CHE 4224

Chemical Engineering Design II

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AIChE 2019 Student Design Competition

Manufacturing Facility for a Biopharmaceutical: Monoclonal Antibody

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Abstract

The objective of this design project was to determine the necessary process requirements in order to operate an industrial-scale Monoclonal Antibody (MAb) production facility. In addition, the economic value of this process was evaluated to ensure that the process is economically attractive and worth the potential investment. MAbs are primarily used for the treatment of cancer as will be the main function of MABs produced by this facility. With clinical studies in several areas of cancer currently being done by our company, as well as glaucoma and macular degeneration studies, there are several prospective uses of MAbs produced by this facility with the potential of new uses becoming available in the future.

The facility will require the purchase of media from an outside vendor and will then go through the designed process to produce the MAb product. The MAb can then be formulated and packaged to be sent to patients. Two different production options were evaluated to ensure plant flexibility with changing production requirements. One option produced concentrations of 1 to 2 g/L while the other produced concentrations of up to 10 g/L.

Currently, the process has a net present value (NPV) of $34.7 billion using a hurdle rate of 15%. The rate of return was found to be 7450%. The payback period is .02 years with capital costs of $38.5 million. Annual operating costs were found to be $20.2 million with an annual revenue of $7.37 billion. It was assumed that the MAb product was sold at $7150 per gram based on competitor market pricing and then marked down to avoid over-estimation. In order to break-even, the MAb product needs to be sold for at least $56.60 per gram, which based on current market pricing is easily attainable. Economic evaluations were done on the conservative 1 to 2 g/L production basis and therefore the process can have even higher returns in the future if the production titer is changed up to 10 g/L. Due to the economic viability of this process, our design team recommends moving forward with the design and startup of this facility.

Introduction

Biopharmaceuticals are currently one of the largest classes of drugs in development. Monoclonal Antibodies (MAbs) are an emerging force in the pharmaceutical industry falling specifically into the biopharmaceutical sector. The first MAbs were developed in 1975 when Kohler and Milstein successfully reported fusing B-cells and myeloma cells together, forming the first hybridomas. The first MAb product available on the market was approved in the United States in 1986 for organ transplant rejection. Today, monoclonal antibodies are used to treat rheumatoid arthritis, Crohn's disease, transplant rejection, and a variety of cancers [1].

The objective of this project was to design a manufacturing facility capable of producing humanized MAbs against Vascular Endothelial Growth Factor for use in cancer treatment. The company currently has clinical studies in colon cancer, breast cancer, neovascular glaucoma, and macular degeneration. With many potential uses for MAbs emerging in the future, the plant was designed to be flexible with not only current, but future applications in mind. The specific MAbs produced by this plant will be humanized monoclonal antibodies. Humanized MAbs are a combination of human and animal antibodies, typically either mouse or rat. Including a human part in the MAb makes rejection of the antibody less likely to occur. The MAbs produced by this manufacturing facility will be 95% human with only the antigen binding region being mouse [1]. The design of this facility allows for production of titers of 1 to 2 g/L and up to 10 g/L of MAb product. Chinese Hamster Ovary Cells (CHO) will be used for the seed train portion of the process as they have the ability to function in humans due to similarity to human cells [2]. The first MAbs were made using hybridomas but the yields tended to be low. For this reason, the CHOs will act as the host for the production of the MAbs.

The production site will be located on the same site as the company's research and development location with a small-scale biopharmaceutical pilot plant. Because of this, utilities and infrastructure are already in place for the production site, requiring minimal additional costs for these areas. The production facility is required to meet both good manufacturing practice (GMP) and European regulations since there is potential that the MAb product will be sold internationally. FDA regulations will also be followed at this site.

Design Basis

The design of this process was based on the block flow diagram provided in the memo from AIChE as seen in Figure 1 below as well as the general process description provided. The project was estimated on a 15 year evaluation life with the design basis specifications discussed below.

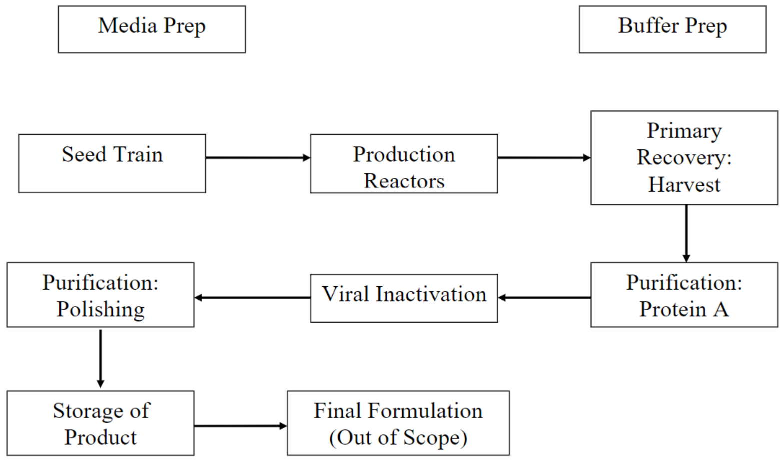

Figure 1: MAb Block Flow Diagram

The intent was to design a facility that could allow for the production of MAbs in titers of 1 to 2 g/L and titers of up to 10 g/L while meeting a production rate of at least 1,000 kg/yr. The reactors in the facility are designed to run both batch and fed batch processes with at least 2 g/L of glucose being fed to the production bioreactors to ensure acceptable growth of the MAb. It was provided that the cells follow the traditional growth curve starting in lag phase before moving to log phase, stationary phase, and the final death stage as can be seen in Figure 2 below. For this process, log phase was used in the bioreactors up until stationary phase began and then the cells were transferred to the next bioreactor to resume log phase. Since it was determined that Chinese Hamster Ovary Cells (CHO) would be used for the seed train, the specified doubling time of 36 hours was used in this portion of the process. The vials used in the seed train portion were provided to be 1 mL in size and contain 10^6 viable cells/mL. Specifications for the culture and production requirements are summarized in Tables 1 and 2.

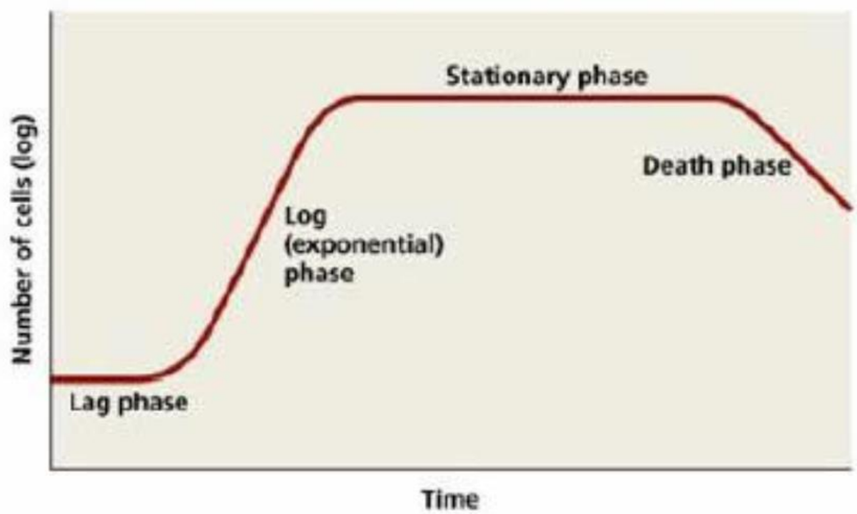

Figure 2: Growth Curve of CHO Cells

Table 1: Design Production Requirements

Production Information	
Titer from Reactors	1-2 g/L
Expected Future Titer	5-10 g/L
Production Rate	>1000 kg/yr

Table 2: Cell Culture Conditions

Culture Information	
Vial Size	1 mL
Contents	10^6 viable cells/mL
CHO Doubling Time	36 hours
Glucose Level in Reactors	>2 g/L

Cost data for the plant was provided and used to estimate the costs of running the plant. Specifications for the utilities costs are provided in Table 3 below. The provided electricity, sewer, and water for injection (WFI) costs were used to estimate the operating costs and thus mainly affected the economics. The details regarding these effects can be found in the economics section.

Table 3: Utilities

Utility Costs	
Electricity	$0.05/kWh
Sewer	$5/1000 gal
Water for Injection	$1000/1000 L

Process Flow Diagram and Material Balances

This process was simulated using SuperPro Designer, available from Intelligen, Inc. Unlike other common chemical engineering simulation software, SuperPro is especially good at modeling batch processes. The PFD and material balances for this process can be seen below.

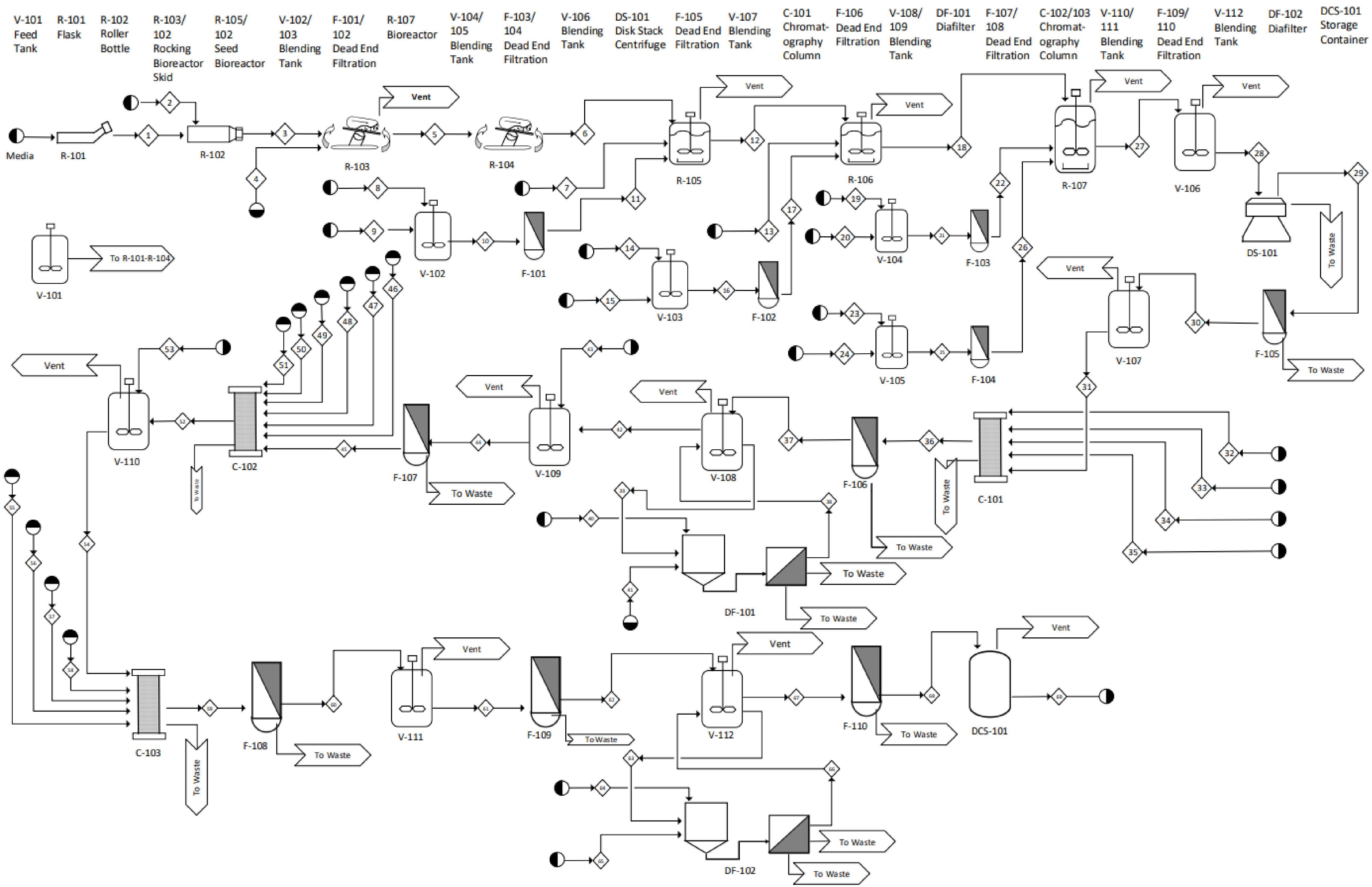

Figure 3: Process Flow Diagram

Table 4: Stream Summary Table

Stream Number:	Media	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Source	Input	R-101	Input	R-102	Input	R-103	R-104	Input	Input	Input	V-101	F-101	R-105	Input	Input	Input
Destination	R-101	R-102	R-102	R-103	R-103	R-104	R-105	R-105	V-102	V-102	F-101	R-105	R-106	R-106	V-103	V-103
Temperature (◦C)	25.00	37.00	25.00	37.00	25.00	37.00	37.00	25.00	25.00	25.00	25.00	25.00	37.00	25.00	25.00	25.00
Pressure (bar)	1.01	10.28	1.01	7.55	1.01	1.01	1.01	1.01	1.01	1.01	10.13	10.13	1.01	1.01	1.01	1.01
Density (g/L)	1000	990	1000	990	1000	990	990	1.18	995	995	995	995	991	1.18	995	995
Specific Enthalpy (kcal/kg)	25.11	37.10	25.11	37.10	25.11	37.10	37.10	6.05	25.11	25.11	25.11	25.11	37.10	6.05	25.11	25.11
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.24	1.00	1.00	1.00	1.00	1.00	0.24	1.00	1.00
Component Flowrates (kg/batch)																
Biomass		0.01		0.04		0.11	0.41						2.96			
Impurities						0.03	0.15						0.83			
MAB						0.01	0.04						0.21			
Media	0.07	0.02	0.23	0.07	0.87	0.28	0.76						0.76			
Nitrogen								398						1594		
Oxygen								121						484		
Serum Free Medium										21.25	21.25	21.25				85.22
Water		0.04		0.18		0.68	2.95						14.85			
WFI	3.53	3.53	11.17	14.70	42.73	57.43	229		683		683	683	913		2740	
TOTAL (kg/batch)	3.60	3.60	11.40	15.00	43.60	58.53	234	518	683	21.25	705	705	937	2078	2740	85.22
TOTAL (L/batch)	3.60	3.63	11.40	15.14	43.60	59.10	236	439427	687	21.36	708	708	946	1761985	2755	85.67

Table 4: Stream Summary Table (cont.)

Stream Number:	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Source	V-102	F-102	R-106	Input	Input	V-104	F-103	Input	Input	V-105	F-104	R-107	V-106	S-101	F-105	V-107
Destination	F-102	R-106	R-107	V-104	V-104	F-103	R-107	V-105	V-105	F-104	R-107	V-106	DS-101	F-105	V-107	C-101
Temperature (◦C)	25.00	25.00	37.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	37.00	37.00	43.46	43.46	43.45
Pressure (bar)	10.13	10.13	1.01	1.01	1.01	10.13	10.13	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Density (g/L)	995	995	991	995	995	995	995	995	995	995	995	991	991	988	988	988
Specific Enthalpy (kcal/kg)	25.11	25.11	37.10	25.11	25.11	25.11	25.11	25.11	25.11	25.11	25.11	37.10	37.10	43.55	43.55	43.54
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)																
Biomass			24.43									150	150	3.00		
Impurities			5.13									17.98	17.98	16.92	16.92	16.92
MAB			3.07									28.79	28.79	27.09	27.09	27.09
Media			0.76									0.76	0.76	0.71	0.71	0.71
Serum Free Medium	85.22	85.22	17.89		173	173	173	174	174		174	43.49	43.49	40.93	40.92	40.92
Water			72.11									297	297	280	280	280
WFI	2740	2740	3653	9947		9947	9947	174		174	174	13774	13774	12963	12960	12960
TOTAL (kg/batch)	2826	2826	3776	9947	173	10120	10120	348	174	174	348	14312	14312	13331	13325	13325
TOTAL (L/batch)	2841	2841	3812	10000	174	10174	10174	350	175	175	350	14443	14443	13493	13487	13487

Table 4: Stream Summary Table (cont.)

Stream Number:	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Source	INPUT	INPUT	INPUT	INPUT	C-101	F-106	DF-101	V-108	INPPUT	INPUT	V-108	INPUT	V-109	F-107	INPUT	INPUT
Destination	C-101	C-101	C-101	C-101	F-106	V-108	V-108	DF-101	DF-101	DF-101	V-109	V-109	F-107	C-102	C-102	C-102
Temperature (◦C)	25.00	25.00	25.00	25.00	25.13	25.13	25.39	25.13	25.00	25.00	25.39	25.00	25.39	25.39	25.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Density (g/L)	1030	1030	1010	1005	995	995	995	995	995	14.51	995	995	995	995	1004	1060
Specific Enthalpy (kcal/kg)	24.96	24.96	25.03	25.07	25.16	25.16	25.49	25.16	25.11	25.11	25.49	25.11	25.49	25.49	25.00	24.09
Heat Capacity (kcal/kg-°C)	0.99	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.96
Component Flowrates (kg/batch)																
Acetic Acid			54.71		21.88	21.88	0.58	21.88			0.58		0.58	0.58		
EDTA Disodium	18.60	22.32														
Impurities					1.02	1.02	0.05	1.02			0.05		0.05	0.05		
KCl															0.03	
KH2PO4															0.03	
MAB					24.38	24.38	23.65	24.38			23.65		23.65	23.65		
Na2HPO4															16.65	50.84
Polysorbate												0.07	0.07	0.07		
Sodium Chloride	9.30	11.16													136	1593
Sodium Citrate				9.80												
TRIS Base	9.30	11.16														
TRIS HCl	27.90	33.48														
WFI	9234	11081	9064	5434	3626	3626	710	3626	1469	497	710	0.07	710	710	14985	14338
TOTAL (kg/batch)	9299	11159	9119	5444	3673	3673	734	3673	1469	497	734	0.15	735	735	15138	15982
TOTAL (L/batch)	9028	10834	9028	5417	3692	3692	738	3692	1477	500	738	0.15	739	739	15077	15077

Table 4: Stream Summary Table (cont.)

Stream Number:	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63
Source	INPUT	INPUT	INPUT	INPUT	C-102	INPUT	V-110	INPUT	INPUT	INPUT	INPUT	C-103	F-108	V-111	F-109	V-112
Destination	C-102	C-103	C-104	C-105	V-110	V-110	C-103	C-103	C-104	C-105	C-106	F-108	V-111	F-109	V-112	DF-102
Temperature (◦C)	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01	1.01	1.01	10.13	1.01	1.01	1.01	1.01	1.01	1.01	10.13	10.13	10.13
Density (g/L)	995	1030	1030	1021	996	1769	1040	1021	1025	1060	1060	1015	1015	1015	1015	1015
Specific Enthalpy (kcal/kg)	25.11	24.41	24.57	24.87	25.07	8.50	23.49	24.87	24.67	24.09	23.18	24.68	24.68	24.68	24.68	24.68
Heat Capacity (kcal/kg-°C)	1.00	0.97	0.98	0.99	1.00	0.34	0.94	0.99	0.98	0.96	0.92	0.98	0.98	0.98	0.98	0.98
Component Flowrates (kg/batch)																
Ammonium Sulfate						645	645									
Impurities					0.01		0.01									
MAB					109		109					97.70	97.70	97.70	97.70	97.70
Media																
Na2HPO4		8.37							39.90	45.76	17.84	15.96	15.96	15.96	15.96	15.96
NaH2PO4					3.35		3.35									
Sodium Chloride		44.10	528		17.64		17.64		545	1434	1128	218	218	218	218	218
Sodium Hydroxid				181				272								
Water			8790	9055				13583								
WFI	14173	801			5990		5990		13324	12905	4608	5330	5330	5330	5330	5330
TOTAL (kg/batch)	14173	854	9318	9236	6119	645	6764	13855	13909	14384	5754	5661	5661	5661	5661	5661
TOTAL (L/batch)	14248	829	9046	9046	6142	364	6506	13570	13570	13570	5428	5578	5578	5578	5578	5578

Table 4: Stream Summary Table (cont.)

Stream Number:	64	65	66	67	68	69
Source	INPUT	INPUT	DF-102	V-112	F-110	DCS-101
Destination	DF-102	DF-102	V-112	F-110	DCS-101	TO SHIP
Temperature (◦C)	25.00	25.00	25.62	25.62	25.62	25.62
Pressure (bar)	1.01	1.01	1.01	2.54	2.54	10.14
Density (g/L)	1000	995	1001	1001	1001	1001
Specific Enthalpy (kcal/kg)	25.01	25.11	25.58	25.58	25.58	25.58
Heat Capacity (kcal/kg-°C)	1.00	1.00	0.99	0.99	0.99	0.99
Component Flowrates (kg/batch)						
Impurities						
KCl	0.01		0.01	0.01	0.01	0.01
KH2PO4	0.01		0.01	0.01	0.01	0.01
MAB			97.70	97.70	97.70	97.70
Media			4.87	4.87	4.87	
Na2HPO4	8.18					4.87
Sodium Chloride	59.50		44.53	44.53	44.53	44.53
Sodium Hydroxid						
Water						
WFI	7370	995	3579	3579	3579	3579
TOTAL (kg/batch)	7438	995	3726	3726	3726	3726
TOTAL (L/batch)	7438	1000	3723	3723	3723	3723

Process Description

Like most biopharmaceutical processes, this process operates in batch and fed-batch modes rather than continuous. This is due to the process' low throughput and the reactants' longer time spent in the bioreactors.

This process can be divided into upstream and downstream portions. The upstream portion of this design includes the media prep, seed train, and production reactor sections. The downstream portion of this design includes the primary recovery and harvest, Protein A purification, viral inactivation, polishing, and storage sections. The facility was required to produce a minimum of 1,000 kg of product per year. Apart from the MAb manufacturing process, production waste "kill tanks" also had to be designed for this facility. These tanks will feed into the existing county sewage facility.

Media Prep

Five media tanks are used for each batch of MAbs produced. The first media prep tank, V-101, produces 234 L of media solution to be used by the T-flasks, roller bottles, and disposable bag bioreactors. 4.52 kilograms of media powder is combined with 234 L of water for injection (WFI) in stirred-tank V-101 to form a solution that is 19.3 g/L per the manufacturer's user guide [3]. This forms a solution that is approximately 2% media and 98% WFI by weight. All media tanks produce this concentration of media, except for the media prep tank that feeds media to the production bioreactor. This media must be more concentrated to minimize the size of the production bioreactor.

The second media prep tank, V-102, produces media solution for disposable seed bioreactor, R-105. 13.7 kg of media powder is combined with 708 L of WFI. This large volume of media solution is passed through dead-end filter F-101 before entering R-105 to remove any impurities.

The third media prep tank, V-103, produces media solution for disposable seed bioreactor, R-106. 54.8 kg of media powder is added to 2,840 L of WFI. After mixing, the media solution passes through dead-end filter F-102 to remove any impurities before entering R-106.

Production bioreactor R-107 requires two media prep tanks to make media solutions of two different concentrations. The fourth media prep tank, V-104, produces media solution to be fully charged into R-107 at the beginning of fermentation. 196 kg of media powder is added to 10,100 L of WFI. This media passes through dead-end filter F-103 before entering R-107. The fifth media prep tank, V-105, is used to feed fresh media solution to R-107 as the fermentation reaction progresses to ensure cells continue growth and MAb production. As there is already a large volume in the reactor, more concentrated media solution is used, as it will be diluted once added to R-107. This minimizes the size of R-107 because it can hold the same amount of media powder at a smaller volume. The feed to R-107 produced by V-105 is a 500 g/L solution which is 50% media and 50% WFI by weight. 175 kg of media powder is combined with 350 L of WFI. This solution is passed through dead-end filter F-104 as it is fed to R-107 to remove any impurities.

This process uses three production bioreactors, R-107 A/B/C, to increase the capacity of the manufacturing plant. As such, three fed media prep tanks, V-105 A/B/C, and three dead-end filters, F-104 A/B/C, are required to provide enough media to each production bioreactor.

Seed Train

In a typical seed train, the CHO cells pass through many cultivation vessels that grow larger in volume with each pass. This seed train is composed of six different vessels that the cells will pass through. Additional media is added to each vessel in the train rather than all at once because high media concentrations inhibit cell growth [4]. An overview of this process can be seen below.

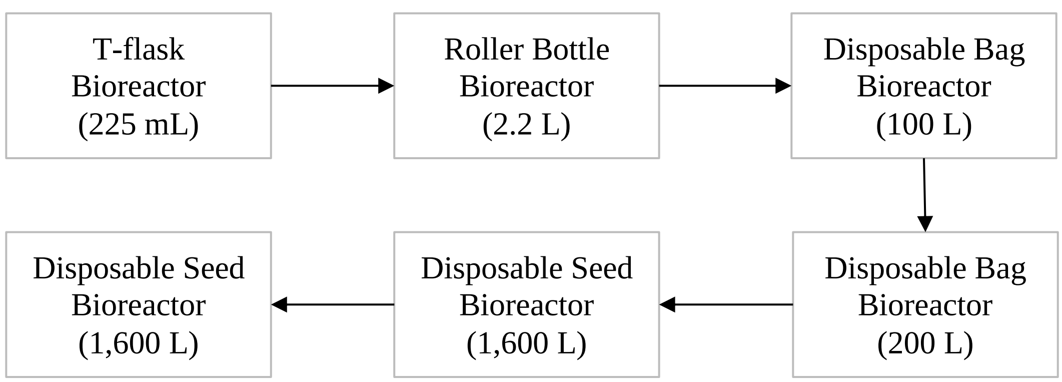

Figure 4: Overview of Seed Train Process

To increase throughput, several of these units are duplicated and run staggered of one another. There are two roller bottle units, two sets of two 100 L disposable bag bioreactors, two sets of three 200 L disposable bag bioreactors, two 1,600 L disposable seed bioreactors and two sets of three 1,600 L disposable seed bioreactors.

A 1 mL vial of CHO cells is thawed and combined with 3.6 L of media solution from V-101. The cells and media are placed in a skid of 18 small 225 mL T-flasks, R-101, for 96 hours (4 days). After fermenting, the cells and media enter a skid of 8 roller bottles that are 2.2 L in size, R-102 A/B, and 11.4 L of fresh media solution is added from V-101. The cells and media remain in R-102 A/B for 120 hours (5 days).

After fermenting, the cell and media solution is split into two equal amounts and enters a skid of two 100 L disposable bag bioreactors R-103 A/B. 43.6 L of fresh media solution from V-101 is added, in total, to R-103 A/B. The cells and media remain in the R-103 A/B for 144 hours (6 days). At this point in the process, 0.026 g of MAbs have been produced.

The cell and media solution from R-103 A/B is combined with 175 L of fresh media solution from V-101 and divided into three equal portions. The cells and media enter a skid of three 200 L disposable bag bioreactors, R-104 A/B and remain to ferment for 144 hours. At this point, 0.41 g of MAbs have been produced.

The cell and media solution from R-104 A/B enters 1,600 L disposable seed bioreactor R-105 A/B where it is combined with 708 L of fresh media from V-102. The cells and media remain in R-105 A/B for 144 hours. At this point, 6.55 g of MAbs have been produced.

The cells and media from R-105 A/B are split into three equal portions and enter a skid of three 1,600 L disposable seed bioreactors, R-106 A/B. 2,840 L of fresh media from V-103 enters R-106 A/B and the cells and media ferment for 144 hours. At this point, 105 g of MAbs have been produced.

Production Reactors

After the cells exit the seed train, they enter one of three production reactors, R-107 A/B/C. These reactors have been designed to run in both batch and fed-batch modes. The product of the second disposable seed bioreactor, R-106 A/B is charged into the production bioreactor along with 10,200 L of fresh media solution from V-104. The fermentation time in the reactor is 12 days. During this time, concentrated media solution from V-105 A/B/C is fed to R-107 A/B/C at a rate of 0.12 L/h to maintain a minimum glucose concentration of 2 g/L.

