rad.com/en-us/product/nuvia-q-resin?ID=LKA6ZM15.

Petrides, Demetri, et al. "Biopharmaceutical process optimization with simulation and scheduling tools." Bioengineering 1.4 (2014): 154-187.

“POROS™ MabCapture™ A Select Affinity Chromatography Resin.” Thermo Fisher Scientific, Thermo Fisher Scientific, 31 Oct.2017, www.thermofisher.com/order/catalog/product/A26460.

“Quattroflow Fluid Systems: Expert Solutions for Biopharma Applications.” Holland Applied Technologies, PSG: A Dover Company, 2016, www.hollandapt.com/Documents/Ctrl_Hyperlink/doccopy11288_uid192018402382.pdf.

Reinhart, David, et al. "Benchmarking of commercially available CHO cell culture media for antibody production." Applied microbiology and biotechnology 99.11 (2015): 4645-4657.

Reis, Robert Van, and Andrew Zydney. “Bioprocess Membrane Technology.” Journal of Membrane Science, 13 Feb. 2007, pp. 16–50.

Samson, Ian. “Considerations for Personal Protective Equipment When Handling Cytotoxic Drugs.” *PharmTech Home*, 27 Nov. 2017, www.pharmtech.com/considerations-personal-protective-equipment-when-handling-cytotoxic-drugs.

Seider, Warren D., Junior D. Seader, and Daniel R. Lewin. PRODUCT & PROCESS DESIGN PRINCIPLES: SYNTHESIS, ANALYSIS AND EVALUATION, (With CD). John Wiley & Sons, 2009.

Sellick, Christopher, et al. “Optimizing CHO Cell Culture Conditions.” GEN, Genetic Engineering and Biotechnology News, 1 Oct. 2009, www.genengnews.com/gen-articles/optimizing-cho-cell-culture-conditions/3057?page=1.

“SMART LYO™ Pharma Freeze Dryer.” GEA Engineering for a Better World, www.gea.com/en/products/smart-lyo-freeze-dryer-sl-10-80.jsp.

Syrop, Jackie. “Bevacizumab May Treat Refractory Brain Edema.” *Center for Biosimilars*, 22 Nov. 2017.

Ulrich, Gael D., and Palligarnai T. Vasudevan. Chemical engineering: process design and economics; a practical guide. Process Publ., 2004.

US EPA, OAR. "Greenhouse Gas Equivalencies Calculator." *US EPA*. -08-28T10:43:23-04:00 2015. Web. Apr 25, 2018 <https://www.epa.gov/energy/greenhouse-gas-equivalencies-calculator>.

Walpole, Sarah Catherine, et al. "The weight of nations: an estimation of adult human biomass." *BMC public health* 12.1 (2012): 439.

Appendices

A. All final Calculations

Appendix A contains the equipment design equations and is submitted to the dropbox

1. Appendix A – Equipment Design Calculations

B. Spread Sheets with explanations

Two Spreadsheets are included with this report.

1. Appendix B.1 – Equipment Design Calculations Spreadsheet
2. Appendix B.2 – Economic Analysis Spreadsheet
3. Appendix B.3 – Mass Balance Calculations

C. Useful/highlighted ASPEN output

ASPEN files and output for heat exchanger design and calculations are submitted to d2l electronically.

1. Appendix C- ASPEN File for HTX Design

D. Hazard and Operability Analysis Documentation

The HAZOPS for four pieces of process equipment are submitted to d2l electronically.

1. Appendix D – HAZOP Forms

E. Group Meeting Logs

Group meeting logs are submitted to d2l electronically.

1. Appendix D – Group Meeting Logs