This cell line doubles every 36 hours and produces product at a rate of 25 pg/(cell*day) [1]. After the total time spent in the seed train and production reactor, each batch produces 26.8 kg of MAbs, producing a titer of 1.8 g/L. Because multiple upstream units have been included in this process and are operated on a staggered schedule, 56 batches of MAbs are able to be produced per year.

Primary Recovery and Harvest

From the upstream production process, waste materials have been produced in addition to the MAb product. Once the process stream leaves the reactors, the desired product must be separated from the impurities, byproducts, remaining media, etc. The separation of MAb product from the byproducts and media mixture takes place in different steps including phase separation, biomolecular separation and filtration to achieve the desired purity.

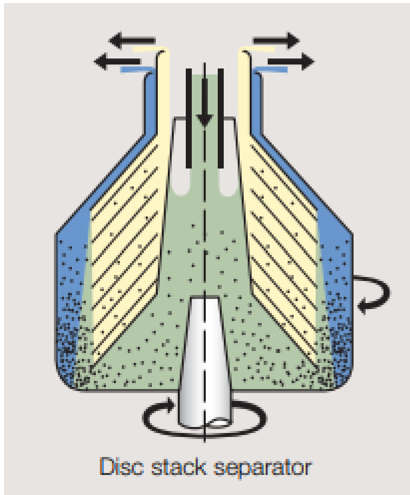

Figure 5: Disc Stack Centrifuge Diagram [5]

After the cells have finished reacting in R-107 A/B/C, the contents must be harvested. The contents of the bioreactors are removed and the broth enters the separation process. First, the process mixture is separated in a disk stack centrifuge, DS-107 [5]. The entering mixture is mostly water (98% wt) with very dilute MAb concentration (2 g/L). To allow for upstream equipment to be emptied for cleaning procedures, the mixture is first placed in surge tank V-106, and then fed into the centrifuge. The final reactor, R-107 A/B/C empties into the surge tank quickly at 14,400 kg/h and the centrifuge runs continuously at a slower flow rate of 1,710 kg/h of mixture into the centrifuge.

Since the denser components of the mixture are separated and sent out in a waste stream, the exiting flow rate is 110 kg/h less than that entering. The centrate stream is then sent through dead-end filter F-105 for additional separation. The filter does not drastically change flow rate or concentration of MAb (<1% increase). However, this is an important preparatory step for the affinity chromatography.

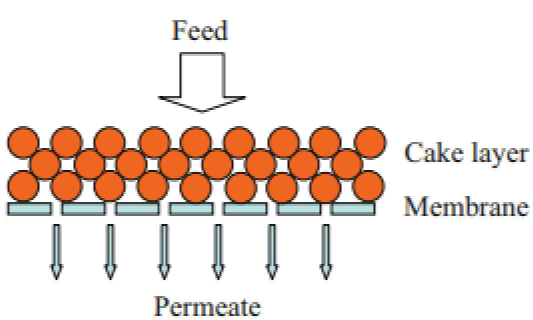

Figure 6: Filter Diagram [6]

Purification: Protein A Chromatography

After the two-step solids separation, the process mixture is purified using protein A chromatography in C-101. This is a crucial step in the downstream parts of the process. The MAb concentration in the mixture entering the column is 2 g/L and 6.6 g/L leaving the column. Removing the excess media and most of the impurities prepares the mixture for more extreme purification and concentration in other chromatography steps and diafiltration. The undesirable materials, buffers, and column regeneration mixture are transferred out in a waste stream to be properly disposed of. Upon being eluted from the column, the MAb stream is sent through a polishing filter, F-106, to ensure the purity of the product.

Virus Inactivation

The first step of virus inactivation is diafiltration. The diafilter, DF-101, is operated as a batch process which returns the retentate to the feed tank, V-108 [7]. The purification steps preceding this have increased purity by removing unwanted materials. In the diafiltration step, the MAb concentration of the mixture increases. The mixture enters at 6.6 g/L and exits at 32 g/L. Though this does increase the purity of the product by almost five times, it also decreases batch size drastically (3670 kg/batch to 735 kg/batch).

Following diafiltration, the concentrated mixture undergoes virus inactivation. To do this, the mixture is moved to a blending tank, V-109, where Polysorbate 80 is added. Polysorbate 80 is commonly used for virus inactivation in biopharmaceutical production [8]. Then the mixture is passed through a polishing filter, F-107, once more before two-step chromatography purification.

Purification/Polishing

After virus inactivation, the product must go through another purification step to remove any remaining impurities. Though Protein A chromatography is able to remove around 99.5% of impurities, this product must be made purer. This polishing step usually involves two purification units in series. Many options were considered including anion and cation exchange chromatography, ceramic hydroxyapatite, hydrophobic interaction chromatography, and membrane chromatography. Currently, membrane chromatography is only utilized at the lab and pilot scale because of limitations such as low binding capacity. Ultimately, ion exchange chromatography followed by hydrophobic interaction chromatography (HIC) was selected because it is a common combination used in the industry [9].

In the ion exchange column, C-102, the Polysorbate 80 and the remainder of the acetic acid are removed completely and the other impurities are decreased by more than 94%. The concentration of MAbs does decrease in this step. This is due to the large amounts of water required to collect the MAbs from the column that end up in the product stream and the 10% loss of MAb product that did not bind to the stationary phase in the column. The stream leaving the column is then fed into a mixing tank, V-110, where ammonium sulfate is added.

Hydrophobic interaction chromatography is the last chromatography step. The main change that occurs in this process is the removal of the last of the impurities. When the product stream leaves the HIC column, C-103, it contains water, disodium phosphate, sodium chloride, and 17.5 g of MAbs/L. This mixture is sent to the viral exclusion process before the final diafiltration.

The viral exclusion step is simple in terms of equipment. A storage tank, V-111, feeds the mixture through a dead end filter, F-109. After the virus filtration, the mixture is concentrated using diafiltration in DF-102, just as earlier in the process. The concentration process of MAb is not as drastic as before (17.5 g/L to 26.2 g/L) but that is due partly to the contents of the product bulk storage (PBS) buffer. 5% of MAbs are assumed to be lost due to denaturation.

Compared to the broth leaving the upstream bioreactors, the MAb concentration in the final product mixture has increased significantly to 26.2 g/L but has lost some of the product during purification. From the beginning of the downstream processing at the centrifuge to the final filtration step, the MAb recovery is 66.6%.

Storage of Product

After production and purification, the MAb product is packaged and frozen in DCS-101 for storage and transport. The 730 L batch of product contains a total of 19.2 kg of MAbs. 1 L plastic bags are used for packaging. The bags are filled to 90% capacity with the mixture. To hold the 730 L of product, 812 plastic bags are filled with 0.9 L each.

Table 5: Final Product Composition

Process Output			
Component	Flow Rate (kg/batch)	%wt	Concentration (g/L)
KCl	0.00123	0.00%	0.00168
KH_2PO_4	0.00123	0.00%	0.00168
MAb	19.2	2.62%	26.2
Na_2HPO_4	0.960	0.13%	1.32
NaCl	8.73	1.19%	12.0
WFI	702	96.1%	961

Production Waste

Liquid production waste is collected from all parts of the process and is sent to storage tank V-113 A/B. There it is steam and acid treated to inactivate all biologic material. After treatment, the waste is fed into the county sewer system. Vapor waste streams are vented from the process and not collected for additional treatment.

Energy Balance and Utility Requirements

This process takes advantage of the electricity and water utilities available. The water for injection is used as a solvent in the process and to make steam for heating the waste materials for treatment. The usage of these utilities and other energy transfer within the process are summarized in Table 6.

Table 6: Utilities Used

Unit	Electricity (kWh/batch)	Steam (kg/batch)	Chilled Water (kg/batch)	Cooling Water (kg/batch)
DF-101	17.7	197	N/A	N/A
DF-102	18.2	228	N/A	N/A
DS-101	215	200	N/A	N/A
R-101	0.14	N/A	N/A	15.3
R-102	N/A	0.27	N/A	N/A
R-103	1.28	N/A	N/A	115
R-104	4.07	N/A	N/A	282
R-105	40.8	59.1	5021	N/A
R-106	110	238	10826	N/A
R-107	684	903	83366	N/A
V-102	N/A	39.4	N/A	N/A
V-103	N/A	158	N/A	N/A
V-104	N/A	565	N/A	N/A
V-105	N/A	19.4	N/A	N/A
V-106	N/A	802	N/A	N/A
V-107	N/A	750	N/A	N/A
V-108	N/A	205	N/A	N/A
V-109	N/A	41.0	N/A	N/A
V-110	N/A	70.9	N/A	N/A
V-111	N/A	60.8	N/A	N/A
V-112	N/A	60.8	N/A	N/A
Total	1091	4597	99213	413

Equipment List and Unit Descriptions

Table 7 below shows the list of equipment for this process with the capacity for each unit. These capacity values were obtained from the built-in sizing tools offered by the SuperPro Designer software.

Table 7: Equipment List for MAb Manufacturing Process

Equipment Name	Description	Quantity	Capacity per Unit
C-101	Protein A Chromatography Column	1	600 L
C-102	IEX Chromatography Column	1	500 L
C-103	HIC Chromatography Column	1	600 L
DCS-101	Product Storage Skid	1	850 L
DF-101	Diafilter	1	50 m^2
DF-102	Diafilter	1	60 m^2
DS-101	Centrifuge	1	38.5 L/min
F-101	Filter	1	10 m^2
F-102	Filter	1	20 m^2
F-103	Filter	1	50 m^2
F-104 A/B/C	Filter	3	10 m^2
F-105	Filter	1	10 m^2
F-106	Filter	1	10 m^2
F-107	Filter	1	10 m^2
F-108	Filter	1	20 m^2
F-109	Filter	1	20 m^2
F-110	Filter	1	20 m^2
R-101	Bioreactor	1	225 mL
R-102 A/B	Bioreactor	2	2.2 L
R-103 A/B	Bioreactor	4	100 L
R-104 A/B	Bioreactor	6	200 L
R-105 A/B	Bioreactor	2	1,600 L
R-106 A/B	Bioreactor	6	1,600 L
R-107 A/B/C	Bioreactor	3	24,500 L
V-101	Media Prep Tank	1	300 L
V-102	Media Prep Tank	1	880 L
V-103	Media Prep Tank	1	4,000 L
V-104	Media Prep Tank	1	12,000 L
V-105 A/B/C	Media Prep Tank	3	7,100 L
V-106	Intermediate Storage Tank	1	25,000 L
V-107	Intermediate Storage Tank	1	17,000 L

V-108	Intermediate Storage Tank	1	24,000 L
V-109	Intermediate Storage Tank	1	4,300 L
V-110	Intermediate Storage Tank	1	8,000 L
V-111	Intermediate Storage Tank	1	7,500 L
V-112	Intermediate Storage Tank	1	7,500 L
V-113 A/B	Waste Disposal Tank	2	50,000 L

Media Prep

Media is used in the upstream manufacturing process to provide nutrients for the growing cells. Media has a complex number of ingredients including many components ranging from amino acids to trace elements [10]. It was common in the past for animal-derived serum to be included in media to meet the nutritional demand of the cells, however, because of the emergence of diseases due to serum, this facility will use serum-free chemically defined media. Media prepared from powder is considered to be the best method because it can be used directly after dissolving in WFI, resulting in a fresher solution. Powder also requires less storage space and is less expensive than pre-made solutions [11].

It was determined that purchasing a pre-made media powder from a vendor would be more efficient for the development of this new process than creating a proprietary formulation specific to the company. Later on, a vendor with expertise in media formulations can be hired to help develop a custom media formulation for this process. This is a common practice when establishing a new manufacturing site [12]. The media selected was CD OptiCHO AGT media manufactured by Gibco [3]. This media was selected for its consistent performance across cell lines compared to other off-the-shelf media formulations [12].

The seed train and production reactors require media addition at each step in the process. Rather than using one large tank to produce all the media required for the upstream units, five media preparation tanks were selected. This allows for easier staggering of unit scheduling in the upstream portion of the process to increase the total number of batches per year and allows for decreased unit sizes.

The reasoning for sizing the media prep vessels as shown in the equipment list can be viewed below. The specific amounts of media selected for each bioreactor will be discussed in the seed train portion following this section.

V-101

V-101 is the first media prep tank in the process that supplies media solution to R-101, R-102 A/B, R-103 A/B and R-104 A/B. A total of 234 L is supplied to these four bioreactors. For this reason, a 300 L stainless-steel stirred tank vessel was selected to produce this media.

V-102 and F-101

V-102 is the second media prep tank that supplies the media solution for R-105 A/B. 708 L of media solution is supplied to R-105 A/B from this vessel. An 880 L stainless-steel stirred tank vessel was selected for V-102. There are two reactors in R-105 A/B that operate staggered of one another. Because of the staggered schedule, V-102 can supply the media for both of these reactors without the need for a second media prep tank.

F-101 was selected to be a 10 m^2 dead-end filter. Dead-end filters were selected for their high collection rate, low cost, and lack of chemical cleaning and backwashing required [13]. High collection rate is important for this part of the process to remove any early impurities and lower downstream purification costs.

V-103 and F-102

V-103 is the third media prep tank which supplies 2,840 L of media solution, total, to the skid of three bioreactors, R-106 A/B. A 4,000 L stainless-steel stirred tank vessel was selected for V-103. There are six total reactor vessels in R-106 A/B. Three bioreactors operate simultaneously on one schedule while the other three operate simultaneously staggered of the first three. Because of the staggered scheduling, V-103 is able to produce media solution for both sets of reactors.

F-102 was selected to be a 20 m^2 dead-end filter to remove any impurities before media solution enters R-106 A/B.

V-104 and F-103

V-104 is the fourth media prep tank which supplies 10,200 L of media solution to production bioreactor, R-107 A/B/C. This media solution is added at the beginning of the fermentation process. V-104 is a 12,000 L stainless-steel stirred tank vessel. Because of staggered scheduling, V-104 is capable of providing media solution to the three production bioreactors of R-107 A/B/C.

F-103 is a 50 m^2 dead-end filter designed to remove any impurities in the media solution before entering R-107 A/B/C.

V-105 A/B/C and F-104 A/B/C

V-105 A/B/C is the fifth and final media prep tank which feeds concentrated media solution to R-107 A/B/C. V-105 A/B/C is a 7,100 L stainless steel tank that supplies 350 L of concentrated media solution to R-107 A/B/C at a rate of 0.12 L/h. The concentration of media was determined by the mass of media required to produce a titer of 10 g/L while still being able to keep the production reactor under 25,000 L in size. This concentration was determined to be 50% (weight) media powder to 50% (weight) WFI.

V-105 A/B/C may appear oversized for the production of a titer of 2 g/L, but 5,000 L of concentrated media solution are required to produce a titer of 10 g/L and, for this reason, V-105 A/B/C is required to be 7,100 L in size.

F-104 A/B/C is a 10 m^2 dead-end filter designed to remove any impurities in the media solution before entering R-107 A/B/C.

Seed Train and Production Reactor

The purpose of a seed train is to generate a desirable amount of cells to inoculate the production bioreactor. Chinese hamster ovary (CHO) cells will be used to produce the MAbs. CHO cells were selected because they are the most commonly used mammalian host cells in MAb manufacturing [14]. The volume of each vial of CHO cells is 1 mL which contains 10^6 viable cells/mL. The doubling time of these cells is 36 hours. This cell line produces product at a rate of 25 pg/(cell*day) [1].

A desired titer of 1-2 g/L had to be obtained with the capability of producing a titer up to 10 g/L in case future economic situations favor increased titers. Using the doubling time and MAb production rate given for this cell line, it was determined that for a volume of 15,000 L exiting the production reactor, 30 kg of product had to be produced to obtain a titer of 2 g/L. This much product could be obtained with 45.2 days of fermentation in the seed train and production reactor. A common fermentation time in the production reactor is 12 days, so this number was fixed [10]. Because of this, the product must remain in the seed train for 33.2 days. This was rounded down to 33 days. It was determined that the cells would spend 4 days in the T-flasks, 5 days in the roller bottles, 6 days each in the disposable bag bioreactors and disposable seed bioreactors, and finally 12 days in the production bioreactor. This was deemed to be a simple timeline for operators to remember and would allow for transfers between vessels to occur in even, 24-hour increments. The final production rate using this reaction time is 26.8 kg/batch producing a titer of 1.8 g/L.

The volume of media required for all vessels in the seed train was determined by recommended viable cell densities and a model provided by the SuperPro Designer [15, 4]. The reaction kinetics used to model the fermentation of cells in the seed train and production bioreactor were determined from published literature [16, 14].

R-101 and R-102 A/B

In this process, T-flasks (R-101) and roller bottles (R-102 A/B) were selected as the first vessels in this train because they are commonly used as the first steps in the process for large-scale manufacturing [17]. Because of their small size and low cost, both of these units are disposable, so no clean-in-place (CIP) and steam-in-place (SIP) procedures are required.

CHO cells are thawed and combined with 3.6 L of media solution and placed into the 225 mL T-flasks of R-101. This is a standard size for T-flasks and, for this reason, 18 T-flasks were required for the R-101 skid to hold 3.6 L total.

After exiting R-101, the 3.6 L of cells and media enter standard-size 2.2 L roller bottles with 11.4 L of additional media solution. 8 roller bottles are required to hold the 15 L of media solution in R-102 A/B. For scheduling purposes, two units are required for R-102 A/B to operate staggered of one another to increase the number of batches per year.

R-103 A/B and R-104 A/B

R-103 A/B and R-104 A/B were selected to be disposable rocking-bag bioreactors. It was preferable to select a disposable vessel for every unit in the seed train to minimize the amount of steam and WFI required to clean these vessels. Rocking-bag bioreactors have the advantage of providing sufficient gas exchange while exerting less shear on the cells, causing less damage to cells than stirred-tank reactors [18].

R-103 A/B is required to hold 60 L of cells and media. It is recommended that capacity of the bags does not exceed 50% to allow for adequate gas exchange. For this reason, 2 rocking-bag bioreactors that are 100 L in size were selected for the skid of R-103 A/B. Two skids of these rocking-bag bioreactors are required for R-103 A/B to operate in a staggered mode to produce the required amount of MAb product per year.

R-104 A/B is required to hold 240 L of media solution and cells. Using the maximum capacity of 50%, 200 L bags had to be used in the system with 3 rocking-bag bioreactors on the skid of R-

104 A/B. Two skids were purchased to allow for staggered scheduling and increased number of batches for R-104 A/B.

R-105 A/B and R-106 A/B

Rocking-bag bioreactors are only available at a max size of 300 L [12]. For this reason, R-105 A/B and R-106 A/B were selected to be single-use batch reactors to minimize the capital costs of this plant by still using disposable reactors. Standard stainless-steel vessels are required to be sterilized before and after each use using SIP and CIP procedures, respectively [19]. These processes are expensive due to the amount of steam that must be generated to clean these vessels. As can be seen below in Figure 7, single-use batch reactors have a much lower net present cost (NPC) than traditional stainless-steel reactors. Because the slopes of the traditional stainless-steel batch and single-use batch curves are similar, this indicates that operating costs for both vessels are approximately equal and the reduction in NPC is almost exclusively due to the decrease in initial capital investment [20]. At higher titers of 10 g/L, the NPC of single-use batch reactors is even lower.

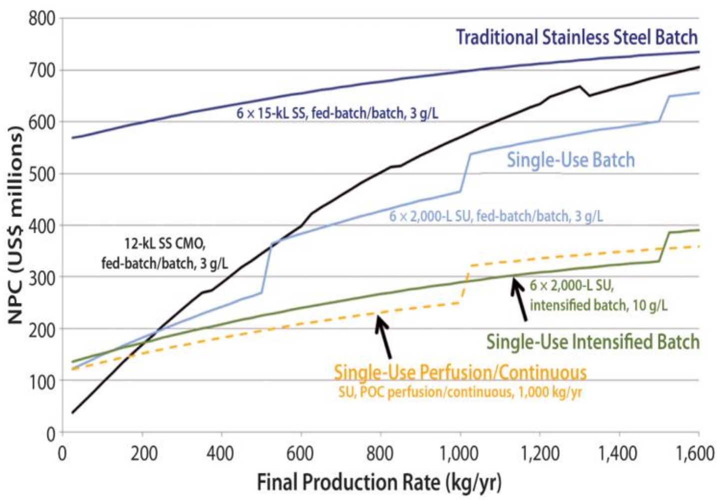

Figure 7: Net Present Cost of Different Bioreactor Types [14]

R-105 A/B is required to hold 950 L of media solution and CHO cells. For this reason, R-105 A/B was selected to be a 1,600 L single-use stirred tank bioreactor. Two bioreactors are required for R-105 A/B for staggered scheduling to enable enough MAb product to be produced.

R-106 A/B is required to hold 3,800 L of media solution and CHO cells. The largest single-use batch reactors currently available only hold a maximum capacity of 2,000 L. For this reason, three 1,600 L reactors were selected for the skid of R-106 A/B to allow for a disposable bioreactor. Two skids were required for R-106 A/B to produce enough MAbs on a staggered schedule.

R-107 A/B/C

The process was developed around R-107 A/B/C which determines the titer achieved and the amount of MAbs produced annually. Initially, a volume of 15,000 L was selected because this is a volume commonly used in large-scale MAb manufacturing facilities [21]. This required the production of 30 kg/batch to produce a titer of 2 g/L. This also required at least 34 batches per year to produce 1,000 kg of MAbs/year. This does not account for the loss of product in downstream purification, which was estimated before simulation to be approximately 66% [4]. This would require at least 50 batches to produce 1,000 kg of MAbs/year.

Since R-107 A/B/C has the longest fermentation time of 12 days, it is the bottleneck of this process. The number of staggered units was increased in the process simulation until a new unit became the bottleneck. Then, the number of staggered units was increased for this unit and this was repeated until 56 batches per year were obtained by the process simulation. This required two seed trains (besides R-101) and three production bioreactors with three fed-batch media vessels and filters (V-105 A/B/C and F-104 A/B/C).

To size the production bioreactor, the total volume required to produce a 10 g/L titer needed to be determined. Using concentrated media solution from V-105 A/B/C, 5,000 L of media is required to produce a titer of 10 g/L, bringing the total volume of R-107 A/B/C to 19,500 L. For this reason, a 24,500 L stainless-steel stirred-tank bioreactor was selected for R-107 A/B/C.

The largest single-use batch reactors currently available only hold a maximum capacity of 2,000 L. Because the production reactor is much larger than this, the production reactor could not be designed as single-use. The option of running several scaled-down disposable production bioreactors with shorter batch times was considered, but it was determined that running larger batches less frequently would result in a more consistent product and would decrease the number of operators required. Additionally, the larger stainless-steel reactors would require less space than several equivalent single-use reactors. Since operating costs are similar for stainless-steel and single-use reactors, the priority was to minimize space for the reactors. The current 24,500 L stainless-steel production reactors also have the potential to produce more batches with the addition of another seed train if demand increases in the future.

Primary Recovery and Harvest

V-106, DS-101, and F-105

The first purification step is centrifugation to remove the larger, more distinct materials. A disk stack centrifuge was selected for DS-101 due to its effectiveness in separating mixtures with lower solid concentrations and small particle size [5]. This type of centrifugation is typical for use in MAb purification [22]. Disk stack centrifuges are operated in a continuous flow which benefits process efficiency. DS-101 is designed for a throughput of 1,710 L/h. Because the centrifuge has limited throughput, the bioreactors from the previous step feed into a storage vessel (V-106) that holds the broth until the centrifugation step. To hold all of the process mixture from the upstream production, the storage vessel was designed with a volume of 25,000 L.

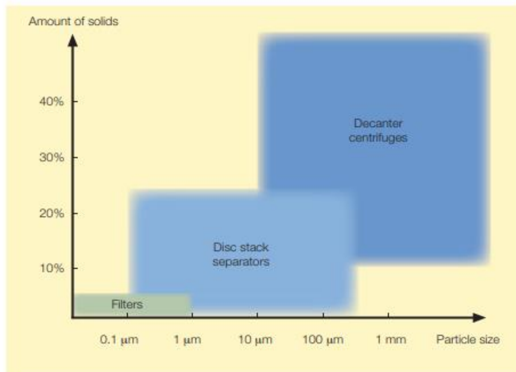

Figure 8: Solid Separation Selection [5]

The second step, polishing filtration, removes the remaining biomass by utilizing dead-end filtration. The centrifugation and this filtration step are important to run in series and in this order. Centrifuges are useful for separating materials with distinct densities but are not an economical way of reaching high purities, thus, the dead-end filters provide increased removal of impurities. However, the filters are not as useful for mixtures with high concentrations of solids. Dead-end filters collect filtrate on the membrane as the broth is forced through it. As the filtrate accumulates, it is more difficult to move the mixture through the filter. Placing them in series, the centrifuge performs the initial separation to prevent excessive filtrate accumulation on the filter and the dead-end filter follows that to remove the finer solids. Filter F-105 is a 10 m^2 dead-end filter with pore size 0.2 μm.

After this filtration, all of the biomass will be removed from the broth. This is very important for the following chromatography step because the biomass could bind to the resin instead of the MAbs resulting in decreased purity leaving the protein A chromatography column. These dead-end filters are operated either with constant permeate flux or with constant pressure drop through the membrane. The filter is fed directly from the continuous centrifuge so the permeate flux must be maintained. Thus, the pressure drop increases as a batch is run and then the filtrate is transferred out after each batch. Between centrifugation and filtration, almost 4% of the MAb product is lost.

Purification: Protein A Chromatography

V-107, C-101, and F-106

Similar to V-105 before DS-101, the mixture is held in a 20,000 L storage vessel (V-106) and fed into the first chromatography step from there. The chromatography column utilizes protein A stationary phase and has a design volume of 500 L. Design considerations for a chromatography column include the amount of material that will be purified and how quickly the desired substance will bind to the resin. Because of this, the amount of resin needed and the time required for MAbs to bind to the resin can be determined. C-101 is designed to run in 4 cycles per batch, and is followed by F-106, a 10 m^2 dead-end filter.

Affinity chromatography has become quite standard in purification of MAbs [9, 23] and is often the first purification step after fermentation [24]. Protein A chromatography is used to selectively remove impurities to produce a high-purity product. In this chromatography step, most of the MAbs will bind to the stationary phase, or resin, while the majority of the impurities will flow through without binding. Before introducing the broth, the column is prepared with a buffer solution containing sodium EDTA, sodium chloride, tris base and tris HCl to maintain a pH that supports MAb binding to the stationary phase. The same buffer solution is used to wash unbound materials out of the column before elution. After the process mixture is run through the column and the unbound substances have been washed out, the bound proteins are treated with an aqueous acetic acid solution to decrease the pH. This causes the adsorbed proteins to be eluted from the stationary phase and carried out of the column [25] and may simultaneously inactivate some viruses. [24] This technique increases the concentration of MAbs to more than three times that of the inlet stream but some MAbs are lost during the separation. Ultimately, the column achieves 90% recovery of MAbs. Afterward, the resin is regenerated using a sodium citrate solution so that it may be reused. Based on industrial use of protein A resin, 50-100 cycles can be expected from a single load of resin [26]. From this, we assumed an 1 year lifetime for the resin which contains 56 batches.

Virus Inactivation

V-108, DF-101, V-109, and F-107

The 5,000 L storage vessel V-108 holds the mixture before and after diafiltration. Diafiltration is a process that utilizes a filter and a series of concentration-dilution steps to purify a component [27]. The initial mixture is filtered using an ultrafiltration membrane which allows only very small molecules to permeate. Thus, larger molecules are retained and concentrated. Before the solution is entirely filtered, more solvent is added. This dilutes the mixture to encourage further filtration. This cycle can be repeated to continue to remove the smaller molecules into the filtrate and concentrate the larger molecules in the retentate as shown in Figure 9 below.

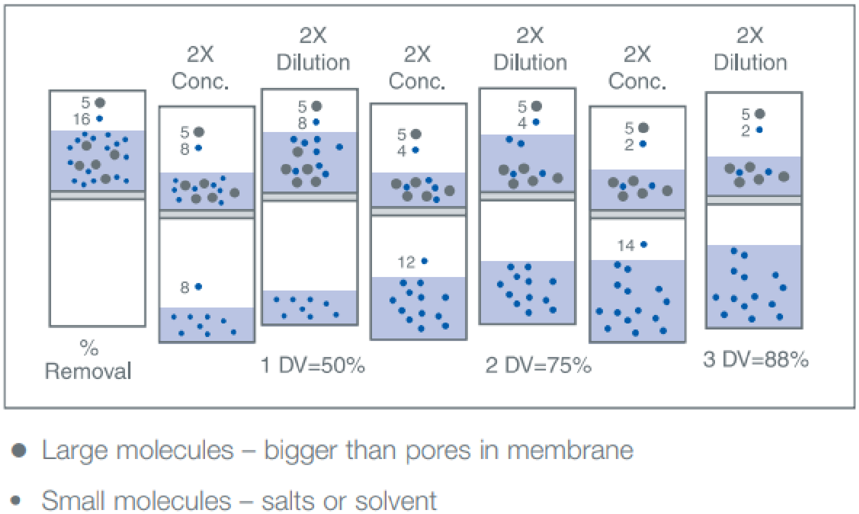

Figure 9: Diafiltration Diagram [27]

The retentate is then removed as a purified and concentrated solution. In this first diafiltration step, the large molecules are the MAb and the smaller molecules are acetic acid and other impurities. Within this process it is assumed that no MAbs are lost in the filtrate but 3% of the MAb denatures to form impurities resulting in a 97% recovery from this process. The product mixture is concentrated by more than five times via diafiltration. DF-101 contains a 20 m^2 filter with 0.45 μm pores.

After diafiltration, the concentrated mixture is treated with polysorbate 80 in the 4,300 L mixing tank V-109 to inactivate viruses before continuing to further purification steps. Polysorbate 80 is a surfactant that is very common in producing protein mixtures within the biopharmaceutical industry [28]. The product is then passed through filter F-107 with an area of 10 m^2.

Purification/Polishing

C-102 and V-110

Next, the mixture enters C-102, an ion exchange chromatography (IEX) column. IEX was selected for use in the purification steps due to its historical success in MAb production as well as lower stationary phase costs. In this chromatography step, the polysorbate 80 and most of the other impurities are removed. IEX is heavily dependent on the charge of the target molecule [29]. The resin and elution buffer are selected based on this charge. The target molecule in this case is the MAb which has a partial charge because of the pH of the system. Entering the column, the MAb has a partial positive charge to bind to the stationary phase. It is then eluted by a buffer containing sodium cations that replace the MAb adsorbed to the stationary phase. In this process the ion exchange elution buffer is a solution of monosodium phosphate, sodium chloride, and water. After the MAb has been eluted and removed, the column is regenerated and rinsed to prepare for cleaning procedures before the next batch. C-102 has a design volume of 500 L and is filled with sepharose stationary phase. To purify an entire batch of MAb mixture, the column undergoes 3 cycles. It is assumed that no MAbs are denatured but 10% are lost in the waste stream.

After the mixture exits the IEX column, it is sent to a mixing tank (V-110) where ammonium sulfate is added in preparation of the hydrophobic interaction chromatography. Ammonium sulfate promotes interaction of the MAbs in the column, thus increasing binding and allowing for improved effectiveness [30]. The tank is sized to 8,000 L in order to hold the solution from the ion exchange and the additional ammonium sulfate.

C-103 and F-109

Following the IEX chromatography, the purification process design includes hydrophobic interaction chromatography (HIC) due to its relatively mild conditions and its common use in conjunction with protein A and ion exchange chromatographies in the purification of Mab [30, 31]. C-103 is a 600 L HIC column that operates at 1 cycle per batch. This step uses a butyl-sepharose resin. Because the product mixture has been treated with ammonium sulfate, the solvation of MAbs has been decreased and, thus, magnifies the hydrophobic characteristics of the proteins. These hydrophobic regions then bind to the stationary phase [32]. The product is eluted by changing the salt concentration with an aqueous solution of disodium phosphate and sodium chloride. The column is then regenerated with sodium hydroxide. It is assumed that no MAbs are denatured in this process. However, 10% exit in the waste stream. After this last chromatography step, the mixture passes through dead-end filter F-109 with a 10 m^2 filter.

V-111 and F-110

Before the final concentration step, the mixture is filtered specifically for virus inactivation. F-110 is a dead-end filter with a 10 m^2 membrane and is fed from storage tank V-111. The membrane is a 0.02 μm virus exclusion filter. Other virus filtration techniques were considered, specifically tangential flow filtration (TFF), however, dead-end filters are easier to use, present lower capital costs, and are the preferred method in industry [33]. With the mixture free from viruses, it is sent to V-112 for holding before the next step.

V-112, DF-102, and F-111

The final process performed on the product mixture before packaging for storage and transport is another diafiltration step. This diafiltration is not as drastic of a concentration step but the final solution is achieved by removing most of the HIC eluent buffer and adding product bulk storage buffer (phosphate buffered saline, PBS) at this step. Through DF-102, the MAb concentration increases from 17.5 g/L to 26.2 g/L and the HIC eluent buffer (disodium phosphate and sodium chloride) decreases by approximately 80%. The PBS contains potassium chloride and monopotassium phosphate.

Storage of Product

DCS-101

After the MAb mixture has finished production and processing, it is dispensed into containers for storage and shipping at DCS-101. The product is loaded into 1 L disposable plastic bags and then chilled. The bags are only filled to 90% capacity to avoid overfilling. Disposable systems are very common in many parts of this process and offer advantages in packaging as well. Storing the product in disposable bags avoids the need for larger storage tanks, which require CIP and SIP, and allows for the product to be frozen for long-term storage immediately after production. Each batch loads 811 bags containing 26.2 g of MAb product.

Production Waste

V-113

All liquid waste streams generated from concentration and purification are collected after being removed from the process. For proper disposal, this waste must be thermally treated to inactivate any biological materials. This is also true for any waste produced in equipment cleaning procedures. To ensure inactivation of all cells, the waste is heated to 80 ℃ for 60 seconds based on the success of this technique in lab-scale experiments [33]. This has shown effectiveness in killing CHO cells, human embryonic kidney cells, and hybridoma cells to the point that no significant growth is observed in 5 days under typical growth conditions. In addition to heat treating biological waste materials, acid treating is very common in industry for sterilization. Traditionally, chlorine has been used to disinfect aqueous waste. More recently, peracetic acid has proven effective as a disinfecting agent as well as having a negligible environmental impact [34]. Approximately 73,700 kg of liquid waste is collected from the production and purification of MAbs. This does not include the materials used for cleaning the process equipment.

Throughout the upstream and downstream parts of the process, gas byproducts are often allowed to vent out of the process vessels. (i.e. bioreactor R-103, storage tank V-106, etc.) The gases released in this process are carbon dioxide, nitrogen, and oxygen. Over 41,000 kg (36 x 10^6 L) of the gases are vented per batch. The upstream processes account for most of the gas flow (>99%). However, only a very small part of that is actually produced from the process. Most of it is from air used to promote reaction. The total gas emissions contain 76.9% N_2, 22.3% O_2, and 0.8% CO_2, by weight. Thus, the gas waste is of little consequence in terms of safety and environmental impact. Because only CO_2 is produced in small amounts, no treatment is performed on the gas waste.

Equipment Specification Sheets

SuperPro Designer automatically sizes equipment based on the maximum capacity the unit encounters during operation. Because values from the simulation have been used in published papers for early-stage design, it was deemed that these values were reliable and could be used for the purpose of this design [4].

Table 8: Equipment Specification Sheet for C-101

C-101	
Description	Chromatography Column
Pressure, bar	1.0
Temperature, °C	25
Height, m	0.25
Diameter, m	1.75
Volume, L	600
MOC	Stainless Steel
Number of Cycles	4

Table 9: Equipment Specification Sheet for C-102

C-102	
Description	Chromatography Column
Pressure, bar	1.0
Temperature, °C	25
Height, m	0.25
Diameter, m	1.6
Volume, L	500
MOC	Stainless Steel
Number of Cycles	3

Table 10: Equipment Specification Sheet for C-103

C-103	
Description	Chromatography Column
Pressure, bar	1.0
Temperature, °C	25
Height, m	0.25
Diameter, m	1.75
Volume, L	600
MOC	Stainless Steel
Number of Cycles	1

Table 11: Equipment Specification Sheet for DF-101

DF-101	
Description	Filter
Type	Diafilter
Pressure, bar	1.0
Temperature, °C	25
Flow Rate, L/min	42
Area, m^2	50.0

Table 12: Equipment Specification Sheet for DF-102

DF-102	
Description	Filter
Type	Diafilter
Pressure, bar	1.0
Temperature, °C	25
Flow Rate, L/min	50
Area, m^2	60.0

Table 13: Equipment Specification Sheet for DS-101

DS-101	
Description	Centrifuge
Type	Disk-stack
Pressure, bar	1.0
Temperature, °C	42
Flow Rate, L/min	38.5
Sigma Factor, m^2	196,000

Table 14: Equipment Specification Sheet for F-101

F-101	
Description	Filter
Type	Dead-end
Pressure, bar	1.0
Temperature, °C	25
Flow Rate, L/min	42
Area, m^2	10.0

Table 15: Equipment Specification Sheet for F-102

F-102	
Description	Filter
Type	Dead-end
Pressure, bar	1.0
Temperature, °C	25
Flow Rate, L/min	84
Area, m^2	20.0

Table 16: Equipment Specification Sheet for F-103

F-103	
Description	Filter
Type	Dead-end
Pressure, bar	1.0
Temperature, °C	25
Flow Rate, L/min	210
Area, m^2	50.0

Table 17: Equipment Specification Sheet for F-104 A/B/C

F-104 A/B/C	
Description	Filter
Type	Dead-end
Pressure, bar	1.0
Temperature, °C	25
Flow Rate, L/min	42
Area, m^2	10.0

Table 18: Equipment Specification Sheet for F-105

F-105	
Description	Filter
Type	Dead-end
Pressure, bar	1.0
Temperature, °C	42
Flow Rate, L/min	42
Area, m^2	10.0

Table 19: Equipment Specification Sheet for F-106

F-106	
Description	Filter
Type	Dead-end
Pressure, bar	1.0
Temperature, °C	25
Flow Rate, L/min	42
Area, m^2	10.0

Table 20: Equipment Specification Sheet for F-107

F-107	
Description	Filter
Type	Dead-end
Pressure, bar	1.0
Temperature, °C	25
Flow Rate, L/min	42
Area, m^2	10.0

Table 21: Equipment Specification Sheet for F-108

F-108	
Description	Filter
Type	Dead-end
Pressure, bar	1.0
Temperature, °C	25
Flow Rate, L/min	84
Area, m^2	20.0

Table 22: Equipment Specification Sheet for F-109

F-109	
Description	Filter
Type	Dead-end
Pressure, bar	10.0
Temperature, °C	25
Flow Rate, L/min	84
Area, m^2	20.0

Table 23: Equipment Specification Sheet for F-110

F-110	
Description	Filter
Type	Dead-end
Pressure, bar	2.5
Temperature, °C	25
Flow Rate, L/min	84
Area, m^2	20.0

Table 24: Equipment Specification Sheet for R-101

R-101	
Description	Seed Bioreactor
Type	T-flask
Pressure, bar	10.3
Temperature, °C	37
Volume per Flask, mL	225
Number of Flasks per Unit	18
Number of Units	1

Table 25: Equipment Specification Sheet for R-102 A/B

R-102 A/B	
Description	Seed Bioreactor
Type	Roller Bottle
Pressure, bar	7.6
Temperature, °C	37
Volume per Bottle, L	2.2
Number of Bottles per Unit	8
Number of Units	2

Table 26: Equipment Specification Sheet for R-103 A/B

R-103 A/B	
Description	Seed Bioreactor
Type	Rocking Bag
Pressure, bar	1.0
Temperature, °C	37
Volume per Bag, L	100
Number of Bags per Unit	2
Number of Units	2

Table 27: Equipment Specification Sheet for R-104 A/B

R-104 A/B	
Description	Seed Bioreactor
Type	Rocking Bag
Pressure, bar	1.0
Temperature, °C	37
Volume per Bag, L	200
Number of Bags per Unit	3
Number of Units	2

Table 28: Equipment Specification Sheet for R-105 A/B

R-105 A/B	
Description	Seed Bioreactor
Type	Single-use Batch
Pressure, bar	1.0
Temperature, °C	37
Height, m	2.0
Diameter, m	1.0
Volume, L	1,600
Number of Reactors per Unit	1
Number of Units	2
MOC (shell)	Stainless Steel

Table 29: Equipment Specification Sheet for R-106 A/B

R-106 A/B	
Description	Seed Bioreactor
Type	Single-use Batch
Pressure, bar	1.0
Temperature, °C	37
Height, m	2.0
Diameter, m	1.0
Volume, L	1,600
Number of Reactors per Unit	3
Number of Units	2
MOC (shell)	Stainless Steel

Table 30: Equipment Specification Sheet for R-107 A/B/C

R-107 A/B/C	
Description	Production Bioreactor
Type	Traditional Batch
Pressure, bar	1.0
Temperature, °C	37
Height, m	5.0
Diameter, m	2.5
Volume, L	24,500
Number of Reactors per Unit	1
Number of Units	3
MOC (shell)	Stainless Steel

Table 31: Equipment Specification Sheet for V-101

V-101	
Description	Media Prep Tank
Pressure, bar	1.0
Temperature, °C	25
Height, m	1.5
Diameter, m	0.5
Volume, L	300
MOC	Stainless Steel

Table 32: Equipment Specification Sheet for V-102

V-102	
Description	Media Prep Tank
Pressure, bar	1.0
Temperature, °C	25
Height, m	2.0
Diameter, m	0.75
Volume, L	880
MOC	Stainless Steel

Table 33: Equipment Specification Sheet for V-103

V-103	
Description	Media Prep Tank
Pressure, bar	1.0
Temperature, °C	25
Height, m	3.25
Diameter, m	1.25
Volume, L	4,000
MOC	Stainless Steel

Table 34: Equipment Specification Sheet for V-104

V-104	
Description	Media Prep Tank
Pressure, bar	1.0
Temperature, °C	25
Height, m	5.0
Diameter, m	1.75
Volume, L	12,000
MOC	Stainless Steel

Table 35: Equipment Specification Sheet for V-105 A/B/C

V-105 A/B/C	
Description	Media Prep Tank
Pressure, bar	1.0
Temperature, °C	25
Height, m	4.0
Diameter, m	1.5
Volume, L	7,100
MOC	Stainless Steel

Table 36: Equipment Specification Sheet for V-106

V-106	
Description	Holding Tank
Pressure, bar	1.0
Temperature, °C	37
Height, m	6.25
Diameter, m	2.25
Volume, L	25,000
MOC	Stainless Steel

Table 37: Equipment Specification Sheet for V-107

V-107	
Description	Holding Tank
Pressure, bar	1.0
Temperature, °C	42
Height, m	5.5
Diameter, m	2.0
Volume, L	17,000
MOC	Stainless Steel

Table 38: Equipment Specification Sheet for V-108

V-108	
Description	Holding Tank
Pressure, bar	1.0
Temperature, °C	25
Height, m	6.0
Diameter, m	2.25
Volume, L	24,000
MOC	Stainless Steel

Table 39: Equipment Specification Sheet for V-109

V-109	
Description	Holding Tank
Pressure, bar	1.0
Temperature, °C	25
Height, m	3.50
Diameter, m	1.25
Volume, L	4,300
MOC	Stainless Steel

Table 40: Equipment Specification Sheet for V-110

V-110	
Description	Holding Tank
Pressure, bar	1.0
Temperature, °C	25
Height, m	4.5
Diameter, m	1.5
Volume, L	8,000
MOC	Stainless Steel

Table 41: Equipment Specification Sheet for V-111

V-111	
Description	Holding Tank
Pressure, bar	1.0
Temperature, °C	25
Height, m	4.25
Diameter, m	1.5
Volume, L	7,500
MOC	Stainless Steel

Table 42: Equipment Specification Sheet for V-112

V-112	
Description	Holding Tank
Pressure, bar	1.0
Temperature, °C	25
Height, m	4.25
Diameter, m	1.5
Volume, L	7,500
MOC	Stainless Steel

Table 43: Equipment Specification Sheet for V-113 A/B

V-113 A/B	
Description	Holding Tank
Pressure, bar	1.0
Temperature, °C	25
Height, m	83.0
Diameter, m	27.75
Volume, L	50,000
MOC	Stainless Steel

Fixed Capital Investment Summary

SuperPro Designer has built-in cost correlations for all pieces of equipment available in the software. These correlations take into account both size and material of construction of each piece of equipment [manual]. SuperPro Designer provides default installation factors for all equipment to calculate the total installed cost. Because equipment cost values from SuperPro Designer have been used in published papers for early-stage design, these values were determined to be valid for use in this design [4]. The purchased cost and cost after installation for each piece of equipment can be seen below in Table 44 below.

Table 44: Fixed Capital Investment Summary for MAb Manufacturing Process

Equipment Name	Description	Quantity	Capacity	Total Purchased Cost	Total Installed Cost
C-101	Protein A Chromatography Column	1	600 L	$661,000	$694,050
C-102	IEX Chromatography Column	1	500 L	$702,000	$737,100

C-103	HIC Chromatography Column	1	600 L	$734,000	$770,700
DCS-101	Product Storage Skid	1	850 L	$500,000	$1,250,000
DF-101	Diafilter	1	50 m^2	$108,000	$162,000
DF-102	Diafilter	1	60 m^2	$132,000	$198,000
DS-101	Centrifuge	1	38.5 L/min	$504,000	$756,000
F-101	Filter	1	10 m^2	$39,000	$58,500
F-102	Filter	1	20 m^2	$69,000	$103,500
F-103	Filter	1	50 m^2	$160,000	$240,000
F-104 A/B/C	Filter	3	10 m^2	$117,000	$175,500
F-105	Filter	1	10 m^2	$39,000	$58,500
F-106	Filter	1	10 m^2	$39,000	$58,500
F-107	Filter	1	10 m^2	$39,000	$58,500
F-108	Filter	1	20 m^2	$80,000	$120,000
F-109	Filter	1	20 m^2	$80,000	$120,000
F-110	Filter	1	20 m^2	$80,000	$120,000
R-101	Bioreactor	1	225 mL	$100,000	$250,000
R-102 A/B	Bioreactor	2	2.2 L	$250,000	$625,000
R-103 A/B	Bioreactor	4	100 L	$888,000	$2,220,000
R-104 A/B	Bioreactor	6	200 L	$1,332,000	$3,330,000
R-105 A/B	Bioreactor	2	1,600 L	$2,310,000	$3,003,000
R-106 A/B	Bioreactor	6	1,600 L	$7,032,000	$9,141,600
R-107 A/B/C	Bioreactor	3	24,500 L	$6,159,000	$8,006,700
V-101	Media Prep Tank	1	300 L	$162,000	$210,600
V-102	Media Prep Tank	1	880 L	$179,000	$232,700
V-103	Media Prep Tank	1	4,000 L	$217,000	$282,100
V-104	Media Prep Tank	1	12,000 L	$247,000	$321,100
V-105 A/B/C	Media Prep Tank	3	7,100 L	$705,000	$916,500
V-106	Intermediate Storage Tank	1	25,000 L	$285,000	$370,500
V-107	Intermediate Storage Tank	1	17,000 L	$261,000	$339,300
V-108	Intermediate Storage Tank	1	24,000 L	$283,000	$367,900
V-109	Intermediate Storage Tank	1	4,300 L	$226,000	$293,800
V-110	Intermediate Storage Tank	1	8,000 L	$281,000	$365,300
V-111	Intermediate Storage Tank	1	7,500 L	$275,000	$357,500
V-112	Intermediate Storage Tank	1	7,500 L	$275,000	$357,500
V-113 A/B	Waste Disposal Tank	2	50,000 L	$1,415,000	$1,839,500
Total Cost				**$26,965,000**	**$38,511,450**

Safety, Health, and Environmental Considerations

A summary of the material properties of compounds used in this process can be seen on the following page in Table 45.

Chromatography resins could not be included in the material properties table since they are a mixture of several chemical components but are still hazardous due to their high flammability. Ethanol is used in Table 45 to demonstrate the hazards associated with the resin. This is due to ethanol being a main component of the resin and having a high flammability.

Most of the chemicals in Table 45 are not flammable or have flash points much greater than the temperatures this process is operating at. Ethanol and carbon dioxide present the greatest hazards to workers if inhaled. However, ethanol is contained in the resin and an atmospheric concentration of >1000 ppm is not expected to be encountered and carbon dioxide will be released to process vents and removed from the plant.

Table 45: Material Properties of Significant Compounds in MAb Manufacturing Process

	Material Properties							
Compound	**MW, g/mol**	**Normal Boiling Point, °C**	**Flammability Limits**	**Flash Point, °C**	**Auto-ignition Temperature, °C**	**Liquid Density, g/cm3**	**Toxicity Limits**	**Reactivity with Water**
Acetic Acid	60.1	118	4.0-19.9%	39	485	1.04	N/A	Stable
Ammonium Sulfate	132.1	N/A	N/A	N/A	N/A	1.78	N/A	Stable
Biomass	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Carbon Dioxide	44.0	N/A	N/A	N/A	N/A	N/A	PEL 5000 ppm	N/A
Disodium Phosphate	142.0	N/A	N/A	N/A	N/A	1.70	1 g/kg	Stable
EDTA Disodium	336.2	N/A	N/A	> 100	N/A	N/A	N/A	Stable
Ethanol	46.0	78	3.3-19.0%	90	363	0.79	PEL 1000 ppm	Stable
Monoclonal Antibody	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Phosphoric Acid	98.0	407	N/A	N/A	N/A	1.90	PEL 1 mg/m3	Stable
Polysorbate 80	604.4	N/A	N/A	150	N/A	1.10	N/A	Stable
Potassium Chloride	74.5	1500	N/A	N/A	N/A	1.99	1 mmol/L/2 H	Stable
Potassium Dihydrogen Phosphate	136.1	N/A	N/A	N/A	N/A	2.34	40 mmol/L/24 H	Stable
Serum-free Media	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Sodium Chloride	58.0	1465	N/A	N/A	N/A	2.17	N/A	Stable
Sodium Citrate	258.1	N/A	N/A	N/A	N/A	N/A	1.5 g/kg	Stable
Sodium Dihydrogen Phosphate	120.0	N/A	N/A	N/A	N/A	2.36	8.29 g/kg	Stable
Sodium Hydroxide	40.0	1388	N/A	N/A	N/A	2.13	N/A	Stable
Tri-n-Butyl Phosphate	266.3	289	N/A	146	410	0.98	N/A	Stable
TRIS Base	121.1	220	N/A	N/A	N/A	N/A	N/A	Stable
TRIS HCl	157.6	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Water for Injection	18.0	100	N/A	N/A	N/A	1.00	N/A	N/A

The interaction matrix can be viewed below in Figure 10 [35]. This shows the compatibility of chemicals with one another and indicates which chemicals cannot be worked with or stored in close proximity to one another.

	ACETIC ACID, GLACIAL	AMMONIUM SULFATE	CARBON DIOXIDE	SODIUM PHOSPHATE, DIBASIC	ETHANOL	PHOSPHORIC ACID	POLYSORBATE 80	POTASSIUM CHLORIDE	SODIUM CHLORIDE	SODIUM HYDROXIDE SOLUTION	TRIBUTYL PHOSPHATE
AMMONIUM SULFATE	Caution Generates heat	AMMONIUM SULFATE									
CARBON DIOXIDE	Compatible	Compatible	CARBON DIOXIDE								
SODIUM PHOSPHATE, DIBASIC	Caution Generates gas Generates heat	Incompatible Generates heat	Caution Generates heat	SODIUM PHOSPHATE, DIBASIC							
ETHANOL	Caution Flammable Generates gas Generates heat Intense or explosive reaction	Compatible	Compatible	Compatible	ETHANOL						
PHOSPHORIC ACID	Compatible	Compatible	Compatible	Caution Generates heat	Compatible	PHOSPHORIC ACID					
POLYSORBATE 80	Compatible	Incompatible Generates heat Intense or explosive reaction	Compatible	Compatible	Compatible	Compatible	POLYSORBATE 80				
POTASSIUM CHLORIDE	Compatible	Compatible	Compatible	Caution Flammable Intense or explosive reaction	Compatible	Compatible	Compatible	POTASSIUM CHLORIDE			
SODIUM CHLORIDE	Compatible	Compatible	Compatible	Caution Flammable Intense or explosive reaction	Compatible	Compatible	Compatible	Compatible	SODIUM CHLORIDE		
SODIUM HYDROXIDE SOLUTION	Incompatible Corrosive Flammable Generates gas Intense or explosive reaction Toxic	Incompatible Corrosive Generates gas Generates heat	Incompatible Corrosive Generates heat	Incompatible Flammable Generates gas Generates heat Intense or explosive reaction	Incompatible Flammable Generates gas Generates heat	Incompatible Corrosive Generates heat	Compatible	Compatible	Compatible	SODIUM HYDROXIDE SOLUTION	
TRIBUTYL PHOSPHATE	Compatible	Compatible	Compatible	Caution Generates heat	Compatible	Compatible	Compatible	Caution	Caution	Incompatible Corrosive Flammable Generates gas Generates heat	TRIBUTYL PHOSPHATE
WATER	Compatible	Caution Corrosive Generates gas Generates heat	Caution Corrosive Generates heat	Incompatible Flammable Generates gas Generates heat Intense or explosive reaction	Compatible	Caution Corrosive Generates heat	Compatible	Compatible	Compatible	Caution Corrosive Generates gas Generates heat Toxic	Caution Corrosive Generates gas

Figure 10: Interaction matrix of chemicals used in MAb manufacturing process [16]

Some chemicals found in Table 45 were not available on the CAMEO website and could not be included in the reactivity matrix. It should be ensured that incompatible chemicals be separated from one another in storage and used in separate areas of the plant to avoid any accidental contact.

This process is operating at relatively safe temperatures and pressures. The highest pressure encountered in this process is 10.3 bar and the highest temperature encountered is 43.3 ^{o}C, however, most of the process is operating at 1.01 bar and 25 ^{o}C. Operators should be aware of units operating above standard conditions and be properly trained on how to operate these units, with additional PPE if required. Appropriate PPE should be worn at all times on the manufacturing floor.

CD OptiCHO AGT media is not immediately dangerous to health but may cause eye and skin irritation and as such eyewash stations should be available near the Media Prep section of the process where the media will be utilized [36]. Gloves and protective eyewear should be utilized when handling the media. If contact should occur the affected area should be rinsed with water for at least 15 minutes according to the SDS. Additionally, the media may be harmful in cases of inhalation and therefore should not be inhaled. If inhalation occurs, the affected person(s) should proceed to fresh air. In all cases, if symptoms persist, a physician should be contacted.

Protein A chromatography resin is a highly flammable liquid with a flash point of 21-22 ^{o}C because it contains ethanol [37]. Resin should be stored in a well-ventilated area and containers should be regularly inspected for leaks. Resin should be kept away from ignition sources and potential static discharge. Contact with Protein A chromatography resin may cause eye, skin, and respiratory irritation as well as gastrointestinal irritation if ingested. A safety shower and eyewash station should be located near the resin handling area in case of any accidental contact. Impervious gloves and safety glasses with side shields or goggles should be worn. In the case of an environmental release, the resin should be prevented from entering drains and should be soaked up with inert absorbent material.

Sepharose high performance resin is used in the ion exchange chromatography (IEX) column and butyl-sepharose resin is used in the hydrophobic interaction chromatography (HIC) column. Like Protein A resin, both resins are flammable and should be kept in a sealed container away from ignition sources [38, 39]. These resins should be used in a well-ventilated area as they can cause skin, respiratory, and eye irritation. Safety glasses and gloves must be worn when handling these resins and safety showers and eyewash stations must be included near the resin handling area. It is recommended by the SDS that this waste be given to a licensed disposal company for incineration.

Several buffer solutions are also introduced in the three chromatography columns to ensure proper binding. Most of these solutions are low concentrations, however, the most concentrated of these buffers is a 20% (mass) NaCl solution which equates to approximately a 4.3 M solution. Gloves and safety goggles should be worn when working with these buffer solutions. Caustic solutions can cause eye, skin, and respiratory irritation and should be washed from the eyes or skin immediately if contact is made [40]. Caustics can also corrode metals and proper metallurgy should be used with regular inspections.

Ammonium sulfate is used in the polishing step before HIC. Ammonium sulfate can cause skin, eye, and respiratory irritation and is harmful if swallowed [41]. It should be handled in a well-ventilated area and gloves and safety goggles should be worn. If contact is made with eyes or skin, the affected area should be washed for ample time in a safety shower.

Since this manufacturing facility is being built on an existing site, the HSE department should review if an additional environmental permit is required to operate. If a permit is required, the HSE department should contact appropriate specialists and the EPA to ensure the correct permit is being obtained.

Approximately 74,500 L (74,700 kg) of waste are being produced per batch in this process. This waste largely includes media, MAb, biomass, buffer solutions, acetic acid, ammonium sulfate, and WFI. The waste will be neutralized in kill tanks using steam and peracetic acid to kill all biological agents, and proper testing will be performed to ensure that the contents of the kill tanks are safe before being released to the county sewer system.

Process Safety Considerations

Inherently Safer Design

Table 46: Inherently Safer Design Application Summary

Inherently Safer Design Application Summary

Hazard	**Inherent Safety Concept**	**How Incorporated in Preliminary Design**
Large release and higher risk of contamination from use of one large media preparation tank	Minimization	Five small media preparation tanks are used rather than one large one to minimize the size of a release and reduce the volume of affected solution if contamination occurs.
High pressures encountered at beginning of process because no pressure relief available	Minimization	The first vessels in the process are no larger than 2.2 L in size. A rupture of one of these vessels would not be catastrophic to the process.

Mildly exothermic fermentation reaction producing CO2 gas	Moderation	Chilled water is used in the larger reactors R-105 A/B, R-106 A/B, and R-107 A/B/C to remove heat from the process.
Contamination of product may occur if SIP and CIP procedures fail	Substitution	The seed train reactors have been designed to be disposable to minimize the risk of product contamination and be more environmentally friendly by limiting the amount of WFI required by the process.
Use of many small disposable production reactors to meet product demand	Simplification	Three large stainless-steel batch reactors were selected for R-107 A/B/C instead of multiple small disposable batch reactors to minimize the number of vessels required by the process.
Many caustic compounds are used for the buffer solutions including sodium chloride and sodium hydroxide.	Moderation	The buffer solutions are diluted, with most of the solutions being >95 wt % WFI. These concentrations are not as hazardous if they come into contact with skin or eyes.
Flammability of chromatography column resins	Moderation/Minimization	Resins are stored in an isolated area of the plant under refrigeration. Since resins only need to be replaced once per year, a very small quantity is stored on site. The product is run through the columns for multiple cycles to minimize the size of the columns and amount of resin required.

This process uses five media preparation tanks per batch. This is inherently safer than using one large tank because it minimizes the amount of solution that could be released and minimizes the volume of affected solution if contamination occurs. The media powder selected contains no substances which at their given concentration, are considered to be hazardous to health.

This process is operating at relatively safe temperatures and pressures. The highest pressures of 10.3 bar and 7.6 bar are encountered at the first stages of the seed train process when vessels are 225 mL and 2.2 L in size. These pressures are high because of the buildup of carbon dioxide from the fermentation reaction with no vent to atmosphere, but because the volumes are relatively small, it is not a large risk if one vessel is to rupture from overpressure. Once the cells move to the disposable bag bioreactors, a pressure relief vent is introduced to avoid the larger volume of 100 L from rupturing due to overpressure. All vessels in the seed train, past the roller bottles, and the production bioreactors include vents to allow carbon dioxide to escape.

The highest temperatures are encountered in the seed train and production bioreactors because cells must be kept in an environment of 37 ^{o}C for proper growth. Fermentation reactions are inherently exothermic and no substitution of chemistry has been discovered at the current time.

The seed train vessels were minimized in size. This will result in smaller batch sizes with a larger frequency of batches to still meet product demand while minimizing the hazards of a release from a vessel. The larger disposable reactors and the production bioreactor also use cooling water and chilled water to control the heat of reaction.

Single-use batch reactors were selected for the seed train process to minimize the risk of contamination of the product by eliminating the dependence on SIP and CIP procedures. Many small single-use batch reactors would have to be purchased to meet the required annual production of the bioreactors, R-107 A/B/C. Although this would decrease the risk of contamination, it was determined to be more important for the process to have a minimum number of vessels. Since there is minimal risk of contamination in the seed train and there are multiple virus inactivation procedures downstream, the risk of contamination in the production bioreactors should be small.

Many different caustic compounds are used in the buffer solutions that can be hazardous to equipment and operators in large concentrations. These solutions have been diluted to minimize the hazards of contact with one of these solutions.

The resins used in the three chromatography columns are extremely flammable and have been stored in a refrigerated space to minimize chances of flashing. These resins will also be stored in small quantities to minimize the size of any fires or explosions that may result from exposure to an ignition source.

Hazard Identification and Risk Analysis

The most significant hazards present in this process are as follows:

- High pressure in R-101 and R-102 A/B due to no pressure relief system availability
- Rupture of R-103 A/B, R-104 A/B, R-105 A/B, R-106 A/B and R-107 A/B/C if pressure relief valves fail and CO_2 from fermentation reaction accumulates
- Flammability of resins used in the chromatography columns
- Contamination of product

Because this process is manufacturing biopharmaceuticals, the hazards of product contamination are large to consumers. For this reason, this facility will be required to follow good manufacturing practice (GMP) to ensure no contamination occurs.

The largest hazards are present in handling the three chromatography resins. These resins contain highly flammable hydrocarbons that must be stored properly in a refrigerated area where no known ignition sources are available.

High pressures of 10.3 and 7.6 bar are encountered in R-101 and R-102 A/B, respectively. These vessels are 225 mL and 2.2 L in size, respectively, and although these high pressures could cause a rupture with potential catastrophic damage in a larger vessel, these vessels are too small to cause damage to surrounding equipment. Minor injury to operators located in close proximity to R-101 or R-102 A/B could occur if one of these vessels ruptures. The disposable vessels should be examined for any cracks or damage to seals before use to ensure no mechanical failure or rupture occur.

The smallest reactors with pressure relief systems in place are 100 L in size. This could result in injury of nearby operators, but because they are disposable bag reactors, the equipment damage would be minimal. The largest reactors in the process are the production bioreactors at 24,500 L in size. This can result in a catastrophic accident if pressure relief systems fail and the reactor ruptures. Control systems should be put in place on R-103 A/B, R-104 A/B, R-105 A/B, R-106 A/B and R-107 A/B/C with high pressure alarms, emergency shutdown systems, and rupture discs to ensure that no vessel is overpressured to the point of failure. Additionally, the reactors are constructed of stainless steel to ensure no rupture occurs.

Siting and Layout of Process and Equipment

The chromatography columns contain highly flammable resin. For this reason, these columns should be located at a safe distance from one another and should be located away from the upstream part of the process which generates heat. Dry chemical fire extinguishers should be located in close proximity to all chromatography columns to quickly respond to any fire.

Resin should be stored in a separate area from CHO cells and media powder to minimize losses in the case of a fire. Buffer solutions should also be stored away from CHO cells and media powder as well as away from resin to minimize any contamination or damage to CHO cells. MAb product should be stored in a separate refrigerated area to minimize loss of product in the case of a fire.

Economic Analysis

Basis for Evaluation

An economic analysis was performed to determine the economic attractiveness of this project. The assumptions used to perform this analysis are summarized in Table 47.

Table 47: Parameters Used for Economic Evaluation

Project Life	15 years
MACRS Depreciation Rate	10 years
Tax Rate	25.7%
Minimum Rate of Return	15%
Escalation Rate	2%

The project life was assumed to be 15 years. When a patent is involved in the manufacturing of a biopharmaceutical product, this is a typical project life. Though this product will not require a patent, Avastin, the molecule this product is modeled after, has obtained a patent. There is no MACRS depreciation rate specific to equipment involved in pharmaceutical manufacturing, so the MACRS depreciation rate for chemical processing equipment was used to depreciate all capital costs.

New tax laws will become effective in 2019, lowering the corporate tax rate from 35% to 21%. Since it is unknown the location of this exact site, an average of state income tax rates was taken and the effective tax rate used in this economic analysis was 25.7% [42]. An annual escalation rate of 2% was assumed as this is the current national average.

A common value used for the minimum rate of return when evaluating a project is 15%. This practice was followed for the evaluation of this project.

Revenue and Operating Expense Estimates

It was recommended that the selling price of the MAb product be based on the drug Avastin, currently available on the market. The patent on Avastin will be expiring in July 2019, opening the market for several generic versions of the pharmaceutical. To stay competitive with the market, the average cost of Avastin was marked down 15%, since this is a typical generic brand markdown. The price of MAb product was set to be $7,150 per gram for this evaluation. This produces an annual revenue of $7.4 billion (in 2019 dollars).

The costs of raw materials are summarized in Table 48. Many operating costs are associated with this process including resins for columns, electricity, WFI, waste disposal, labor, and various other costs. A summary of these annual operating costs can be seen in Table 49. Figure 11 presents the individual operating costs as percentages of the total operating costs. Any of these costs that do not make up more than 1% of the total operating costs were omitted from this figure.

Table 48: Summary of Raw Material Costs

Raw Material Cost	Annual Amount (2019 Dollars)
CHO Cells	$30,000
Media Powder	$30,700,000
Total	**$30,730,000**

Table 49: Summary of Annual Operating Costs

Operating Cost	Annual Amount (2019 Dollars)
Protein A Resin	$2,620,000
Sepharose Resin	$304,000
Butyl-Sepharose Resin	$1,930,000
Waste Disposal	$5,510
Electricity	$3,050
WFI	$8,550,000
Labor	$2,050,000
Consumables	$322,000
Lab Charges	$157,000
Insurance	$1,150,000
Plant Overhead	$742,000
Administration	$185,000
Maintenance	$2,150,000
Total	**$20,168,560**

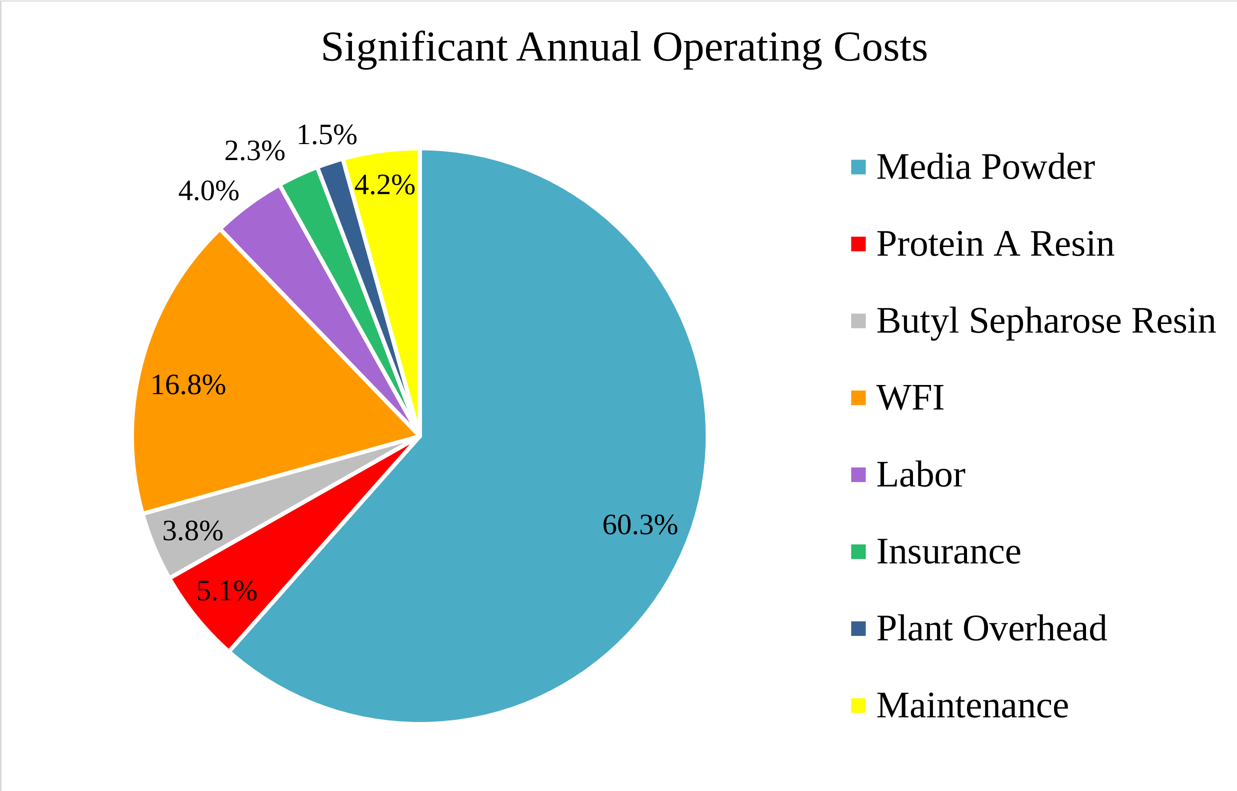

Figure 11: Annual Operating Cost Summary

As can be seen in Figure 11 above, the largest costs in this process are media powder and water for injection (WFI). This WFI is used in the process for both media solution preparation and cleaning and sanitizing vessels that are non-disposable. To produce 56 batches of MAb product (1,070 kg), 290 kg of media powder are used. Though this is not a large quantity of media powder, the cost of media powder per batch is approximately $580,000, resulting in the large operating cost.

The price of WFI was provided as $1/liter. The costs of all chemicals used in the process, as well as CHO cells, were estimated from chemical vendor websites such as Thermofisher and Sigma Aldrich. Consumables account for the disposable reactor vessels in this process. Data on these costs is not readily available to the public, and vendors should be contacted during the detailed design stage to obtain more realistic costs.

The SuperPro Designer software gives values for total electricity usage by the facility as well as estimated labor hours. The cost of electricity is $0.05/kWh. Labor costs were estimated using the labor hours obtained from the software and an estimated wage of $17/hour [43].

Lab charges, insurance, plant overhead, administration, and maintenance costs are typically estimated by using a multiplier with the fixed capital costs of the plant and labor costs. The remaining operating costs were estimated using common multipliers from published sources [4].

DCFROR Analysis

A summary of the key values obtained from the economic analysis are shown below in Table 50.

Table 50: Summary of Values Obtained from Economic Analysis

MAb Product Selling Price	$7,150/gram
Annual Revenue	$7,370,000,000
NPV at ROR = 15%	$34,700,000,000
DCFROR	7450%
MAb Breakeven Price	$56.60/gram
Payback Period	0.02 years

This project is remarkably favorable. As can be seen by the breakeven price, this process is profitable above a product selling price of $56.60 per gram. Because the product will be sold at a price much higher than this one, the process will be extremely profitable. This project has a rate of return of 7450%. This is well over the hurdle rate of 15% and, for this reason, this project is economically attractive. Any NPV greater than 0 indicates an economically attractive project. Since the NPV of this project is $34.7 billion, this project is extremely economically attractive. The payback period of this project is 0.02 years after startup, which is equivalent to 7 days.

A full cash flow table can be viewed on the following page in Table 51.

Table 51: Cash Flow Diagram

End of Year	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029
Net Revenue	0	3,835,975,000	7,825,389,000	7,981,896,780	8,141,534,716	8,304,365,410	8,470,452,718	8,639,861,772	8,812,659,008	8,988,912,188
- Raw Material Costs	0	(16,308,942)	(32,617,884)	(33,270,242)	(33,935,646)	(34,614,359)	(35,306,647)	(36,012,779)	(36,733,035)	(37,467,696)
- Other Operating Costs	0	(15,482,587)	(21,402,825)	(21,830,881)	(22,267,499)	(22,712,849)	(23,167,106)	(23,630,448)	(24,103,057)	(24,585,118)
- Depreciation	0	(3,726,100)	(6,706,980)	(5,365,584)	(4,292,467)	(3,435,464)	(2,746,136)	(2,440,596)	(2,440,596)	(2,444,322)
Taxable Income	0	3,800,457,371	7,764,661,311	7,921,430,073	8,081,039,103	8,243,602,737	8,409,232,830	8,577,777,950	8,749,382,320	8,924,415,053
- Tax @ 25.7%	0	(976,717,544)	(1,995,517,957)	(2,035,807,529)	(2,076,827,049)	(2,118,605,904)	(2,161,172,837)	(2,204,488,933)	(2,248,591,256)	(2,293,574,669)
Net Income	0	2,823,739,827	5,769,143,354	5,885,622,544	6,004,212,054	6,124,996,834	6,248,059,993	6,373,289,016	6,500,791,064	6,630,840,384
+ Depreciation	0	3,726,100	6,706,980	5,365,584	4,292,467	3,435,464	2,746,136	2,440,596	2,440,596	2,444,322
-Fixed Capital	(38,511,450)	0	0	0	0	0	0	0	0	0
Cash Flow	(38,511,450)	2,827,465,927	5,775,850,334	5,890,988,128	6,008,504,521	6,128,432,298	6,250,806,128	6,375,729,612	6,503,231,660	6,633,284,706
Discount Factor (P/F i*,n)	1.000	0.870	0.756	0.658	0.572	0.497	0.432	0.376	0.327	0.284
Discounted Cash Flow	(38,511,450)	2,458,666,023	4,367,372,654	3,873,420,319	3,435,381,961	3,046,913,962	2,702,395,986	2,396,872,918	2,125,917,965	1,885,593,510
NPV @ i*	34,659,278,858									
DCFROR	7445.75%									

End of Year	2030	2031	2032	2033	2034	2035	2036
Net Revenue	9,168,690,432	9,352,064,240	9,539,105,525	9,729,887,636	9,924,485,389	10,122,975,096	10,325,434,598
- Raw Material Costs	(38,217,050)	(38,981,391)	(39,761,019)	(40,556,239)	(41,367,364)	(42,194,711)	(43,038,605)
- Other Operating Costs	(25,076,820)	(25,578,357)	(26,089,924)	(26,611,722)	(27,143,957)	(27,686,836)	(28,240,573)
- Depreciation	(2,440,596)	(1,222,161)	0	0	0	0	0
Taxable Income	9,102,955,966	9,286,282,332	9,473,254,583	9,662,719,674	9,855,974,068	10,053,093,549	10,254,155,420
- Tax @ 25.7%	(2,339,459,683)	(2,386,574,559)	(2,434,626,428)	(2,483,318,956)	(2,532,985,335)	(2,583,645,042)	(2,635,317,943)
Net Income	6,763,496,283	6,899,707,773	7,038,628,155	7,179,400,718	7,322,988,732	7,469,448,507	7,618,837,477
+ Depreciation	2,440,596	1,222,161	0	0	0	0	0
-Fixed Capital	0	0	0	0	0	0	0
Cash Flow	6,765,936,878	6,900,929,934	7,038,628,155	7,179,400,718	7,322,988,732	7,469,448,507	7,618,837,477
Discount Factor (P/F i*,n)	0.247	0.215	0.187	0.163	0.141	0.123	0.107
Discounted Cash Flow	1,672,436,119	1,483,308,120	1,315,569,930	1,166,853,329	1,034,948,170	917,954,029	814,185,313

Sensitivity Analysis

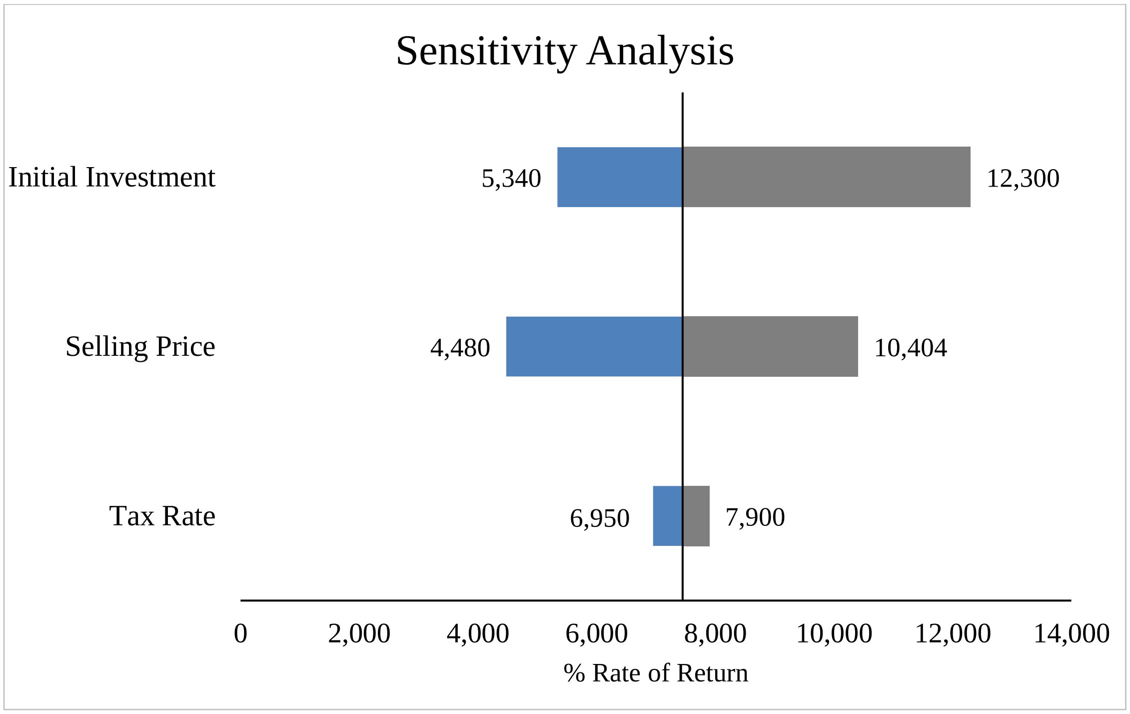

Figure 12: Sensitivity Analysis of Rate of Return to Various Parameters

A sensitivity analysis was performed on three key parameters to determine how a variation can affect the rate of return of this project. Both the initial investment and selling price of the MAb product were varied by +/- 40%. Since this is a preliminary design, a large error is expected for the capital cost estimates. An increase in capital cost results in a smaller change in rate of return than a decrease in capital costs, which means the rate of return will not be affected as much if the capital costs for this project have been underestimated.

Since the patent on Avastin is expiring, a competitive market price for the MAb product is not known. An estimate has been used based on typical generic brand markdowns, but competitors could choose to sell this product for less than the prediction used in this design. Changes in selling price will affect the rate of return of this project, but since they aren't expected to approach the breakeven price, this project is expected to remain economically attractive.

Since the state this plant will be operating in is unknown, a sensitivity analysis was performed to determine how state tax rates impact the rate of return of this project. The baseline was calculated using a tax rate of 25.7% which accounts for an average of all state tax rates. The state with the highest tax rate is California, with an effective tax rate of 34.3%. Six states have no state tax rate, so the lower bound was set to the federal rate of 21%. As can be seen in Figure 12 above, tax rate does not have a large impact on the rate of return for this project relative to selling price and initial investment.

Conclusions and Recommendations

Given the technical feasibility and projected return of this project, it is certainly attractive from a development and economics standpoint with an NPV of $34.7 billion and DCFROR of 7450%. This design meets the production requirements of 2 g/L and up to 10 g/L titers with an annual production of 1,070 kg MAb. Hazards in the process have been identified which could result in loss of containment and damage to property and/or persons. Despite these safety concerns, it is recommended that this project be continued and further design work and considerations be made.

Currently, membrane technology is being explored as a purification step to replace chromatography in research lab environments. Membrane technology is currently not recommended for use in large-scale manufacturing plants due to problems with low throughput, but it is recommended that as this technology develops, the plant look into replacing the chromatography columns with membrane filters.

The patent for Avastin will be expiring in July 2019, opening the market for other companies to begin selling generic versions of this pharmaceutical. Though a general markdown of the Avastin price was assumed for purposes of calculating the revenue for this preliminary design, it is recommended that a careful market study is conducted to determine a competitive price for this MAb product.

Acknowledgements

We would like to acknowledge our Chemical Engineering Design professors for teaching us the fundamentals of process design, as well as the rest of the Chemical Engineering faculty for guiding us over the course of our undergraduate program.

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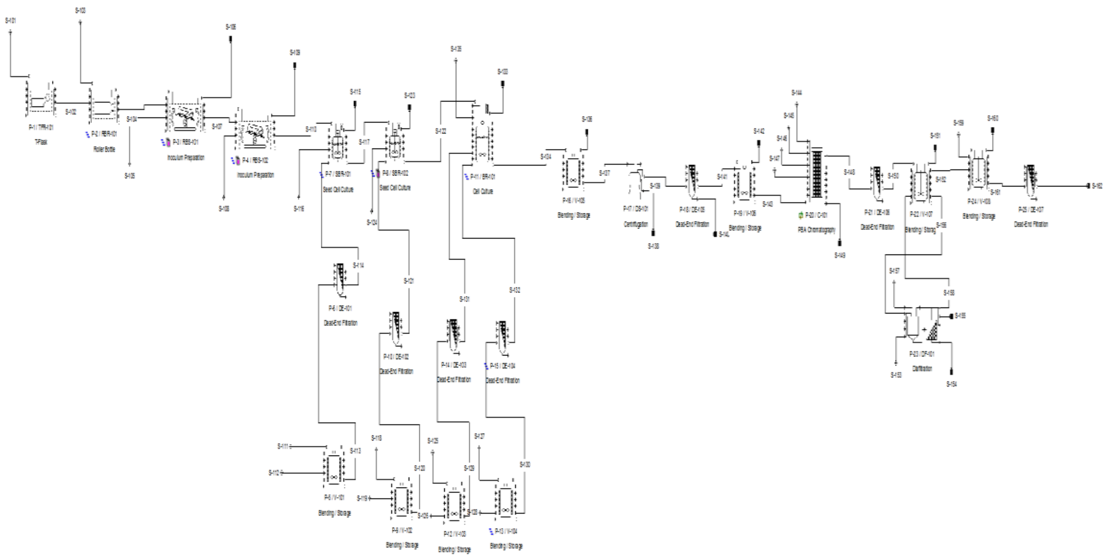
Appendix

The following can be found in the appendix:
Upstream Design for 2 g/L Titer
Downstream Design for 2 g/L Titer
Upstream Design for 10 g/L Titer

Upstream Design for 2 g/L Titer



Materials & Streams Report

for Final Design1 (1)

March 13, 2019

1. OVERALL PROCESS DATA

Annual Operating Time	7,859.08 h
Recipe Batch Time	1,162.83 h
Recipe Cycle Time	121.75 h
Number of Batches per Year	56.00

MP = Undefine

2.1 STARTING MATERIAL REQUIREMENTS (per Section)

Section	Starting Material	Active Product	Amount Needed (kg Sin/kg MP)	Molar Yield (%)	Mass Yield (%)	Gross Mass Yield (%)
Main Section	(none)	(none)	Unknown	Unknown	Unknown	Unknown
Section #1	(none)	(none)	Unknown	Unknown	Unknown	Unknown

Sin = Section Starting Material, Aout = Section Active Product

2.2 BULK MATERIALS (Entire Process)

Material	kg/yr	kg/batch	kg/kg MP
Air	1,004,987	17,946.19	
H3PO4 (5% w/w)	520,976	9,303.14	
Media Solution	13,104	234.00	
NaOH (0.1 M)	186,906	3,337.60	
NaOH (0.5 M)	285,378	5,096.04	
polysorbate 80	4	0.07	
Protein A Eluti	510,649	9,118.74	
Protein A Equil	1,145,675	20,458.48	
Protein A Reg B	304,873	5,444.16	
SerumFree Media	25,396	453.50	
WFI	2,346,527	41,902.26	
TOTAL	6,344,474	113,294	

2.3 BULK MATERIALS (per Section)

SECTIONS IN: Main Branch

Main Section

Material	kg/yr	kg/batch	kg/kg MP
Air	1,004,987	17,946.19	
H3PO4 (5% w/w)	520,976	9,303.14	
Media Solution	13,104	234.00	
NaOH (0.1 M)	186,906	3,337.60	
NaOH (0.5 M)	285,378	5,096.04	
polysorbate 80	4	0.07	
Protein A Eluti	510,649	9,118.74	
Protein A Equil	1,145,675	20,458.48	
Protein A Reg B	304,873	5,444.16	
SerumFree Media	25,396	453.50	
WFI	2,346,527	41,902.26	
TOTAL	6,344,474	113,294.19	

2.4 BULK MATERIALS (per Material)

Air

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-7	2.89	29,018	518.18	
P-8	11.58	116,355	2,077.77	
P-11	85.53	859,613	15,350.24	
TOTAL	100.00	1,004,987	17,946.19	

H3PO4 (5% w/w)

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-5	5.11	26,620	475.36	
P-7	6.70	34,895	623.13	
P-8	12.67	66,014	1,178.82	
P-9	8.12	42,293	755.23	
P-11	16.62	86,583	1,546.12	
P-12	7.10	36,976	660.29	
P-13	2.31	12,026	214.75	
P-16	13.96	72,725	1,298.66	
P-17	2.79	14,538	259.62	
P-19	7.80	40,619	725.34	
P-22	8.86	46,153	824.16	
P-23	2.79	14,538	259.62	
P-24	5.18	26,994	482.03	

TOTAL	100.00	520,976	9,303.14	

Media Solution

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	1.54	202	3.60	
P-2	4.87	638	11.40	
P-3	18.63	2,442	43.60	
P-4	74.96	9,822	175.40	
TOTAL	100.00	13,104	234.00	

NaOH (0.1 M)

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-20	100.00	186,906	3,337.60	
TOTAL	100.00	186,906	3,337.60	

NaOH (0.5 M)

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-5	4.59	13,086	233.69	
P-7	6.01	17,154	306.33	
P-8	11.37	32,452	579.50	
P-9	7.29	20,791	371.27	
P-11	14.91	42,564	760.06	
P-12	6.37	18,177	324.59	
P-13	2.07	5,912	105.57	
P-16	12.53	35,751	638.41	
P-17	5.01	14,294	255.25	
P-19	12.24	34,944	624.01	
P-22	7.95	22,689	405.15	
P-23	5.01	14,294	255.25	
P-24	4.65	13,270	236.96	
TOTAL	100.00	285,378	5,096.04	

polysorbate 80

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-24	100.00	4	0.07	
TOTAL	100.00	4	0.07	

Protein A Eluti

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-20	100.00	510,649	9,118.74	
TOTAL	100.00	510,649	9,118.74	

Protein A Equil

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-20	100.00	1,145,675	20,458.48	
TOTAL	100.00	1,145,675	20,458.48	

Protein A Reg B

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-20	100.00	304,873	5,444.16	
TOTAL	100.00	304,873	5,444.16	

SerumFree Media

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-5	4.69	1,190	21.25	
P-9	18.79	4,772	85.22	
P-12	38.14	9,686	172.96	
P-13	38.38	9,748	174.07	
TOTAL	100.00	25,396	453.50	

WFI

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-5	3.80	89,266	1,594.03	
P-7	4.27	100,275	1,790.62	
P-8	9.43	221,314	3,952.03	
P-9	9.99	234,485	4,187.23	
P-11	7.07	165,870	2,961.96	
P-12	29.02	680,997	12,160.67	
P-13	2.13	50,065	894.02	
P-16	5.94	139,322	2,487.89	
P-17	3.56	83,555	1,492.06	
P-19	5.80	136,177	2,431.74	
P-20	4.75	111,407	1,989.41	
P-22	3.77	88,417	1,578.87	
P-23	8.25	193,661	3,458.23	
P-24	2.20	51,716	923.51	
TOTAL	100.00	2,346,527	41,902.26	

2.5 BULK MATERIALS: SECTION TOTALS (kg/batch)

Raw Material	Main Section	Section #1
Air	17,946.19	0.00
H3PO4 (5% w/w)	9,303.14	0.00
Media Solution	234.00	0.00
NaOH (0.1 M)	3,337.60	0.00
NaOH (0.5 M)	5,096.04	0.00
polysorbate 80	0.07	0.00
Protein A Eluti	9,118.74	0.00
Protein A Equil	20,458.48	0.00
Protein A Reg B	5,444.16	0.00
SerumFree Media	453.50	0.00
WFI	41,902.26	0.00
TOTAL	113,294.19	0.00

2.6 BULK MATERIALS: SECTION TOTALS (kg/yr)

Raw Material	Main Section	Section #1
Air	1,004,987	0
H3PO4 (5% w/w)	520,976	0
Media Solution	13,104	0
NaOH (0.1 M)	186,906	0
NaOH (0.5 M)	285,378	0
polysorbate 80	4	0
Protein A Eluti	510,649	0
Protein A Equil	1,145,675	0
Protein A Reg B	304,873	0
SerumFree Media	25,396	0
WFI	2,346,527	0
TOTAL	6,344,474	0

3. STREAM DETAILS

Stream Name	S-125	S-126	S-129	S-118
Source	INPUT	INPUT	P-12	INPUT
Destination	P-12	P-12	P-14	P-9
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	25.00
Pressure (bar)	1.01	1.01	10.13	1.01
Density (g/L)	994.70	994.70	994.70	994.70
Total Enthalpy (kW-h)	290.28	5.05	295.32	79.97
Specific Enthalpy (kcal/kg)	25.11	25.11	25.11	25.11
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
SerumFree Media	0.00	172.96	172.96	0.00
WFI	9,947.04	0.00	9,947.04	2,740.41
TOTAL (kg/batch)	9,947.04	172.96	10,120.00	2,740.41
TOTAL (L/batch)	10,000.00	173.88	10,173.88	2,755.00

Stream Name	S-119	S-120	S-113	S-114
Source	INPUT	P-9	P-5	P-6
Destination	P-9	P-10	P-6	P-7
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	25.00
Pressure (bar)	1.01	10.13	10.13	10.13
Density (g/L)	994.70	994.70	994.70	994.70
Total Enthalpy (kW-h)	2.49	82.46	20.56	20.56
Specific Enthalpy (kcal/kg)	25.11	25.11	25.11	25.11
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
SerumFree Media	85.22	85.22	21.25	21.25
WFI	0.00	2,740.41	683.36	683.36
TOTAL (kg/batch)	85.22	2,825.63	704.61	704.61
TOTAL (L/batch)	85.67	2,840.67	708.36	708.36

Stream Name	S-101	S-102	S-103	S-104
Source	INPUT	P-1	INPUT	P-2
Destination	P-1	P-2	P-2	P-3
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	37.00	25.00	37.00
Pressure (bar)	1.01	10.28	1.01	7.55
Density (g/L)	1,000.00	990.49	1,000.00	990.48
Total Enthalpy (kW-h)	0.11	0.16	0.33	0.65
Specific Enthalpy (kcal/kg)	25.11	37.10	25.11	37.10
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
Biomass	0.00	0.01	0.00	0.04
Impurities	0.00	0.00	0.00	0.00
MAB	0.00	0.00	0.00	0.00
Media	0.07	0.02	0.23	0.07
Water	0.00	0.04	0.00	0.18
WFI	3.53	3.53	11.17	14.70
TOTAL (kg/batch)	3.60	3.60	11.40	15.00
TOTAL (L/batch)	3.60	3.63	11.40	15.14

Stream Name	S-105	S-106	S-107	S-108
Source	INPUT	P-3	P-3	INPUT
Destination	P-3	OUTPUT	P-4	P-4
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	37.00	37.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1,000.00	1.23	990.43	1,000.00
Total Enthalpy (kW-h)	1.27	0.00	2.52	5.12
Specific Enthalpy (kcal/kg)	25.11	20.54	37.10	25.11
Heat Capacity (kcal/kg-°C)	1.00	0.23	1.00	1.00
Component Flowrates (kg/batch)				
Biomass	0.00	0.00	0.11	0.00
Carb. Dioxide	0.00	0.03	0.00	0.00
Impurities	0.00	0.00	0.03	0.00
MAB	0.00	0.00	0.01	0.00
Media	0.87	0.00	0.28	3.51
Nitrogen	0.00	0.08	0.00	0.00
Oxygen	0.00	0.02	0.00	0.00
Water	0.00	0.00	0.68	0.00
WFI	42.73	0.00	57.43	171.89
TOTAL (kg/batch)	43.60	0.13	58.53	175.40
TOTAL (L/batch)	43.60	105.48	59.10	175.40

Stream Name	S-109	S-110	S-116	S-115
Source	P-4	P-4	INPUT	P-7
Destination	OUTPUT	P-7	P-7	OUTPUT
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	37.00	37.00	25.00	37.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1.26	990.43	1.18	1.13
Total Enthalpy (kW-h)	0.02	10.07	3.64	5.53
Specific Enthalpy (kcal/kg)	24.80	37.10	6.05	9.13
Heat Capacity (kcal/kg-°C)	0.23	1.00	0.24	0.24
Component Flowrates (kg/batch)				
Biomass	0.00	0.41	0.00	0.00
Carb. Dioxide	0.17	0.00	0.00	1.70
Impurities	0.00	0.15	0.00	0.00
MAB	0.00	0.04	0.00	0.00
Media	0.00	0.76	0.00	0.00
Nitrogen	0.30	0.00	397.51	398.37
Oxygen	0.09	0.00	120.68	120.94
Water	0.00	2.95	0.00	0.00
WFI	0.00	229.32	0.00	0.00
TOTAL (kg/batch)	0.55	233.63	518.18	521.01
TOTAL (L/batch)	435.47	235.89	439,427.25	459,089.60

Stream Name	S-117	S-111	S-112	S-121
Source	P-7	INPUT	INPUT	P-10
Destination	P-8	P-5	P-5	P-8
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	37.00	25.00	25.00	25.00
Pressure (bar)	1.01	1.01	1.01	10.13
Density (g/L)	990.51	994.70	994.70	994.70
Total Enthalpy (kW-h)	40.38	19.94	0.62	82.46
Specific Enthalpy (kcal/kg)	37.10	25.11	25.11	25.11
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
Biomass	2.96	0.00	0.00	0.00
Impurities	0.83	0.00	0.00	0.00
MAB	0.21	0.00	0.00	0.00
Media	0.76	0.00	0.00	0.00
SerumFree Media	4.25	0.00	21.25	85.22
Water	14.85	0.00	0.00	0.00
WFI	912.68	683.36	0.00	2,740.41
TOTAL (kg/batch)	936.54	683.36	21.25	2,825.63
TOTAL (L/batch)	945.52	687.00	21.36	2,840.67

Stream Name	S-124	S-123	S-122	S-135
Source	INPUT	P-8	P-8	INPUT
Destination	P-8	OUTPUT	P-11	P-11
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	37.00	37.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1.18	1.14	990.69	1.18
Total Enthalpy (kW-h)	14.61	25.11	162.83	107.93
Specific Enthalpy (kcal/kg)	6.05	10.45	37.10	6.05
Heat Capacity (kcal/kg-°C)	0.24	0.24	1.00	0.24
Component Flowrates (kg/batch)				
Biomass	0.00	0.00	24.43	0.00
Carb. Dioxide	0.00	57.23	0.00	0.00
Impurities	0.00	0.00	5.13	0.00
MAB	0.00	0.00	3.07	0.00
Media	0.00	0.00	0.76	0.00
Nitrogen	1,593.90	1,597.37	0.00	11,775.44
Oxygen	483.88	413.39	0.00	3,574.80
SerumFree Media	0.00	0.00	17.89	0.00
Water	0.00	0.00	72.11	0.00
WFI	0.00	0.00	3,653.09	0.00
TOTAL (kg/batch)	2,077.77	2,067.99	3,776.49	15,350.24
TOTAL (L/batch)	1,761,985.22	1,813,091.25	3,811.96	13,017,263.35

Stream Name	S-131	S-132	S-133	S-134
Source	P-14	P-15	P-11	P-11
Destination	P-11	P-11	OUTPUT	P-16
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	37.00	37.00
Pressure (bar)	10.13	1.01	1.01	1.01
Density (g/L)	994.70	994.70	1.14	990.92
Total Enthalpy (kW-h)	295.32	10.16	175.13	617.07
Specific Enthalpy (kcal/kg)	25.11	25.11	9.85	37.10
Heat Capacity (kcal/kg-°C)	1.00	1.00	0.24	1.00
Component Flowrates (kg/batch)				
Biomass	0.00	0.00	0.00	149.79
Carb. Dioxide	0.00	0.00	253.87	0.00
Impurities	0.00	0.00	0.00	17.98
MAB	0.00	0.00	0.00	28.79
Media	0.00	0.00	0.00	0.76
Nitrogen	0.00	0.00	11,788.61	0.00
Oxygen	0.00	0.00	3,257.44	0.00
SerumFree Media	172.96	174.07	0.00	43.49
Water	0.00	0.00	0.00	297.12
WFI	9,947.04	174.07	0.00	13,774.21
TOTAL (kg/batch)	10,120.00	348.15	15,299.92	14,312.14
TOTAL (L/batch)	10,173.88	350.00	13,447,549.75	14,443.29

Stream Name	S-127	S-128	S-130	S-141
Source	INPUT	INPUT	P-13	P-18
Destination	P-13	P-13	P-15	P-19
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	43.46
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	994.70	994.70	994.70	987.98
Total Enthalpy (kW-h)	5.08	5.08	10.16	674.41
Specific Enthalpy (kcal/kg)	25.11	25.11	25.11	43.55
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
Impurities	0.00	0.00	0.00	16.92
MAB	0.00	0.00	0.00	27.09
Media	0.00	0.00	0.00	0.71
SerumFree Media	0.00	174.07	174.07	40.92
Water	0.00	0.00	0.00	279.55
WFI	174.07	0.00	174.07	12,959.94
TOTAL (kg/batch)	174.07	174.07	348.15	13,325.13
TOTAL (L/batch)	175.00	175.00	350.00	13,487.31

Stream Name	S-142	S-143	S-136	S-137
Source	P-19	P-19	P-16	P-16
Destination	OUTPUT	P-20	OUTPUT	P-17
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	20.00	43.45	20.00	37.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1.20	987.98	1.20	990.92
Total Enthalpy (kW-h)	0.09	674.29	0.10	617.04
Specific Enthalpy (kcal/kg)	4.84	43.54	4.84	37.10
Heat Capacity (kcal/kg-°C)	0.24	1.00	0.24	1.00
Component Flowrates (kg/batch)				
Biomass	0.00	0.00	0.00	149.79
Impurities	0.00	16.92	0.00	17.98
MAB	0.00	27.09	0.00	28.79
Media	0.00	0.71	0.00	0.76
Nitrogen	12.28	0.00	13.12	0.00
Oxygen	3.73	0.00	3.98	0.00
SerumFree Media	0.00	40.92	0.00	43.49
Water	0.00	279.55	0.00	297.12
WFI	0.00	12,959.94	0.00	13,774.21
TOTAL (kg/batch)	16.01	13,325.13	17.11	14,312.14
TOTAL (L/batch)	13,346.96	13,487.27	14,262.10	14,443.28

Stream Name	S-139	S-138	S-140	S-144
Source	P-17	P-17	P-18	INPUT
Destination	P-18	OUTPUT	OUTPUT	P-20
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	43.46	43.46	43.46	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	987.99	996.78	1,018.99	1,030.00
Total Enthalpy (kW-h)	674.71	49.64	0.29	269.80
Specific Enthalpy (kcal/kg)	43.55	43.53	43.49	24.96
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	0.99
Component Flowrates (kg/batch)				
Biomass	3.00	146.80	3.00	0.00
EDTA Disodium	0.00	0.00	0.00	18.60
Impurities	16.92	1.06	0.00	0.00
MAB	27.09	1.70	0.01	0.00
Media	0.71	0.04	0.00	0.00
SerumFree Media	40.93	2.56	0.01	0.00
Sodium Chloride	0.00	0.00	0.00	9.30
TRIS Base	0.00	0.00	0.00	9.30
TRIS HCl	0.00	0.00	0.00	27.90
Water	279.61	17.51	0.06	0.00
WFI	12,962.68	811.53	2.74	9,234.21
TOTAL (kg/batch)	13,330.94	981.19	5.81	9,299.31
TOTAL (L/batch)	13,493.02	984.36	5.71	9,028.46

Stream Name	S-145	S-146	S-147	S-148
Source	INPUT	INPUT	INPUT	P-20
Destination	P-20	P-20	P-20	P-21
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	25.13
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1,030.00	1,010.00	1,005.00	994.93
Total Enthalpy (kW-h)	323.75	265.29	158.61	107.40
Specific Enthalpy (kcal/kg)	24.96	25.03	25.07	25.16
Heat Capacity (kcal/kg-°C)	0.99	1.00	1.00	1.00
Component Flowrates (kg/batch)				
Acetic-Acid	0.00	54.71	0.00	21.88
EDTA Disodium	22.32	0.00	0.00	0.00
Impurities	0.00	0.00	0.00	1.02
MAB	0.00	0.00	0.00	24.38
Sodium Chloride	11.16	0.00	0.00	0.00
Sodium Citrate	0.00	0.00	9.80	0.00
TRIS Base	11.16	0.00	0.00	0.00
TRIS HCl	33.48	0.00	0.00	0.00
WFI	11,081.06	9,064.03	5,434.36	3,625.61
TOTAL (kg/batch)	11,159.17	9,118.74	5,444.16	3,672.89
TOTAL (L/batch)	10,834.15	9,028.46	5,417.07	3,691.61

Stream Name	S-149	S-150	S-158	S-151
Source	P-20	P-21	P-23	P-22
Destination	OUTPUT	P-22	P-22	OUTPUT
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	30.51	25.13	25.39	20.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	994.49	994.93	994.60	1.20
Total Enthalpy (kW-h)	1,584.33	107.39	21.75	0.03
Specific Enthalpy (kcal/kg)	30.51	25.16	25.49	4.84
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	0.24
Component Flowrates (kg/batch)				
Acetic-Acid	32.83	21.88	0.58	0.00
EDTA Disodium	40.92	0.00	0.00	0.00
Impurities	15.91	1.02	0.05	0.00
MAB	2.71	24.38	23.65	0.00
Media	0.71	0.00	0.00	0.00
Nitrogen	0.00	0.00	0.00	4.01
Oxygen	0.00	0.00	0.00	1.22
SerumFree Media	40.92	0.00	0.00	0.00
Sodium Chloride	20.46	0.00	0.00	0.00
Sodium Citrate	9.80	0.00	0.00	0.00
TRIS Base	20.46	0.00	0.00	0.00
TRIS HCl	61.38	0.00	0.00	0.00
Water	279.55	0.00	0.00	0.00
WFI	44,147.99	3,625.61	710.17	0.00
TOTAL (kg/batch)	44,673.62	3,672.89	734.43	5.23
TOTAL (L/batch)	44,920.93	3,691.61	738.42	4,358.51

Stream Name	S-152	S-156	S-157	S-153
Source	P-22	P-22	INPUT	INPUT
Destination	P-24	P-23	P-23	P-23
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.39	25.13	25.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	994.60	994.93	994.70	994.70
Total Enthalpy (kW-h)	21.76	107.39	42.86	14.51
Specific Enthalpy (kcal/kg)	25.49	25.16	25.11	25.11
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
Acetic-Acid	0.58	21.88	0.00	0.00
Impurities	0.05	1.02	0.00	0.00
MAB	23.65	24.38	0.00	0.00
WFI	710.17	3,625.61	1,468.83	497.35
TOTAL (kg/batch)	734.43	3,672.89	1,468.83	497.35
TOTAL (L/batch)	738.42	3,691.62	1,476.65	500.00

Stream Name	S-155	S-154	S-159	S-160
Source	P-23	P-23	INPUT	P-24
Destination	OUTPUT	OUTPUT	P-24	OUTPUT
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.39	25.00	20.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	994.70	994.78	994.70	1.20
Total Enthalpy (kW-h)	14.51	130.27	0.00	0.00
Specific Enthalpy (kcal/kg)	25.11	25.43	25.11	4.84
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	0.24
Component Flowrates (kg/batch)				
Acetic-Acid	0.00	21.31	0.00	0.00
Impurities	0.00	1.70	0.00	0.00
Nitrogen	0.00	0.00	0.00	0.67
Oxygen	0.00	0.00	0.00	0.20
polysorbate 80	0.00	0.00	0.07	0.00
WFI	497.35	4,384.27	0.07	0.00
TOTAL (kg/batch)	497.35	4,407.28	0.15	0.87
TOTAL (L/batch)	500.00	4,430.41	0.15	726.29

Stream Name	S-161	S-162
Source	P-24	P-25
Destination	P-25	OUTPUT
Stream Properties		
Activity (U/ml)	0.00	0.00
Temperature (°C)	25.39	25.39
Pressure (bar)	1.01	1.01
Density (g/L)	994.60	994.60
Total Enthalpy (kW-h)	21.76	21.76
Specific Enthalpy (kcal/kg)	25.49	25.49
Heat Capacity (kcal/kg-°C)	1.00	1.00
Component Flowrates (kg/batch)		
Acetic-Acid	0.58	0.58
Impurities	0.05	0.05
MAB	23.65	23.65
polysorbate 80	0.07	0.07
WFI	710.24	710.24
TOTAL (kg/batch)	734.58	734.58
TOTAL (L/batch)	738.57	738.57

4. OVERALL COMPONENT BALANCE (kg/batch)

COMPONENT	INITIAL	INPUT	OUTPUT	FINAL	IN-OUT
Acetic-Acid	0.00	54.71	54.71	0.00	0.00
Biomass	0.00	0.00	149.79	0.00	- 149.79
Carb. Dioxide	0.00	0.00	312.99	0.28	- 313.26
EDTA Disodium	0.00	40.92	40.92	0.00	0.00
Impurities	0.00	0.00	18.71	0.00	- 18.71
MAB	0.00	0.00	28.06	0.00	- 28.06
Media	0.00	4.68	0.76	0.00	3.92
Nitrogen	69.13	13,766.84	13,814.80	49.23	- 28.05
Oxygen	20.99	4,179.35	3,801.02	14.82	384.50
Phosphoric Acid	0.00	465.16	465.16	0.00	0.00
polysorbate 80	0.00	0.07	0.07	0.00	0.00
SerumFree Media	0.00	453.50	43.49	0.00	410.01
Sodium Chloride	0.00	20.46	20.46	0.00	0.00
Sodium Citrate	0.00	9.80	9.80	0.00	0.00
Sodium Hydroxid	0.00	129.05	129.05	0.00	0.00
TRIS Base	0.00	20.46	20.46	0.00	0.00
TRIS HCl	0.00	61.38	61.38	0.00	0.00
Water	0.00	4,996.16	5,293.28	0.00	- 297.12
WFI	0.00	89,091.65	89,091.65	0.00	0.00
TOTAL	90.11	113,294.19	113,356.55	64.32	36.57
				Overall Error:	0.032%

5. EQUIPMENT CONTENTS

TFR-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-1	START	0.00	0.00	0.00
P-1	HOLD-1 (Holding)	1.00	0.00	0.00
P-1	CHARGE-1 (Charge)	1.50	3.62	0.00
P-1	REACT-1 (Batch Stoich. Reaction)	97.50	3.63	0.00
P-1	TRANSFER-OUT-1 (Transfer Out)	98.00	0.00	0.00

RBR-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-2	START	0.00	0.00	0.02
P-2	HOLD-1 (Holding)	1.00	0.00	0.02
P-2	TRANSFER-IN-1 (Transfer In)	98.00	3.63	0.02
P-2	CHARGE-1 (Charge)	98.50	15.10	0.02
P-2	REACT-1 (Batch Stoich. Reaction)	242.50	15.14	0.02
P-2	TRANSFER-OUT-1 (Transfer Out)	243.50	0.00	0.02

RBS-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-3	START	240.50	0.00	0.24
P-3	HOLD-1 (Holding)	242.50	0.00	0.24
P-3	TRANSFER-IN-1 (Transfer In)	243.50	15.14	0.24
P-3	CHARGE-1 (Charge)	244.50	58.98	0.24
P-3	REACT-1 (Batch Stoich. Reaction)	388.50	59.10	0.17(*)
P-3	TRANSFER-OUT-1 (Transfer Out)	389.00	0.00	0.17(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

RBS-102

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-4	START	386.50	0.00	0.71
P-4	HOLD-1 (Holding)	388.50	0.00	0.71
P-4	TRANSFER-IN-1 (Transfer In)	389.00	59.10	0.71
P-4	CHARGE-1 (Charge)	389.50	235.43	0.71
P-4	REACT-1 (Batch Stoich. Reaction)	533.50	235.89	0.46(*)
P-4	TRANSFER-OUT-1 (Transfer Out)	534.00	0.00	0.46(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

V-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-5	START	532.17	0.00	0.93
P-5	SIP-1 (In-Place-Steaming)	533.00	0.00	0.93
P-5	CHARGE-1 (Charge)	533.50	687.00	0.93
P-5	PULL-IN-1 (Pull In)	534.00	708.36	0.93
P-5	TRANSFER-OUT-1 (Transfer Out)	535.00	0.00	0.93
P-5	CIP-1 (In-Place-Cleaning)	536.83	0.00	0.93

DE-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-6	START	0.00	0.00	0.00
P-6	HOLD-1 (Holding)	0.50	0.00	0.00
P-6	FILTER-1 (Dead-End Filtration)	1.00	0.00	0.00

SBR-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-7	START	509.17	0.00	1.39
P-7	SIP-1 (In-Place-Steaming)	510.00	0.00	1.39
P-7	HOLD-1 (Holding)	534.00	0.00	1.39
P-7	TRANSFER-IN-1 (Transfer In)	535.00	708.36	1.39
P-7	TRANSFER-IN-2 (Transfer In)	534.00	944.25	1.39
P-7	FERMENT-1 (Batch Stoich. Fermentation)	678.00	945.52	0.27(*)
P-7	TRANSFER-OUT-1 (Transfer Out)	679.89	0.00	0.27(*)
P-7	CIP-1 (In-Place-Cleaning)	681.73	0.00	0.27(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

SBR-102

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-8	START	678.00	0.00	5.62
P-8	SIP-1 (In-Place-Steaming)	678.83	0.00	5.62
P-8	HOLD-1 (Holding)	702.00	0.00	5.62
P-8	TRANSFER-IN-1 (Transfer In)	679.00	2,840.67	5.62
P-8	TRANSFER-IN-2 (Transfer In)	679.89	3,786.18	5.62
P-8	FERMENT-1 (Batch Stoich. Fermentation)	823.89	3,811.96	1.09(*)
P-8	TRANSFER-OUT-1 (Transfer Out)	825.23	0.00	1.09(*)
P-8	CIP-1 (In-Place-Cleaning)	828.06	0.00	1.09(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

V-102

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-9	START	0.00	0.00	3.72
P-9	SIP-1 (In-Place-Steaming)	0.83	0.00	3.72
P-9	CHARGE-1 (Charge)	1.83	2,755.00	3.72
P-9	PULL-IN-1 (Pull In)	1.98	2,840.67	3.72
P-9	TRANSFER-OUT-1 (Transfer Out)	2.98	0.00	3.72
P-9	CIP-1 (In-Place-Cleaning)	4.81	0.00	3.72

DE-102

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-10	START	678.00	0.00	0.00
P-10	HOLD-1 (Holding)	678.50	0.00	0.00
P-10	FILTER-1 (Dead-End Filtration)	679.00	0.00	0.00

BR-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-11	START	798.06	0.00	21.29
P-11	SIP-1 (In-Place-Steaming)	798.89	0.00	21.29
P-11	HOLD-1 (Holding)	822.89	0.00	21.29
P-11	TRANSFER-IN-1 (Transfer In)	823.89	10,173.88	21.29
P-11	TRANSFER-IN-2 (Transfer In)	825.23	13,985.85	21.29
P-11	FERMENT-1 (Batch Stoich. Fermentation)	1,113.23	14,443.29	4.11(*)
P-11	TRANSFER-OUT-1 (Transfer Out)	1,114.56	0.00	4.11(*)
P-11	CIP-1 (In-Place-Cleaning)	1,116.39	0.00	4.11(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

V-103

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-12	START	0.00	0.00	13.33
P-12	SIP-1 (In-Place-Steaming)	0.83	0.00	13.33
P-12	CHARGE-1 (Charge)	1.00	10,000.00	13.33
P-12	PULL-IN-1 (Pull In)	1.62	10,173.88	13.33
P-12	TRANSFER-OUT-1 (Transfer Out)	2.62	0.00	13.33
P-12	CIP-1 (In-Place-Cleaning)	4.45	0.00	13.33

V-104

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-13	START	822.39	0.00	0.46
P-13	SIP-1 (In-Place-Steaming)	823.23	0.00	0.46
P-13	CHARGE-1 (Charge)	824.23	175.00	0.46
P-13	CHARGE-2 (Charge)	825.23	350.00	0.46
P-13	PULL-OUT-1 (Pull Out)	1,113.23	0.00	0.46
P-13	CIP-1 (In-Place-Cleaning)	1,115.06	0.00	0.46

DE-103

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-14	START	822.39	0.00	0.00
P-14	HOLD-1 (Holding)	822.89	0.00	0.00
P-14	FILTER-1 (Dead-End Filtration)	823.89	0.00	0.00

DE-104

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-15	START	824.73	0.00	0.00
P-15	HOLD-1 (Holding)	825.23	0.00	0.00
P-15	FILTER-1 (Dead-End Filtration)	1,113.23	0.00	0.00

V-105

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-16	START	1,113.23	0.00	18.92
P-16	SIP-1 (In-Place-Steaming)	1,114.06	0.00	18.92
P-16	TRANSFER-IN-1 (Transfer In)	1,114.56	14,443.28	1.82
P-16	TRANSFER-OUT-1 (Transfer Out)	1,123.00	0.00	18.19
P-16	CIP-1 (In-Place-Cleaning)	1,124.83	0.00	18.19

DE-105

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-18	START	1,114.56	0.00	0.00
P-18	HOLD-1 (Holding)	1,115.06	0.00	0.00
P-18	FILTER-1 (Dead-End Filtration)	1,123.00	5.71	0.00
P-18	TRANSFER-OUT-1 (Transfer Out)	1,123.00	0.00	0.00

V-106

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-19	START	1,110.73	0.00	17.67
P-19	SIP-1 (In-Place-Steaming)	1,111.56	0.00	17.67
P-19	HOLD-1 (Holding)	1,114.56	0.00	17.67
P-19	TRANSFER-IN-1 (Transfer In)	1,123.00	13,487.27	1.66
P-19	TRANSFER-OUT-1 (Transfer Out)	1,136.61	0.00	16.64
P-19	CIP-1 (In-Place-Cleaning)	1,138.44	0.00	16.64

C-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-20	START	1,122.58	0.00	0.00
P-20	EQUILIBRATE-1 (Column Equilibration (Simplified))	1,135.68	0.00	0.00
P-20	HOLD-1 (Holding)	1,137.68	3,371.82	0.00
P-20	LOAD-1 (PBA Column Loading (Simplified))	1,136.61	0.00	0.00
P-20	WASH-1 (Column Wash (Simplified))	1,137.36	0.00	0.00
P-20	ELUTE-1 (Column Elution (Simplified))	1,137.98	0.00	0.00
P-20	REGENERATE-1 (Column Regeneration (Simplified))	1,138.23	0.00	0.00

P-20	CIP-1 (In-Place-Cleaning)	1,139.48	0.00	0.00

DE-106

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-21	START	1,124.18	0.00	0.00
P-21	HOLD-1 (Holding)	1,124.68	0.00	0.00
P-21	FILTER-1 (Dead-End Filtration)	1,137.98	0.00	0.00

V-107

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-22	START	1,124.68	0.00	4.84
P-22	SIP-1 (In-Place-Steaming)	1,125.52	0.00	4.84
P-22	HOLD-1 (Holding)	1,127.68	0.00	4.84
P-22	TRANSFER-IN-1 (Transfer In)	1,137.98	3,691.62	0.48
P-22	TRANSFER-OUT-1 (Transfer Out)	1,142.49	0.00	4.83
P-22	TRANSFER-IN-2 (Transfer In)	1,142.49	738.42	3.96
P-22	TRANSFER-OUT-2 (Transfer Out)	1,157.66	0.00	4.83
P-22	CIP-1 (In-Place-Cleaning)	1,159.50	0.00	4.83

DF-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-23	START	1,136.15	0.00	0.00
	AFTER AUTO-INIT	1,136.15	3,691.62	0.00
P-23	SIP-1 (In-Place-Steaming)	1,136.98	3,691.62	0.00
P-23	FLUSH-1 (Flush)	1,137.98	3,691.62	0.00
P-23	DIAFILTER-1 (Diafiltration)	1,142.49	738.42	0.00
P-23	CIP-1 (In-Place-Cleaning)	1,144.07	738.42	0.00
P-23	END	1,144.07	0.00	0.00

V-108

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-24	START	1,141.66	0.00	0.97
P-24	SIP-1 (In-Place-Steaming)	1,142.49	0.00	0.97
P-24	TRANSFER-IN-1 (Transfer In)	1,143.74	738.42	0.10
P-24	PULL-IN-1 (Pull In)	1,144.07	738.57	0.10
P-24	HOLD-1 (Holding)	1,145.57	738.57	0.10
P-24	TRANSFER-OUT-1 (Transfer Out)	1,161.00	0.00	0.10
P-24	CIP-1 (In-Place-Cleaning)	1,162.83	0.00	0.10

DE-107

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-25	START	1,145.07	0.00	0.00
P-25	HOLD-1 (Holding)	1,145.57	0.00	0.00
P-25	FILTER-1 (Dead-End Filtration)	1,161.00	0.00	0.00

Equipment Report

for Final Design1 (1)

March 13, 2019

1. EQUIPMENT SUMMARY (2019 prices)

Name	Type	Units	Standby/ Staggered	Size (Capacity)	Material of Construction	Purchase Cost ($/Unit)
TFR-101	T-Flask Rack	1	0/0	4.05 L	CS	0
RBR-101	Roller Bottle Rack	1	0/1	17.60 L	CS	0
RBS-101	Rocking Bioreactor Skid	2	0/2	100.00 L	CS	222,000
RBS-102	Rocking Bioreactor Skid	3	0/3	200.00 L	CS	222,000
V-101	Blending Tank	1	0/0	787.07 L	SS316	179,000
DE-101	Dead-End Filter	1	0/0	10.00 m2	SS316	39,000
SBR-101	Seed Bioreactor	1	0/1	1,181.90 L	SS316	1,155,000
SBR-102	Seed Bioreactor	3	0/3	1,588.32 L	SS316	1,172,000
V-102	Blending Tank	1	0/0	3,156.30 L	SS316	217,000
DE-102	Dead-End Filter	1	0/0	20.00 m2	SS316	69,000
BR-101	Bioreactor	1	0/2	18,054.11 L	SS316	1,835,000
V-103	Blending Tank	1	0/0	11,304.31 L	SS316	247,000
V-104	Blending Tank	1	0/2	388.89 L	SS316	162,000
DE-103	Dead-End Filter	1	0/0	50.00 m2	SS316	160,000
DE-104	Dead-End Filter	1	0/2	10.00 m2	SS316	39,000
V-105	Blending Tank	1	0/0	16,048.09 L	SS316	266,000
DS-101	Disk-Stack Centrifuge	1	0/0	1,711.46 L/h	SS316	429,000
DE-105	Dead-End Filter	1	0/0	10.00 m2	SS316	39,000
V-106	Blending Tank	1	0/0	14,985.85 L	SS316	262,000
C-101	PBA Chromatography Column	1	0/0	451.42 L	SS316	576,000
DE-106	Dead-End Filter	1	0/0	10.00 m2	SS316	39,000
V-107	Blending Tank	1	0/0	4,101.80 L	SS316	225,000
DF-101	Diafilter	1	0/0	19.66 m2	SS316	62,000
V-108	Blending Tank	1	0/0	820.63 L	SS316	180,000
DE-107	Dead-End Filter	1	0/0	10.00 m2	SS316	39,000

2. ITEMIZED EQUIPMENT LIST

TFR-101 (T-Flask Rack)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	1.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		CS
Purchase Cost (system model for T-Flask Rack)	0.00	$/unit Unit
Cost of Consumable: 225 mL T-Flask	2.45	$/item Disposal
Cost of Consumable: 225 mL T-Flask	0.00	$/item Number of
Containers	0.00	per unit Equipment
Heat Capacity	0.00	cal/°C

RBR-101 (Roller Bottle Rack)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	1.00	
Installation Factor	1.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		CS
Purchase Cost (system model for Roller Bottle Rack)	0.00	$/unit Unit
Cost of Consumable: 2.2 L Roller Bottle	6.00	$/item Disposal
Cost of Consumable: 2.2 L Roller Bottle	0.00	$/item Number of
Containers	0.00	per unit Equipment
Heat Capacity	0.00	cal/°C

RBS-101 (Rocking Bioreactor Skid)

Equipment size was calculated		
Number of Units	2.00	
Number of Standby Units	0.00	
Number of Staggered Units	2.00	
Installation Factor	1.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h

Availability Rate	100.00	$/h
Material of Construction		CS
Purchase Cost (system model for Rocking Bioreactor Skid)	222,000.00	$/unit
Unit Cost of Consumable: 100 L Cell Bag	300.00	$/item
Disposal Cost of Consumable: 100 L Cell Bag	0.00	$/item
Holding Capacity	100.00	L
Equipment Heat Capacity	0.00	cal/°C

RBS-102 (Rocking Bioreactor Skid)

Equipment size was calculated		
Number of Units	3.00	
Number of Standby Units	0.00	
Number of Staggered Units	3.00	
Installation Factor	1.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		CS
Purchase Cost (system model for Rocking Bioreactor Skid)	222,000.00	$/unit
Unit Cost of Consumable: 200 L Cell Bag	490.56	$/item
Disposal Cost of Consumable: 200 L Cell Bag	0.00	$/item
Holding Capacity	200.00	L
Equipment Heat Capacity	0.00	cal/°C

V-101 (Blending Tank)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Blending Tank)	179,000.00	$/unit
Max Volume	80,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	787.07	L
Height	2.08	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		

Diameter	0.69	m

DE-101 (Dead-End Filter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Dead-End Filter)	39,000.00	$/unit Unit
Cost of Consumable: Dft DEF Cartridge	1,000.00	$/item Disposal
Cost of Consumable: Dft DEF Cartridge	0.00	$/item Max Number
of Cartridge Slots	5.00	%

SBR-101 (Seed Bioreactor)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	1.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Seed Bioreactor)	1,155,000.00	$/unit
Max Volume	2,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	1,181.90	L
Height	1.82	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	0.91	m

SBR-102 (Seed Bioreactor)

Equipment size was calculated		
Number of Units	3.00	
Number of Standby Units	0.00	
Number of Staggered Units	3.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Seed Bioreactor)	1,172,000.00	$/unit
Max Volume	2,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	1,588.32	L
Height	2.01	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	1.00	m

V-102 (Blending Tank)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Blending Tank)	217,000.00	$/unit
Max Volume	80,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	3,156.30	L
Height	3.31	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	1.10	m

DE-102 (Dead-End Filter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Dead-End Filter)	69,000.00	$/unit Unit
Cost of Consumable: Dft DEF Cartridge	1,000.00	$/item Disposal
Cost of Consumable: Dft DEF Cartridge	0.00	$/item Max Number
of Cartridge Slots	5.00	%

BR-101 (Bioreactor)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	2.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Bioreactor)	1,835,000.00	$/unit
Max Volume	30,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	18,054.11	L
Height	4.51	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	2.26	m

V-103 (Blending Tank)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h

Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Blending Tank)	247,000.00	$/unit
Max Volume	80,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	11,304.31	L
Height	5.06	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	1.69	m

V-104 (Blending Tank)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	2.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Blending Tank)	162,000.00	$/unit
Max Volume	80,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	388.89	L
Height	1.65	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	0.55	m

DE-103 (Dead-End Filter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Dead-End Filter)	160,000.00	$/unit

Unit Cost of Consumable: Dft DEF Cartridge	1,000.00	$/item

Disposal Cost of Consumable: Dft DEF Cartridge	0.00	$/item
Max Number of Cartridge Slots	5.00	%

DE-104 (Dead-End Filter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	2.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Dead-End Filter)	39,000.00	$/unit Unit
Cost of Consumable: Dft DEF Cartridge	1,000.00	$/item Disposal
Cost of Consumable: Dft DEF Cartridge	0.00	$/item Max Number
of Cartridge Slots	5.00	%

V-105 (Blending Tank)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Blending Tank)	266,000.00	$/unit
Max Volume	80,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	16,048.08	L
Height	5.69	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	1.90	m

DS-101 (Disk-Stack Centrifuge)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Disk-Stack Centrifuge)	429,000.00	$/unit
Sigma Factor	145,383.76	m2

The unit is aseptic

DE-105 (Dead-End Filter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Dead-End Filter)	39,000.00	$/unit Unit
Cost of Consumable: Dft DEF Cartridge	1,000.00	$/item Disposal
Cost of Consumable: Dft DEF Cartridge	0.00	$/item Max Number
of Cartridge Slots	5.00	%

V-106 (Blending Tank)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Blending Tank)	262,000.00	$/unit

Max Volume	80,000.00	L
Min Working/Vessel Volume	0.00	%

Max Working/Vessel Volume	90.00	%
Volume	14,985.85	L
Height	5.56	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	1.85	m

C-101 (PBA Chromatography Column)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.05	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for PBA Chromatography Column)	576,000.00	$/unit
Unit Cost of Consumable: Protein A	7,358.35	$/L
Disposal Cost of Consumable: Protein A	0.00	$/L
Column Diameter	1.52	m
Bed Height	0.25	m
Column Height	0.25	m
Bed Volume	451.42	L
Column Volume	451.42	L

DE-106 (Dead-End Filter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Dead-End Filter)	39,000.00	$/unit Unit
Cost of Consumable: Dft DEF Cartridge	1,000.00	$/item Disposal
Cost of Consumable: Dft DEF Cartridge	0.00	$/item Max Number
of Cartridge Slots	5.00	m

V-107 (Blending Tank)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Blending Tank)	225,000.00	$/unit
Max Volume	80,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	4,101.79	L
Height	3.61	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	1.20	m

DF-101 (Diafilter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Diafilter)	62,000.00	$/unit
Unit Cost of Consumable: Dft Membrane	400.00	$/m2
Disposal Cost of Consumable: Dft Membrane	0.00	$/m2
Number of Available Cartridge Slots	1.00	%

V-108 (Blending Tank)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.30	
Maintenance Factor	0.10	

Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h

Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Blending Tank)	180,000.00	$/unit
Max Volume	80,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	820.63	L
Height	2.11	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	0.70	m

DE-107 (Dead-End Filter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Dead-End Filter)	39,000.00	$/unit Unit
Cost of Consumable: Dft DEF Cartridge	1,000.00	$/item Disposal
Cost of Consumable: Dft DEF Cartridge	0.00	$/item Max Number
of Cartridge Slots	5.00	%

3. CIP SKID LIST

CIP.SKD-101				
Equipment	Procedure	Operation	Start (abs h)	End (abs h)
V-101	P-5	CIP-1	535.00	536.83
SBR-101	P-7	CIP-1	679.89	681.73

CIP.SKD-102				
Equipment	Procedure	Operation	Start (abs h)	End (abs h)
V-102	P-9	CIP-1	2.98	4.81
V-104	P-13	CIP-1	1,113.23	1,115.06

CIP.SKD-103				
Equipment	Procedure	Operation	Start (abs h)	End (abs h)
V-103	P-12	CIP-1	2.62	4.45

CIP.SKD-104				
Equipment	Procedure	Operation	Start (abs h)	End (abs h)
SBR-102	P-8	CIP-1	826.23	828.06
BR-101	P-11	CIP-1	1,114.56	1,116.39
V-105	P-16	CIP-1	1,123.00	1,124.83

CIP.SKD-105				
Equipment	Procedure	Operation	Start (abs h)	End (abs h)
DS-101	P-17	CIP-1	1,123.00	1,124.83

CIP.SKD-106				
Equipment	Procedure	Operation	Start (abs h)	End (abs h)
V-106	P-19	CIP-1	1,136.61	1,138.4

4. SIP PANEL LIST

No SIP panels are present in the flowsheet

5. EQUIPMENT CONSUMABLES

TFR-101 (T-Flask Rack)

Name	Consumption Rate	Amount per Use	Replac. Frequency
225 mL T-Flask	N/A	18.00 item	1.00 Cycle(s)

RBR-101 (Roller Bottle Rack)

Name	Consumption Rate	Amount per Use	Replac. Frequency
2.2 L Roller Bottle	N/A	8.00 item	1.00 Cycle(s)

RBS-101 (Rocking Bioreactor Skid)

Name	Consumption Rate	Amount per Use	Replac. Frequency
100 L Cell Bag	N/A	2.00 item	1.00 Cycle(s)

RBS-102 (Rocking Bioreactor Skid)

Name	Consumption Rate	Amount per Use	Replac. Frequency
200 L Cell Bag	N/A	3.00 item	1.00 Cycle(s)

DE-101 (Dead-End Filter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft DEF Cartridge	N/A	1.00 item	1.00 Cycle(s)

DE-102 (Dead-End Filter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft DEF Cartridge	N/A	2.00 item	1.00 Cycle(s)

DE-103 (Dead-End Filter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft DEF Cartridge	N/A	5.00 item	1.00 Cycle(s)

DE-104 (Dead-End Filter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft DEF Cartridge	N/A	1.00 item	1.00 Cycle(s)

DE-105 (Dead-End Filter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft DEF Cartridge	N/A	1.00 item	1.00 Cycle(s)

C-101 (PBA Chromatography Column)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Protein A	N/A	451.42 L	80.00 Cycle(s)

DE-106 (Dead-End Filter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft DEF Cartridge	N/A	1.00 item	1.00 Cycle(s)

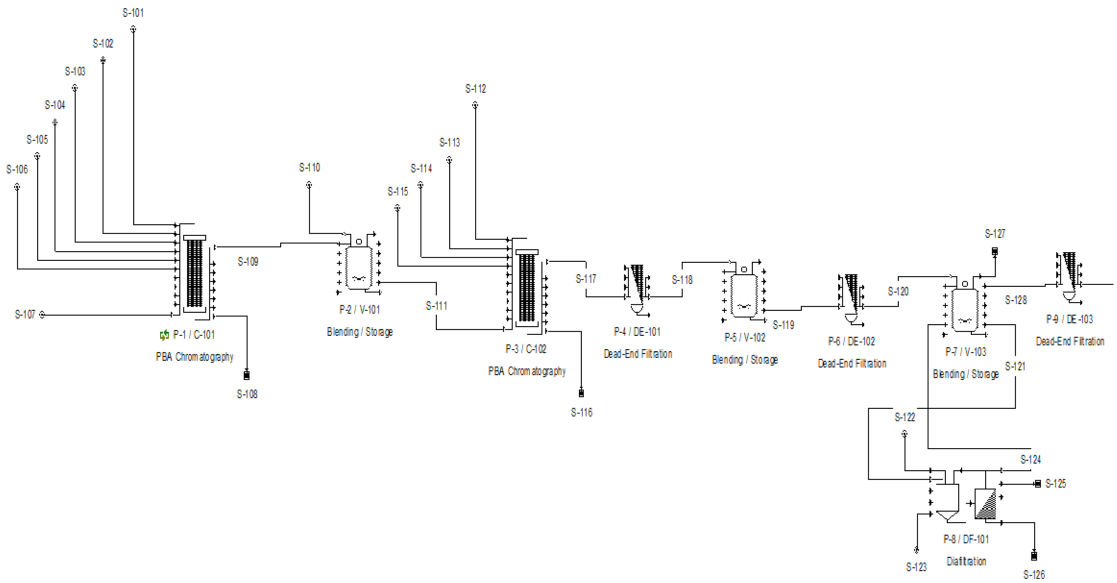
DF-101 (Diafilter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft Membrane	N/A	19.66 m2	1,000.00 hrs

DE-107 (Dead-End Filter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft DEF Cartridge	N/A	1.00 item	1.00 Cycle(s)

Downstream Design for 2 g/L Titer



1. OVERALL PROCESS DATA

Annual Operating Time	7,910.62 h
Recipe Batch Time	1,172.42 h
Recipe Cycle Time	22.31 h
Number of Batches per Year	303.00

MP = Undefined

2.1 STARTING MATERIAL REQUIREMENTS (per Section)

Section	Starting Material	Active Product	Amount Needed (kg Sin/kg MP)	Molar Yield (%)	Mass Yield (%)	Gross Mass Yield (%)
Main Section	(none)	(none)	Unknown	Unknown	Unknown	Unknown
Section #1	(none)	(none)	Unknown	Unknown	Unknown	Unknown

Sin = Section Starting Material, Aout = Section Active Product

2.2 BULK MATERIALS (Entire Process)

Material	kg/yr	kg/batch	kg/kg MP
Acetic-Acid	175	0.58	
Amm. Sulfate	38,307	126.43	
H3PO4 (5% w/w)	762,115	2,515.23	
HIC EI Buff	826,391	2,727.36	
HIC Eq Buff	341,844	1,128.20	
HIC Wash Buff	1,804,175	5,954.37	
IEX EI Buff	50,736	167.45	
IEX Eq Buff	899,400	2,968.32	
Impurities	14	0.05	
MAB	7,167	23.65	
NaCI (1 M)	553,615	1,827.11	
NaOH (0.1 M)	948,087	3,129.00	
NaOH (0.5 M)	1,699,116	5,607.64	
NaOH (1 M)	316,029	1,043.00	
PBS	441,919	1,458.48	
polysorbate 80	22	0.07	
WFI	3,059,999	10,099.01	
TOTAL	11,749,111	38,775.95	

2.3 BULK MATERIALS (per Section)

SECTIONS IN: Main Branch

Main Section

Material	kg/yr	kg/batch	kg/kg MP
Acetic-Acid	175	0.58	
Amm. Sulfate	38,307	126.43	
H3PO4 (5% w/w)	762,115	2,515.23	
HIC El Buff	826,391	2,727.36	
HIC Eq Buff	341,844	1,128.20	
HIC Wash Buff	1,804,175	5,954.37	
IEX El Buff	50,736	167.45	
IEX Eq Buff	899,400	2,968.32	
Impurities	14	0.05	
MAB	7,167	23.65	
NaCl (1 M)	553,615	1,827.11	
NaOH (0.1 M)	948,087	3,129.00	
NaOH (0.5 M)	1,699,116	5,607.64	
NaOH (1 M)	316,029	1,043.00	
PBS	441,919	1,458.48	
polysorbate 80	22	0.07	
WFI	3,059,999	10,099.01	
TOTAL	11,749,111	38,775.95	

2.4 BULK MATERIALS (per Material)

Acetic-Acid

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	100.00	175	0.58	
TOTAL	100.00	175	0.58	

Amm. Sulfate

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-2	100.00	38,307	126.43	
TOTAL	100.00	38,307	126.43	

H3PO4 (5% w/w)

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-2	45.99	350,484	1,156.71	
P-5	21.84	166,483	549.45	
P-7	21.84	166,483	549.45	
P-8	10.32	78,663	259.62	
TOTAL	100.00	762,115	2,515.23	

HIC El Buff

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-3	100.00	826,391	2,727.36	
TOTAL	100.00	826,391	2,727.36	

HIC Eq Buff

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-3	100.00	341,844	1,128.20	
TOTAL	100.00	341,844	1,128.20	

HIC Wash Buff

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	52.63	949,566	3,133.88	
P-3	47.37	854,609	2,820.49	
TOTAL	100.00	1,804,175	5,954.37	

IEX El Buff

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	100.00	50,736	167.45	
TOTAL	100.00	50,736	167.45	

IEX Eq Buff

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	100.00	899,400	2,968.32	
TOTAL	100.00	899,400	2,968.32	

Impurities

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	100.00	14	0.05	
TOTAL	100.00	14	0.05	

MAB				
Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	100.00	7,167	23.65	
TOTAL	100.00	7,167	23.65	

NaCI (1 M)				
Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	100.00	553,615	1,827.11	
TOTAL	100.00	553,615	1,827.11	

NaOH (0.1 M)				
Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	100.00	948,087	3,129.00	
TOTAL	100.00	948,087	3,129.00	

NaOH (0.5 M)				
Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	32.30	548,777	1,811.15	
P-2	5.07	86,148	284.32	
P-3	48.45	823,166	2,716.72	
P-5	4.82	81,842	270.11	
P-7	4.82	81,842	270.11	
P-8	4.55	77,341	255.25	
TOTAL	100.00	1,699,116	5,607.64	

NaOH (1 M)				
Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-3	100.00	316,029	1,043.00	
TOTAL	100.00	316,029	1,043.00	

PBS				
Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-8	100.00	441,919	1,458.48	
TOTAL	100.00	441,919	1,458.48	

polysorbate 80				
Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	100.00	22	0.07	
TOTAL	100.00	22	0.07	

WFI				
Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	46.37	1,419,012	4,683.21	
P-2	9.14	279,764	923.31	
P-3	3.94	120,558	397.88	
P-5	10.42	318,937	1,052.60	
P-7	10.42	318,937	1,052.60	
P-8	19.70	602,791	1,989.41	
TOTAL	100.00	3,059,999	10,099.01	

2.5 BULK MATERIALS: SECTION TOTALS (kg/batch)

Raw Material	Main Section	Section #1
Acetic-Acid	0.58	0.00
Amm. Sulfate	126.43	0.00
H3PO4 (5% w/w)	2,515.23	0.00
HIC El Buff	2,727.36	0.00
HIC Eq Buff	1,128.20	0.00
HIC Wash Buff	5,954.37	0.00
IEX El Buff	167.45	0.00
IEX Eq Buff	2,968.32	0.00
Impurities	0.05	0.00
MAB	23.65	0.00
NaCI (1 M)	1,827.11	0.00
NaOH (0.1 M)	3,129.00	0.00
NaOH (0.5 M)	5,607.64	0.00
NaOH (1 M)	1,043.00	0.00
PBS	1,458.48	0.00
polysorbate 80	0.07	0.00
WFI	10,099.01	0.00
TOTAL	38,775.95	0.00

2.6 BULK MATERIALS: SECTION TOTALS (kg/yr)

Raw Material	Main Section	Section #1
Acetic-Acid	175	0
Amm. Sulfate	38,307	0
H3PO4 (5% w/w)	762,115	0
HIC EI Buff	826,391	0
HIC Eq Buff	341,844	0
HIC Wash Buff	1,804,175	0
IEX EI Buff	50,736	0
IEX Eq Buff	899,400	0
Impurities	14	0
MAB	7,167	0
NaCI (1 M)	553,615	0
NaOH (0.1 M)	948,087	0
NaOH (0.5 M)	1,699,116	0
NaOH (1 M)	316,029	0
PBS	441,919	0
polysorbate 80	22	0
WFI	3,059,999	0
TOTAL	11,749,111	0

3. STREAM DETAILS

Stream Name	S-101	S-102	S-103	S-104
Source	INPUT	INPUT	INPUT	INPUT
Destination	P-1	P-1	P-1	P-1
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1,004.00	1,060.00	994.70	1,030.00
Total Enthalpy (kW-h)	86.24	87.76	81.10	4.75
Specific Enthalpy (kcal/kg)	25.00	24.09	25.11	24.41
Heat Capacity (kcal/kg-°C)	1.00	0.96	1.00	0.97
Component Flowrates (kg/batch)				
KCl	0.01	0.00	0.00	0.00
KH2PO4	0.01	0.00	0.00	0.00
Na2HPO4	3.27	9.97	0.00	0.00
NaH2PO4	0.00	0.00	0.00	1.64
Sodium Chloride	26.71	312.34	0.00	8.65
WFI	2,938.33	2,811.58	2,779.13	157.16
TOTAL (kg/batch)	2,968.32	3,133.88	2,779.13	167.45
TOTAL (L/batch)	2,956.49	2,956.49	2,793.92	162.57

Stream Name	S-105	S-106	S-107	S-109
Source	INPUT	INPUT	INPUT	P-1
Destination	P-1	P-1	P-1	P-2
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1,030.00	1,021.00	994.74	996.38
Total Enthalpy (kW-h)	52.18	52.35	21.43	34.96
Specific Enthalpy (kcal/kg)	24.57	24.87	25.10	25.07
Heat Capacity (kcal/kg-°C)	0.98	0.99	1.00	1.00
Component Flowrates (kg/batch)				
Acetic-Acid	0.00	0.00	0.58	0.00
Impurities	0.00	0.00	0.05	0.00
MAB	0.00	0.00	23.65	21.29
NaH2PO4	0.00	0.00	0.00	0.66
polysorbate 80	0.00	0.00	0.07	0.00
Sodium Chloride	103.60	0.00	0.00	3.46
Sodium Hydroxid	0.00	35.50	0.00	0.00
Water	1,723.51	1,775.65	0.00	0.00
WFI	0.00	0.00	710.43	1,174.51
TOTAL (kg/batch)	1,827.11	1,811.15	734.78	1,199.92
TOTAL (L/batch)	1,773.89	1,773.89	738.67	1,204.28

Stream Name	S-108	S-110	S-111	S-112
Source	P-1	INPUT	P-2	INPUT
Destination	OUTPUT	P-2	P-3	P-3
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	25.00
Pressure (bar)	1.01	1.01	10.13	1.01
Density (g/L)	1,014.68	1,769.00	1,039.66	1,060.00
Total Enthalpy (kW-h)	350.85	1.25	36.21	30.40
Specific Enthalpy (kcal/kg)	24.70	8.50	23.49	23.18
Heat Capacity (kcal/kg-°C)	0.98	0.34	0.94	0.92
Component Flowrates (kg/batch)				
Acetic-Acid	0.58	0.00	0.00	0.00
Amm. Sulfate	0.00	126.43	126.43	0.00
Impurities	0.04	0.00	0.00	0.00
KCl	0.01	0.00	0.00	0.00
KH2PO4	0.01	0.00	0.00	0.00
MAB	2.37	0.00	21.29	0.00
Na2HPO4	13.23	0.00	0.00	3.50
NaH2PO4	0.98	0.00	0.66	0.00
polysorbate 80	0.07	0.00	0.00	0.00
Sodium Chloride	447.84	0.00	3.46	221.13
Sodium Hydroxid	35.50	0.00	0.00	0.00
Water	3,499.16	0.00	0.00	0.00
WFI	8,222.11	0.00	1,174.51	903.57
TOTAL (kg/batch)	12,221.89	126.43	1,326.35	1,128.20
TOTAL (L/batch)	12,045.08	71.47	1,275.75	1,064.34

Stream Name	S-113	S-114	S-115	S-117
Source	INPUT	INPUT	INPUT	P-3
Destination	P-3	P-3	P-3	P-4
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1,060.00	1,025.00	1,021.00	1,014.85
Total Enthalpy (kW-h)	78.98	78.21	78.53	31.84
Specific Enthalpy (kcal/kg)	24.09	24.67	24.87	24.68
Heat Capacity (kcal/kg-°C)	0.96	0.98	0.99	0.98
Component Flowrates (kg/batch)				
MAB	0.00	0.00	0.00	19.16
Na2HPO4	8.97	7.82	0.00	3.13
Sodium Chloride	281.10	106.80	0.00	42.72
Sodium Hydroxid	0.00	0.00	53.25	0.00
Water	0.00	0.00	2,663.47	0.00
WFI	2,530.42	2,612.74	0.00	1,045.10
TOTAL (kg/batch)	2,820.49	2,727.36	2,716.72	1,110.10
TOTAL (L/batch)	2,660.84	2,660.84	2,660.84	1,093.86

Stream Name	S-116	S-120	S-124	S-128
Source	P-3	P-6	P-8	P-7
Destination	OUTPUT	P-7	P-7	P-9
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.62	25.62
Pressure (bar)	1.01	10.13	1.01	2.54
Density (g/L)	1,033.99	1,014.85	1,000.83	1,000.83
Total Enthalpy (kW-h)	270.49	31.84	21.72	21.72
Specific Enthalpy (kcal/kg)	24.22	24.68	25.58	25.58
Heat Capacity (kcal/kg-°C)	0.97	0.98	0.99	0.99
Component Flowrates (kg/batch)				
Amm. Sulfate	126.43	0.00	0.00	0.00
Impurities	0.00	0.00	0.00	0.00
KCl	0.00	0.00	0.00	0.00
KH2PO4	0.00	0.00	0.00	0.00
MAB	2.13	19.16	19.16	19.16
Na2HPO4	17.16	3.13	0.96	0.96
NaH2PO4	0.66	0.00	0.00	0.00
Sodium Chloride	569.77	42.72	8.73	8.73
Sodium Hydroxid	53.25	0.00	0.00	0.00
Water	2,663.47	0.00	0.00	0.00
WFI	6,176.15	1,045.10	701.75	701.75
TOTAL (kg/batch)	9,609.02	1,110.10	730.60	730.60
TOTAL (L/batch)	9,293.11	1,093.86	730.00	730.00

Stream Name	S-121	S-118	S-119	S-122
Source	P-7	P-4	P-5	INPUT
Destination	P-8	P-5	P-6	P-8
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	25.00
Pressure (bar)	10.13	1.01	10.13	1.01
Density (g/L)	1,014.85	1,014.85	1,014.85	1,000.00
Total Enthalpy (kW-h)	31.84	31.84	31.84	42.39
Specific Enthalpy (kcal/kg)	24.68	24.68	24.68	25.01
Heat Capacity (kcal/kg-°C)	0.98	0.98	0.98	1.00
Component Flowrates (kg/batch)				
KCl	0.00	0.00	0.00	0.00
KH2PO4	0.00	0.00	0.00	0.00
MAB	19.16	19.16	19.16	0.00
Na2HPO4	3.13	3.13	3.13	1.60
Sodium Chloride	42.72	42.72	42.72	11.67
WFI	1,045.10	1,045.10	1,045.10	1,445.20
TOTAL (kg/batch)	1,110.10	1,110.10	1,110.10	1,458.48
TOTAL (L/batch)	1,093.86	1,093.86	1,093.86	1,458.48

Stream Name	S-123	S-125	S-126	S-129
Source	INPUT	P-8	P-8	P-9
Destination	P-8	OUTPUT	OUTPUT	P-10
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.62	25.62
Pressure (bar)	1.01	1.01	1.01	2.54
Density (g/L)	994.70	994.70	1,007.49	1,000.83
Total Enthalpy (kW-h)	14.51	14.51	54.34	21.72
Specific Enthalpy (kcal/kg)	25.11	25.11	25.44	25.58
Heat Capacity (kcal/kg-°C)	1.00	1.00	0.99	0.99
Component Flowrates (kg/batch)				
KCl	0.00	0.00	0.00	0.00
KH2PO4	0.00	0.00	0.00	0.00
MAB	0.00	0.00	0.00	19.16
Na2HPO4	0.00	0.00	3.78	0.96
Sodium Chloride	0.00	0.00	45.66	8.73
WFI	497.35	497.35	1,788.54	701.75
TOTAL (kg/batch)	497.35	497.35	1,837.98	730.60
TOTAL (L/batch)	500.00	500.00	1,824.32	730.00

Stream Name	S-130
Source	P-10
Destination	OUTPUT
Stream Properties	
Activity (U/ml)	0.00
Temperature (°C)	25.62
Pressure (bar)	10.05
Density (g/L)	1,000.83
Total Enthalpy (kW-h)	21.72
Specific Enthalpy (kcal/kg)	25.58
Heat Capacity (kcal/kg-°C)	0.99
Component Flowrates (kg/batch)	
KCl	0.00
KH2PO4	0.00
MAB	19.16
Na2HPO4	0.96
Sodium Chloride	8.73
WFI	701.75
TOTAL (kg/batch)	730.60
TOTAL (L/batch)	730.00

4. OVERALL COMPONENT BALANCE (kg/batch)

COMPONENT	INITIAL	INPUT	OUTPUT	FINAL	IN-OUT
Acetic-Acid	0.00	0.58	0.58	0.00	0.00
Amm. Sulfate	0.00	126.43	126.43	0.00	0.00
Impurities	0.00	0.05	0.05	0.00	0.00
KCl	0.00	0.01	0.01	0.00	0.00
KH2PO4	0.00	0.01	0.01	0.00	0.00
MAB	0.00	23.65	23.65	0.00	0.00
Na2HPO4	0.00	35.13	35.13	0.00	0.00
NaH2PO4	0.00	1.64	1.64	0.00	0.00
Nitrogen	4.22	0.00	0.00	4.22	0.00
Oxygen	1.28	0.00	0.00	1.28	0.00
Phosphoric Acid	0.00	125.76	125.76	0.00	0.00
polysorbate 80	0.00	0.07	0.07	0.00	0.00
Sodium Chloride	0.00	1,071.99	1,071.99	0.00	0.00
Sodium Hydroxid	0.00	177.31	177.31	0.00	0.00
Water	0.00	8,224.20	8,224.20	0.00	0.00
WFI	0.00	28,989.12	28,989.12	0.00	0.00
TOTAL	5.50	38,775.95	38,775.95	5.50	0.00

5. EQUIPMENT CONTENTS

C-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-1	START	1,139.88	0.00	0.00
P-1	EQUILIBRATE-1 (Column Equilibration (Simplified))	1,155.37	246.22	0.00
P-1	HOLD-1 (Holding)	1,157.37	246.22	0.00
P-1	LOAD-1 (PBA Column Loading (Simplified))	1,157.69	0.00	0.00
P-1	WASH-1 (Column Wash (Simplified))	1,158.94	0.00	0.00
P-1	ELUTE-1 (Column Elution (Simplified))	1,160.19	0.00	0.00
P-1	REGENERATE-1 (Column Regeneration (Simplified))	1,160.56	0.00	0.00
P-1	WASH-2 (Column Wash (Simplified))	1,160.94	0.00	0.00
P-1	CIP-1 (In-Place-Cleaning)	1,162.19	0.00	0.00

V-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-2	START	1,143.23	0.00	1.67
P-2	SIP-1 (In-Place-Steaming)	1,144.06	0.00	1.67
P-2	TRANSFER-IN-1 (Transfer In)	1,160.19	1,204.28	1.67
P-2	PULL-IN-1 (Pull In)	1,161.19	1,275.75	1.67
P-2	TRANSFER-OUT-1 (Transfer Out)	1,163.19	0.00	1.67
P-2	CIP-1 (In-Place-Cleaning)	1,165.02	0.00	1.67

C-102

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-3	START	1,158.02	0.00	0.00
P-3	EQUILIBRATE-1 (Column Equilibration (Simplified))	1,158.19	1,275.75	0.00
P-3	HOLD-1 (Holding)	1,160.19	1,275.75	0.00
P-3	LOAD-1 (PBA Column Loading (Simplified))	1,160.79	0.00	0.00
P-3	WASH-1 (Column Wash (Simplified))	1,162.04	0.00	0.00
P-3	ELUTE-1 (Column Elution (Simplified))	1,163.29	0.00	0.00
P-3	REGENERATE-1 (Column Regeneration (Simplified))	1,163.91	0.00	0.00
P-3	CIP-1 (In-Place-Cleaning)	1,165.16	0.00	0.00

DE-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-4	START	1,161.54	0.00	0.00
P-4	HOLD-1 (Holding)	1,162.04	0.00	0.00
P-4	FILTER-1 (Dead-End Filtration)	1,163.29	0.00	0.00

V-102

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-5	START	1,162.04	0.00	1.43
P-5	SIP-1 (In-Place-Steaming)	1,162.87	0.00	1.43
P-5	TRANSFER-IN-1 (Transfer In)	1,163.29	1,093.86	1.43
P-5	TRANSFER-OUT-1 (Transfer Out)	1,164.54	0.00	1.43
P-5	CIP-1 (In-Place-Cleaning)	1,166.37	0.00	1.43

DE-102

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-6	START	1,162.79	0.00	0.00
P-6	HOLD-1 (Holding)	1,163.29	0.00	0.00
P-6	FILTER-1 (Dead-End Filtration)	1,165.59	0.00	0.00

V-103

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-7	START	1,159.45	0.00	1.43
P-7	SIP-1 (In-Place-Steaming)	1,160.29	0.00	1.43
P-7	HOLD-1 (Holding)	1,163.29	0.00	1.43
P-7	TRANSFER-IN-1 (Transfer In)	1,165.59	1,093.86	1.43
P-7	TRANSFER-OUT-1 (Transfer Out)	1,169.59	0.00	1.43
P-7	TRANSFER-IN-2 (Transfer In)	1,169.59	730.00	1.43
P-7	TRANSFER-OUT-2 (Transfer Out)	1,170.59	0.00	1.43
P-7	CIP-1 (In-Place-Cleaning)	1,172.42	0.00	1.43

DF-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-8	START	1,165.59	0.00	0.00
	AFTER AUTO-INIT	1,165.59	1,093.86	0.00
P-8	SIP-1 (In-Place-Steaming)	1,166.59	1,093.86	0.00
P-8	FLUSH-1 (Flush)	1,166.59	1,093.86	0.00
P-8	DIAFILTER-1 (Diafiltration)	1,169.59	730.00	0.00
P-8	CIP-1 (In-Place-Cleaning)	1,171.42	730.00	0.00
P-8	END	1,171.42	0.00	0.00

DE-103

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-9	START	1,169.09	0.00	0.00
P-9	HOLD-1 (Holding)	1,169.59	0.00	0.00
P-9	FILTER-1 (Dead-End Filtration)	1,170.59	0.00	0.00

DCS-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-10	START	1,169.59	0.00	0.96
P-10	TRANSFER-IN-1 (Transfer In)	1,170.59	730.00	0.96
P-10	END	1,170.59	0.00	0.96

Equipment Report

for Final Design2

March 13, 2019

1. EQUIPMENT SUMMARY (2019 prices)

Name	Type	Units	Standby/ Staggered	Size (Capacity)	Material of Construction	Purchase Cost ($/Unit)
C-101	PBA Chromatography Column	1	0/0	197.10 L	SS316	426,000
V-101	Blending Tank	1	0/0	1,417.50 L	SS316	194,000
C-102	PBA Chromatography Column	1	0/0	532.17 L	SS316	631,000
DE-101	Dead-End Filter	1	0/0	10.00 m2	SS316	39,000
V-102	Blending Tank	1	0/0	1,215.40 L	SS316	190,000
DE-102	Dead-End Filter	1	0/0	10.00 m2	SS316	39,000
V-103	Blending Tank	1	0/0	1,215.40 L	SS316	190,000
DF-101	Diafilter	1	0/0	22.80 m2	SS316	68,000
DE-103	Dead-End Filter	1	0/0	10.00 m2	SS316	39,000
DCS-101	Disposable Generic Container Skid	1	0/0	812.00 L	CS	0

2. ITEMIZED EQUIPMENT LIST

C-101 (PBA Chromatography Column)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.05	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for PBA Chromatography Column)	426,000.00	$/unit
Unit Cost of Consumable: Gel Filtration Resin	981.11	$/L
Disposal Cost of Consumable: Gel Filtration Resin	0.00	$/L
Column Diameter	1.00	m
Bed Height	0.25	m
Column Height	0.25	m
Bed Volume	197.10	L
Column Volume	197.10	L

V-101 (Blending Tank)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Blending Tank)	194,000.00	$/unit
Max Volume	80,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	1,417.50	L
Height	2.53	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	0.84	m

C-102 (PBA Chromatography Column)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.05	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for PBA Chromatography Column)	631,000.00	$/unit
Unit Cost of Consumable: HIC Biotech Resin	2,452.78	$/L
Disposal Cost of Consumable: HIC Biotech Resin	98.11	$/L
Column Diameter	1.65	m
Bed Height	0.25	m
Column Height	0.25	m
Bed Volume	532.17	L
Column Volume	532.17	L

DE-101 (Dead-End Filter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Dead-End Filter)	39,000.00	$/unit Unit
Cost of Consumable: Dft DEF Cartridge	1,000.00	$/item Disposal
Cost of Consumable: Dft DEF Cartridge	0.00	$/item Max Number
of Cartridge Slots	5.00	m

V-102 (Blending Tank)

Equipment size was calculated	
Number of Units	1.00
Number of Standby Units	0.00
Number of Staggered Units	0.00
Installation Factor	0.30
Maintenance Factor	0.10
Cost Allocation Factor	1.00

Usage Rate	100.00	$/equipment-h

Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Blending Tank)	190,000.00	$/unit
Max Volume	80,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	1,215.40	L
Height	2.41	m
Design Pressure	1.52	bar
Vessel is constructed according to ASME standards		
Diameter	0.80	m

DE-102 (Dead-End Filter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Dead-End Filter)	39,000.00	$/unit Unit
Cost of Consumable: Dft DEF Cartridge	1,000.00	$/item Disposal
Cost of Consumable: Dft DEF Cartridge	0.00	$/item Max Number
of Cartridge Slots	5.00	%

V-103 (Blending Tank)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.30	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Blending Tank)	190,000.00	$/unit
Max Volume	80,000.00	L
Min Working/Vessel Volume	0.00	%
Max Working/Vessel Volume	90.00	%
Volume	1,215.40	L
Height	2.41	m
Design Pressure	1.52	bar

Vessel is constructed according to ASME standards		
Diameter	0.80	m

DF-101 (Diafilter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Diafilter)	68,000.00	$/unit
Unit Cost of Consumable: Dft Membrane	400.00	$/m2
Disposal Cost of Consumable: Dft Membrane	0.00	$/m2
Number of Available Cartridge Slots	1.00	%

DE-103 (Dead-End Filter)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	0.50	
Maintenance Factor	0.10	
Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h
Availability Rate	100.00	$/h
Material of Construction		SS316
Purchase Cost (system model for Dead-End Filter)	39,000.00	$/unit Unit
Cost of Consumable: Dft DEF Cartridge	1,000.00	$/item Disposal
Cost of Consumable: Dft DEF Cartridge	0.00	$/item Max Number
of Cartridge Slots	5.00	%

DCS-101 (Disposable Generic Container Skid)

Equipment size was calculated		
Number of Units	1.00	
Number of Standby Units	0.00	
Number of Staggered Units	0.00	
Installation Factor	1.50	
Maintenance Factor	0.10	

Cost Allocation Factor	1.00	
Usage Rate	100.00	$/equipment-h

Availability Rate	100.00	$/h
Material of Construction		CS
Purchase Cost (system model for Disposable Generic Container Skid)	0.00	$/unit
Unit Cost of Consumable: 1 L Plastic Bag	0.20	$/item
Disposal Cost of Consumable: 1 L Plastic Bag	0.00	$/item
Number of Containers	0.00	per unit
Equipment Heat Capacity	0.00	cal/°C

3. CIP SKID LIST

CIP.SKD-101				
Equipment	Procedure	Operation	Start (abs h)	End (abs h)
V-102	P-5	CIP-1	1,164.54	1,166.37

CIP.SKD-102				
Equipment	Procedure	Operation	Start (abs h)	End (abs h)
V-103	P-7	CIP-1	1,170.59	1,172.42

CIP.SKD-103				
Equipment	Procedure	Operation	Start (abs h)	End (abs h)
DF-101	P-8	CIP-1	1,169.59	1,171.42

4. SIP PANEL LIST

No SIP panels are present in the flowsheet.

C-101 (PBA Chromatography Column)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Gel Filtration Resin	N/A	197.10 L	100.00 Cycle(s)

C-102 (PBA Chromatography Column)

Name	Consumption Rate	Amount per Use	Replac. Frequency
HIC Biotech Resin	N/A	532.17 L	100.00 Cycle(s)

DE-101 (Dead-End Filter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft DEF Cartridge	N/A	1.00 item	1.00 Cycle(s)

DE-102 (Dead-End Filter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft DEF Cartridge	N/A	1.00 item	1.00 Cycle(s)

DF-101 (Diafilter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft Membrane	N/A	22.80 m2	20.00 Cycle(s)

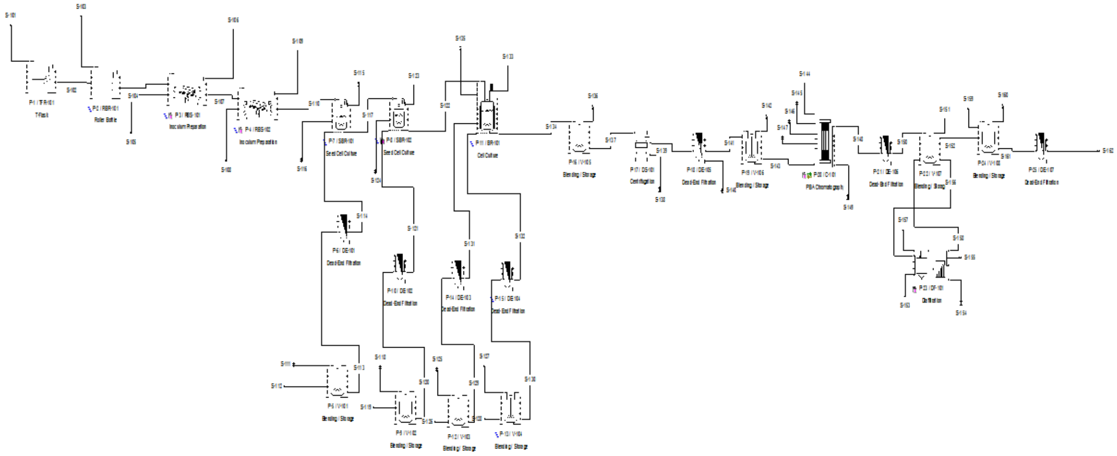
DE-103 (Dead-End Filter)

Name	Consumption Rate	Amount per Use	Replac. Frequency
Dft DEF Cartridge	N/A	1.00 item	1.00 Cycle(s)

DCS-101 (Disposable Generic Container Skid)

Name	Consumption Rate	Amount per Use	Replac. Frequency
1 L Plastic Bag	N/A	812.00 item	1.00 Cycle(s)

Upstream Design for 10 g/L Titer



1. OVERALL PROCESS DATA

Annual Operating Time	7,856.07 h
Recipe Batch Time	1,159.82 h
Recipe Cycle Time	121.75 h
Number of Batches per Year	56.00

MP = Undefined

2.1 STARTING MATERIAL REQUIREMENTS (per Section)

Section	Starting Material	Active Product	Amount Needed (kg Sin/kg MP)	Molar Yield (%)	Mass Yield (%)	Gross Mass Yield (%)
Main Section	(none)	(none)	Unknown	Unknown	Unknown	Unknown
Section #1	(none)	(none)	Unknown	Unknown	Unknown	Unknown

Sin = Section Starting Material, Aout = Section Active Product

2.2 BULK MATERIALS (Entire Process)

Material	kg/yr	kg/batch	kg/kg MP
Air	1,004,987	17,946.19	
H3PO4 (5% w/w)	621,875	11,104.91	
Media Solution	13,104	234.00	
NaOH (0.1 M)	747,622	13,350.40	
NaOH (0.5 M)	342,027	6,107.62	
polysorbate 80	21	0.37	
Protein A Eluti	2,600,220	46,432.49	
Protein A Equil	5,833,760	104,174.29	
Protein A Reg B	1,552,408	27,721.58	
SerumFree Media	154,907	2,766.19	
WFI	3,447,946	61,570.47	
TOTAL	16,318,877	291,408.52	

2.3 BULK MATERIALS (per Section)

SECTIONS IN: Main Branch

Main Section

Material	kg/yr	kg/batch	kg/kg MP
Air	1,004,987	17,946.19	
H3PO4 (5% w/w)	621,875	11,104.91	
Media Solution	13,104	234.00	
NaOH (0.1 M)	747,622	13,350.40	
NaOH (0.5 M)	342,027	6,107.62	
polysorbate 80	21	0.37	
Protein A Eluti	2,600,220	46,432.49	
Protein A Equil	5,833,760	104,174.29	
Protein A Reg B	1,552,408	27,721.58	
SerumFree Media	154,907	2,766.19	
WFI	3,447,946	61,570.47	
TOTAL	16,318,877	291,408.52	

2.4 BULK MATERIALS (per Material)

Air

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-7	2.89	29,018	518.18	
P-8	11.58	116,355	2,077.77	
P-11	85.53	859,613	15,350.24	
TOTAL	100.00	1,004,987	17,946.19	

H3PO4 (5% w/w)

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-5	4.28	26,620	475.36	
P-7	5.61	34,895	623.13	
P-8	10.62	66,014	1,178.82	
P-9	6.80	42,293	755.23	
P-11	15.39	95,705	1,709.03	
P-12	5.95	36,976	660.29	
P-13	4.69	29,180	521.07	
P-16	12.93	80,387	1,435.49	
P-17	2.34	14,538	259.62	
P-19	6.49	40,349	720.51	
P-22	12.77	79,401	1,417.87	
P-23	4.68	29,077	519.23	
P-24	7.47	46,439	829.26	

TOTAL	100.00	621,875	11,104.91	

Media Solution

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-1	1.54	202	3.60	
P-2	4.87	638	11.40	
P-3	18.63	2,442	43.60	
P-4	74.96	9,822	175.40	
TOTAL	100.00	13,104	234.00	

NaOH (0.1 M)

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-20	100.00	747,622	13,350.40	
TOTAL	100.00	747,622	13,350.40	

NaOH (0.5 M)

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-5	3.83	13,086	233.69	
P-7	5.02	17,154	306.33	
P-8	9.49	32,452	579.50	
P-9	6.08	20,791	371.27	
P-11	13.76	47,048	840.14	
P-12	5.31	18,177	324.59	
P-13	4.19	14,345	256.15	
P-16	11.55	39,518	705.68	
P-17	4.18	14,294	255.25	
P-19	10.15	34,712	619.85	
P-22	11.41	39,033	697.01	
P-23	8.36	28,588	510.50	
P-24	6.67	22,829	407.66	
TOTAL	100.00	342,027	6,107.62	

polysorbate 80

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-24	100.00	21	0.37	
TOTAL	100.00	21	0.37	

Protein A Eluti

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-20	100.00	2,600,220	46,432.49	
TOTAL	100.00	2,600,220	46,432.49	

Protein A Equil

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-20	100.00	5,833,760	104,174.29	
TOTAL	100.00	5,833,760	104,174.29	

Protein A Reg B

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-20	100.00	1,552,408	27,721.58	
TOTAL	100.00	1,552,408	27,721.58	

SerumFree Media

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-5	0.77	1,190	21.25	
P-9	3.08	4,772	85.22	
P-12	6.25	9,686	172.96	
P-13	89.90	139,259	2,486.76	
TOTAL	100.00	154,907	2,766.19	

WFI

Procedure	% Total	kg/yr	kg/batch	kg/kg MP
Main Section (Main Branch)				
P-5	2.59	89,266	1,594.03	
P-7	2.91	100,275	1,790.62	
P-8	6.42	221,314	3,952.03	
P-9	6.80	234,485	4,187.23	
P-11	5.32	183,346	3,274.03	
P-12	19.75	680,997	12,160.67	
P-13	6.88	237,084	4,233.65	
P-16	4.47	154,001	2,750.01	
P-17	2.42	83,555	1,492.06	
P-19	3.92	135,270	2,415.54	
P-20	12.92	445,628	7,957.63	
P-22	4.41	152,110	2,716.25	
P-23	18.61	641,631	11,457.69	
P-24	2.58	88,985	1,589.02	
TOTAL	100.00	3,447,946	61,570.47	

2.5 BULK MATERIALS: SECTION TOTALS (kg/batch)

Raw Material	Main Section	Section #1
Air	17,946.19	0.00
H3PO4 (5% w/w)	11,104.91	0.00
Media Solution	234.00	0.00
NaOH (0.1 M)	13,350.40	0.00
NaOH (0.5 M)	6,107.62	0.00
polysorbate 80	0.37	0.00
Protein A Eluti	46,432.49	0.00
Protein A Equil	104,174.29	0.00
Protein A Reg B	27,721.58	0.00
SerumFree Media	2,766.19	0.00
WFI	61,570.47	0.00
TOTAL	291,408.52	0.00

2.6 BULK MATERIALS: SECTION TOTALS (kg/yr)

Raw Material	Main Section	Section #1
Air	1,004,987	0
H3PO4 (5% w/w)	621,875	0
Media Solution	13,104	0
NaOH (0.1 M)	747,622	0
NaOH (0.5 M)	342,027	0
polysorbate 80	21	0
Protein A Eluti	2,600,220	0
Protein A Equil	5,833,760	0
Protein A Reg B	1,552,408	0
SerumFree Media	154,907	0
WFI	3,447,946	0
TOTAL	16,318,877	0

3. STREAM DETAILS

Stream Name	S-125	S-126	S-129	S-118
Source	INPUT	INPUT	P-12	INPUT
Destination	P-12	P-12	P-14	P-9
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	25.00
Pressure (bar)	1.01	1.01	10.13	1.01
Density (g/L)	994.70	994.70	994.70	994.70
Total Enthalpy (kW-h)	290.28	5.05	295.32	79.97
Specific Enthalpy (kcal/kg)	25.11	25.11	25.11	25.11
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
SerumFree Media	0.00	172.96	172.96	0.00
WFI	9,947.04	0.00	9,947.04	2,740.41
TOTAL (kg/batch)	9,947.04	172.96	10,120.00	2,740.41
TOTAL (L/batch)	10,000.00	173.88	10,173.88	2,755.00

Stream Name	S-119	S-120	S-113	S-114
Source	INPUT	P-9	P-5	P-6
Destination	P-9	P-10	P-6	P-7
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	25.00
Pressure (bar)	1.01	10.13	10.13	10.13
Density (g/L)	994.70	994.70	994.70	994.70
Total Enthalpy (kW-h)	2.49	82.46	20.56	20.56
Specific Enthalpy (kcal/kg)	25.11	25.11	25.11	25.11
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
SerumFree Media	85.22	85.22	21.25	21.25
WFI	0.00	2,740.41	683.36	683.36
TOTAL (kg/batch)	85.22	2,825.63	704.61	704.61
TOTAL (L/batch)	85.67	2,840.67	708.36	708.36

Stream Name	S-101	S-102	S-103	S-104
Source	INPUT	P-1	INPUT	P-2
Destination	P-1	P-2	P-2	P-3
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	37.00	25.00	37.00
Pressure (bar)	1.01	10.28	1.01	7.55
Density (g/L)	1,000.00	990.49	1,000.00	990.48
Total Enthalpy (kW-h)	0.11	0.16	0.33	0.65
Specific Enthalpy (kcal/kg)	25.11	37.10	25.11	37.10
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
Biomass	0.00	0.01	0.00	0.04
Impurities	0.00	0.00	0.00	0.00
MAB	0.00	0.00	0.00	0.00
Media	0.07	0.02	0.23	0.07
Water	0.00	0.04	0.00	0.18
WFI	3.53	3.53	11.17	14.70
TOTAL (kg/batch)	3.60	3.60	11.40	15.00
TOTAL (L/batch)	3.60	3.63	11.40	15.14

Stream Name	S-105	S-106	S-107	S-108
Source	INPUT	P-3	P-3	INPUT
Destination	P-3	OUTPUT	P-4	P-4
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	37.00	37.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1,000.00	1.23	990.43	1,000.00
Total Enthalpy (kW-h)	1.27	0.00	2.52	5.12
Specific Enthalpy (kcal/kg)	25.11	20.54	37.10	25.11
Heat Capacity (kcal/kg-°C)	1.00	0.23	1.00	1.00
Component Flowrates (kg/batch)				
Biomass	0.00	0.00	0.11	0.00
Carb. Dioxide	0.00	0.03	0.00	0.00
Impurities	0.00	0.00	0.03	0.00
MAB	0.00	0.00	0.01	0.00
Media	0.87	0.00	0.28	3.51
Nitrogen	0.00	0.08	0.00	0.00
Oxygen	0.00	0.02	0.00	0.00
Water	0.00	0.00	0.68	0.00
WFI	42.73	0.00	57.43	171.89
TOTAL (kg/batch)	43.60	0.13	58.53	175.40
TOTAL (L/batch)	43.60	105.48	59.10	175.40

Stream Name	S-109	S-110	S-116	S-115
Source	P-4	P-4	INPUT	P-7
Destination	OUTPUT	P-7	P-7	OUTPUT
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	37.00	37.00	25.00	37.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1.26	990.43	1.18	1.13
Total Enthalpy (kW-h)	0.02	10.07	3.64	5.53
Specific Enthalpy (kcal/kg)	24.80	37.10	6.05	9.13
Heat Capacity (kcal/kg-°C)	0.23	1.00	0.24	0.24
Component Flowrates (kg/batch)				
Biomass	0.00	0.41	0.00	0.00
Carb. Dioxide	0.17	0.00	0.00	1.70
Impurities	0.00	0.15	0.00	0.00
MAB	0.00	0.04	0.00	0.00
Media	0.00	0.76	0.00	0.00
Nitrogen	0.30	0.00	397.51	398.37
Oxygen	0.09	0.00	120.68	120.94
Water	0.00	2.95	0.00	0.00
WFI	0.00	229.32	0.00	0.00
TOTAL (kg/batch)	0.55	233.63	518.18	521.01
TOTAL (L/batch)	435.47	235.89	439,427.25	459,089.60

Stream Name	S-117	S-111	S-112	S-121
Source	P-7	INPUT	INPUT	P-10
Destination	P-8	P-5	P-5	P-8
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	37.00	25.00	25.00	25.00
Pressure (bar)	1.01	1.01	1.01	10.13
Density (g/L)	990.51	994.70	994.70	994.70
Total Enthalpy (kW-h)	40.38	19.94	0.62	82.46
Specific Enthalpy (kcal/kg)	37.10	25.11	25.11	25.11
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
Biomass	2.96	0.00	0.00	0.00
Impurities	0.83	0.00	0.00	0.00
MAB	0.21	0.00	0.00	0.00
Media	0.76	0.00	0.00	0.00
SerumFree Media	4.25	0.00	21.25	85.22
Water	14.85	0.00	0.00	0.00
WFI	912.68	683.36	0.00	2,740.41
TOTAL (kg/batch)	936.54	683.36	21.25	2,825.63
TOTAL (L/batch)	945.52	687.00	21.36	2,840.67

Stream Name	S-124	S-123	S-122	S-135
Source	INPUT	P-8	P-8	INPUT
Destination	P-8	OUTPUT	P-11	P-11
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	37.00	37.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1.18	1.14	990.69	1.18
Total Enthalpy (kW-h)	14.61	25.11	162.83	107.93
Specific Enthalpy (kcal/kg)	6.05	10.45	37.10	6.05
Heat Capacity (kcal/kg-°C)	0.24	0.24	1.00	0.24
Component Flowrates (kg/batch)				
Biomass	0.00	0.00	24.43	0.00
Carb. Dioxide	0.00	57.23	0.00	0.00
Impurities	0.00	0.00	5.13	0.00
MAB	0.00	0.00	3.07	0.00
Media	0.00	0.00	0.76	0.00
Nitrogen	1,593.90	1,597.37	0.00	11,775.44
Oxygen	483.88	413.39	0.00	3,574.80
SerumFree Media	0.00	0.00	17.89	0.00
Water	0.00	0.00	72.11	0.00
WFI	0.00	0.00	3,653.09	0.00
TOTAL (kg/batch)	2,077.77	2,067.99	3,776.49	15,350.24
TOTAL (L/batch)	1,761,985.22	1,813,091.25	3,811.96	13,017,263.35

Stream Name	S-131	S-132	S-133	S-134
Source	P-14	P-15	P-11	P-11
Destination	P-11	P-11	OUTPUT	P-16
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	37.00	37.00
Pressure (bar)	10.13	1.01	1.01	1.01
Density (g/L)	994.70	994.70	1.17	993.12
Total Enthalpy (kW-h)	295.32	145.14	273.00	835.13
Specific Enthalpy (kcal/kg)	25.11	25.11	15.80	37.09
Heat Capacity (kcal/kg-°C)	1.00	1.00	0.24	1.00
Component Flowrates (kg/batch)				
Biomass	0.00	0.00	0.00	957.07
Carb. Dioxide	0.00	0.00	1,888.47	0.00
Impurities	0.00	0.00	0.00	100.78
MAB	0.00	0.00	0.00	194.38
Media	0.00	0.00	0.00	0.76
Nitrogen	0.00	0.00	11,792.99	0.00
Oxygen	0.00	0.00	1,189.66	0.00
SerumFree Media	172.96	2,486.76	0.00	286.23
Water	0.00	0.00	0.00	1,746.08
WFI	9,947.04	2,486.76	0.00	16,086.90
TOTAL (kg/batch)	10,120.00	4,973.52	14,871.11	19,372.20
TOTAL (L/batch)	10,173.88	5,000.00	12,752,198.89	19,506.43

Stream Name	S-127	S-128	S-130	S-141
Source	INPUT	INPUT	P-13	P-18
Destination	P-13	P-13	P-15	P-19
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	42.38
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	994.70	994.70	994.70	988.37
Total Enthalpy (kW-h)	72.57	72.57	145.14	644.86
Specific Enthalpy (kcal/kg)	25.11	25.11	25.11	42.47
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
Impurities	0.00	0.00	0.00	71.51
MAB	0.00	0.00	0.00	137.92
Media	0.00	0.00	0.00	0.54
SerumFree Media	0.00	2,486.76	2,486.76	203.09
Water	0.00	0.00	0.00	1,238.88
WFI	2,486.76	0.00	2,486.76	11,413.97
TOTAL (kg/batch)	2,486.76	2,486.76	4,973.52	13,065.90
TOTAL (L/batch)	2,500.00	2,500.00	5,000.00	13,219.64

Stream Name	S-142	S-143	S-136	S-137
Source	P-19	P-19	P-16	P-16
Destination	OUTPUT	P-20	OUTPUT	P-17
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	20.00	42.37	20.00	37.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1.20	988.37	1.20	993.12
Total Enthalpy (kW-h)	0.09	644.75	0.13	835.10
Specific Enthalpy (kcal/kg)	4.84	42.46	4.84	37.09
Heat Capacity (kcal/kg-°C)	0.24	1.00	0.24	1.00
Component Flowrates (kg/batch)				
Biomass	0.00	0.00	0.00	957.07
Impurities	0.00	71.51	0.00	100.78
MAB	0.00	137.92	0.00	194.38
Media	0.00	0.54	0.00	0.76
Nitrogen	12.03	0.00	17.72	0.00
Oxygen	3.65	0.00	5.38	0.00
SerumFree Media	0.00	203.09	0.00	286.23
Water	0.00	1,238.88	0.00	1,746.08
WFI	0.00	11,413.97	0.00	16,086.90
TOTAL (kg/batch)	15.68	13,065.90	23.10	19,372.20
TOTAL (L/batch)	13,077.41	13,219.60	19,261.74	19,506.42

Stream Name	S-139	S-138	S-140	S-144
Source	P-17	P-17	P-18	INPUT
Destination	P-18	OUTPUT	OUTPUT	P-20
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	42.38	42.38	42.38	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	988.46	997.13	1,019.19	1,030.00
Total Enthalpy (kW-h)	646.69	309.29	1.83	1,373.79
Specific Enthalpy (kcal/kg)	42.47	42.45	42.41	24.96
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	0.99
Component Flowrates (kg/batch)				
Biomass	19.14	937.93	19.14	0.00
EDTA Disodium	0.00	0.00	0.00	94.70
Impurities	71.60	29.18	0.10	0.00
MAB	138.11	56.27	0.19	0.00
Media	0.54	0.22	0.00	0.00
SerumFree Media	203.37	82.86	0.28	0.00
Sodium Chloride	0.00	0.00	0.00	47.35
TRIS Base	0.00	0.00	0.00	47.35
TRIS HCl	0.00	0.00	0.00	142.06
Water	1,240.59	505.49	1.71	0.00
WFI	11,429.71	4,657.18	15.74	47,020.49
TOTAL (kg/batch)	13,103.06	6,269.14	37.16	47,351.95
TOTAL (L/batch)	13,256.10	6,287.20	36.46	45,972.77

Stream Name	S-145	S-146	S-147	S-148
Source	INPUT	INPUT	INPUT	P-20
Destination	P-20	P-20	P-20	P-21
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.00	25.00	25.12
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	1,030.00	1,010.00	1,005.00	994.93
Total Enthalpy (kW-h)	1,648.55	1,350.83	807.62	546.67
Specific Enthalpy (kcal/kg)	24.96	25.03	25.07	25.15
Heat Capacity (kcal/kg-°C)	0.99	1.00	1.00	1.00
Component Flowrates (kg/batch)				
Acetic-Acid	0.00	278.59	0.00	111.44
EDTA Disodium	113.64	0.00	0.00	0.00
Impurities	0.00	0.00	0.00	4.29
MAB	0.00	0.00	0.00	124.13
Sodium Chloride	56.82	0.00	0.00	0.00
Sodium Citrate	0.00	0.00	49.90	0.00
TRIS Base	56.82	0.00	0.00	0.00
TRIS HCl	170.47	0.00	0.00	0.00
WFI	56,424.58	46,153.90	27,671.68	18,461.56
TOTAL (kg/batch)	56,822.34	46,432.49	27,721.58	18,701.41
TOTAL (L/batch)	55,167.32	45,972.77	27,583.66	18,796.71

Stream Name	S-149	S-150	S-158	S-151
Source	P-20	P-21	P-23	P-22
Destination	OUTPUT	P-22	P-22	OUTPUT
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	26.31	25.12	25.38	20.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	996.60	994.93	994.60	1.20
Total Enthalpy (kW-h)	5,278.87	546.62	110.74	0.15
Specific Enthalpy (kcal/kg)	26.30	25.15	25.48	4.84
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	0.24
Component Flowrates (kg/batch)				
Acetic-Acid	167.16	111.44	2.94	0.00
EDTA Disodium	208.35	0.00	0.00	0.00
Impurities	67.22	4.29	0.21	0.00
MAB	13.79	124.13	120.40	0.00
Media	0.54	0.00	0.00	0.00
Nitrogen	0.00	0.00	0.00	20.42
Oxygen	0.00	0.00	0.00	6.20
SerumFree Media	203.09	0.00	0.00	0.00
Sodium Chloride	104.17	0.00	0.00	0.00
Sodium Citrate	49.90	0.00	0.00	0.00
TRIS Base	104.17	0.00	0.00	0.00
TRIS HCl	312.52	0.00	0.00	0.00
Water	1,238.88	0.00	0.00	0.00
WFI	170,223.06	18,461.56	3,615.99	0.00
TOTAL (kg/batch)	172,692.85	18,701.41	3,739.54	26.62
TOTAL (L/batch)	173,281.79	18,796.71	3,759.84	22,192.53

Stream Name	S-152	S-156	S-157	S-153
Source	P-22	P-22	INPUT	INPUT
Destination	P-24	P-23	P-23	P-23
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.38	25.12	25.00	25.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	994.60	994.93	994.70	994.70
Total Enthalpy (kW-h)	110.75	546.65	218.25	29.03
Specific Enthalpy (kcal/kg)	25.48	25.15	25.11	25.11
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	1.00
Component Flowrates (kg/batch)				
Acetic-Acid	2.94	111.44	0.00	0.00
Impurities	0.21	4.29	0.00	0.00
MAB	120.40	124.13	0.00	0.00
WFI	3,615.99	18,461.56	7,478.87	994.70
TOTAL (kg/batch)	3,739.54	18,701.41	7,478.87	994.70
TOTAL (L/batch)	3,759.85	18,796.72	7,518.69	1,000.00

Stream Name	S-155	S-154	S-159	S-160
Source	P-23	P-23	INPUT	P-24
Destination	OUTPUT	OUTPUT	P-24	OUTPUT
Stream Properties				
Activity (U/ml)	0.00	0.00	0.00	0.00
Temperature (°C)	25.00	25.38	25.00	20.00
Pressure (bar)	1.01	1.01	1.01	1.01
Density (g/L)	994.70	994.78	994.70	1.20
Total Enthalpy (kW-h)	29.03	663.18	0.02	0.02
Specific Enthalpy (kcal/kg)	25.11	25.43	25.11	4.84
Heat Capacity (kcal/kg-°C)	1.00	1.00	1.00	0.24
Component Flowrates (kg/batch)				
Acetic-Acid	0.00	108.50	0.00	0.00
Impurities	0.00	7.80	0.00	0.00
Nitrogen	0.00	0.00	0.00	3.40
Oxygen	0.00	0.00	0.00	1.03
polysorbate 80	0.00	0.00	0.37	0.00
WFI	994.70	22,324.44	0.37	0.00
TOTAL (kg/batch)	994.70	22,440.74	0.75	4.44
TOTAL (L/batch)	1,000.00	22,558.44	0.75	3,698.04

Stream Name	S-161	S-162
Source	P-24	P-25
Destination	P-25	OUTPUT
Stream Properties		
Activity (U/ml)	0.00	0.00
Temperature (°C)	25.38	25.38
Pressure (bar)	1.01	1.01
Density (g/L)	994.60	994.60
Total Enthalpy (kW-h)	110.75	110.75
Specific Enthalpy (kcal/kg)	25.48	25.48
Heat Capacity (kcal/kg-°C)	1.00	1.00
Component Flowrates (kg/batch)		
Acetic-Acid	2.94	2.94
Impurities	0.21	0.21
MAB	120.40	120.40
polysorbate 80	0.37	0.37
WFI	3,616.36	3,616.36
TOTAL (kg/batch)	3,740.29	3,740.29
TOTAL (L/batch)	3,760.59	3,760.59

4. OVERALL COMPONENT BALANCE (kg/batch)

COMPONENT	INITIAL	INPUT	OUTPUT	FINAL	IN-OUT
Acetic-Acid	0.00	278.59	278.59	0.00	0.00
Biomass	0.00	0.00	957.07	0.00	- 957.07
Carb. Dioxide	0.00	0.00	1,947.59	0.93	- 1,948.52
EDTA Disodium	0.00	208.35	208.35	0.00	0.00
Impurities	0.00	0.00	104.50	0.00	- 104.50
MAB	0.00	0.00	190.66	0.00	- 190.66
Media	0.00	4.68	0.76	0.00	3.92
Nitrogen	102.56	13,766.84	13,842.67	75.39	- 48.66
Oxygen	31.14	4,179.35	1,740.36	21.94	2,448.18
Phosphoric Acid	0.00	555.25	555.25	0.00	0.00
polysorbate 80	0.00	0.37	0.37	0.00	0.00
SerumFree Media	0.00	2,766.19	286.23	0.00	2,479.96
Sodium Chloride	0.00	104.17	104.17	0.00	0.00
Sodium Citrate	0.00	49.90	49.90	0.00	0.00
Sodium Hydroxid	0.00	236.39	236.39	0.00	0.00
TRIS Base	0.00	104.17	104.17	0.00	0.00
TRIS HCl	0.00	312.52	312.52	0.00	0.00
Water	0.00	5,987.91	7,733.99	0.00	- 1,746.08
WFI	0.00	262,853.82	262,853.82	0.00	0.00
TOTAL	133.70	291,408.52	291,507.39	98.26	63.43
				Overall Error:	0.022%

5. EQUIPMENT CONTENTS

TFR-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-1	START	0.00	0.00	0.00
P-1	HOLD-1 (Holding)	1.00	0.00	0.00
P-1	CHARGE-1 (Charge)	1.50	3.62	0.00
P-1	REACT-1 (Batch Stoich. Reaction)	97.50	3.63	0.00
P-1	TRANSFER-OUT-1 (Transfer Out)	98.00	0.00	0.00

RBR-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-2	START	0.00	0.00	0.02
P-2	HOLD-1 (Holding)	1.00	0.00	0.02
P-2	TRANSFER-IN-1 (Transfer In)	98.00	3.63	0.02
P-2	CHARGE-1 (Charge)	98.50	15.10	0.02
P-2	REACT-1 (Batch Stoich. Reaction)	242.50	15.14	0.02
P-2	TRANSFER-OUT-1 (Transfer Out)	243.50	0.00	0.02

RBS-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-3	START	240.50	0.00	0.24
P-3	HOLD-1 (Holding)	242.50	0.00	0.24
P-3	TRANSFER-IN-1 (Transfer In)	243.50	15.14	0.24
P-3	CHARGE-1 (Charge)	244.50	58.98	0.24
P-3	REACT-1 (Batch Stoich. Reaction)	388.50	59.10	0.17(*)
P-3	TRANSFER-OUT-1 (Transfer Out)	389.00	0.00	0.17(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

RBS-102

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-4	START	386.50	0.00	0.71
P-4	HOLD-1 (Holding)	388.50	0.00	0.71
P-4	TRANSFER-IN-1 (Transfer In)	389.00	59.10	0.71
P-4	CHARGE-1 (Charge)	389.50	235.43	0.71
P-4	REACT-1 (Batch Stoich. Reaction)	533.50	235.89	0.46(*)
P-4	TRANSFER-OUT-1 (Transfer Out)	534.00	0.00	0.46(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

V-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-5	START	532.17	0.00	0.93
P-5	SIP-1 (In-Place-Steaming)	533.00	0.00	0.93
P-5	CHARGE-1 (Charge)	533.50	687.00	0.93
P-5	PULL-IN-1 (Pull In)	534.00	708.36	0.93
P-5	TRANSFER-OUT-1 (Transfer Out)	535.00	0.00	0.93
P-5	CIP-1 (In-Place-Cleaning)	536.83	0.00	0.93

DE-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-6	START	0.00	0.00	0.00
P-6	HOLD-1 (Holding)	0.50	0.00	0.00
P-6	FILTER-1 (Dead-End Filtration)	1.00	0.00	0.00

SBR-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-7	START	509.17	0.00	1.39
P-7	SIP-1 (In-Place-Steaming)	510.00	0.00	1.39
P-7	HOLD-1 (Holding)	534.00	0.00	1.39
P-7	TRANSFER-IN-1 (Transfer In)	535.00	708.36	1.39
P-7	TRANSFER-IN-2 (Transfer In)	534.00	944.25	1.39
P-7	FERMENT-1 (Batch Stoich. Fermentation)	678.00	945.52	0.27(*)
P-7	TRANSFER-OUT-1 (Transfer Out)	679.89	0.00	0.27(*)
P-7	CIP-1 (In-Place-Cleaning)	681.73	0.00	0.27(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

SBR-102

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-8	START	678.00	0.00	5.62
P-8	SIP-1 (In-Place-Steaming)	678.83	0.00	5.62
P-8	HOLD-1 (Holding)	702.00	0.00	5.62
P-8	TRANSFER-IN-1 (Transfer In)	679.00	2,840.67	5.62
P-8	TRANSFER-IN-2 (Transfer In)	679.89	3,786.18	5.62
P-8	FERMENT-1 (Batch Stoich. Fermentation)	823.89	3,811.96	1.09(*)
P-8	TRANSFER-OUT-1 (Transfer Out)	825.23	0.00	1.09(*)
P-8	CIP-1 (In-Place-Cleaning)	828.06	0.00	1.09(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

V-102

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-9	START	0.00	0.00	3.72
P-9	SIP-1 (In-Place-Steaming)	0.83	0.00	3.72
P-9	CHARGE-1 (Charge)	1.83	2,755.00	3.72
P-9	PULL-IN-1 (Pull In)	1.98	2,840.67	3.72
P-9	TRANSFER-OUT-1 (Transfer Out)	2.98	0.00	3.72
P-9	CIP-1 (In-Place-Cleaning)	4.81	0.00	3.72

DE-102

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-10	START	678.00	0.00	0.00
P-10	HOLD-1 (Holding)	678.50	0.00	0.00
P-10	FILTER-1 (Dead-End Filtration)	679.00	0.00	0.00

BR-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-11	START	798.06	0.00	28.75
P-11	SIP-1 (In-Place-Steaming)	798.89	0.00	28.75
P-11	HOLD-1 (Holding)	822.89	0.00	28.75
P-11	TRANSFER-IN-1 (Transfer In)	823.89	10,173.88	28.75
P-11	TRANSFER-IN-2 (Transfer In)	825.23	13,985.85	28.75
P-11	FERMENT-1 (Batch Stoich. Fermentation)	1,113.23	19,506.43	5.69(*)
P-11	TRANSFER-OUT-1 (Transfer Out)	1,114.56	0.00	5.69(*)
P-11	CIP-1 (In-Place-Cleaning)	1,116.39	0.00	5.69(*)

(*) Contains material in vapor phase other than Oxygen & Nitrogen

V-103

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-12	START	0.00	0.00	13.33
P-12	SIP-1 (In-Place-Steaming)	0.83	0.00	13.33
P-12	CHARGE-1 (Charge)	1.00	10,000.00	13.33
P-12	PULL-IN-1 (Pull In)	1.62	10,173.88	13.33
P-12	TRANSFER-OUT-1 (Transfer Out)	2.62	0.00	13.33
P-12	CIP-1 (In-Place-Cleaning)	4.45	0.00	13.33

V-104

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-13	START	822.39	0.00	6.55
P-13	SIP-1 (In-Place-Steaming)	823.23	0.00	6.55
P-13	CHARGE-1 (Charge)	824.23	2,500.00	6.55
P-13	CHARGE-2 (Charge)	825.23	5,000.00	6.55
P-13	PULL-OUT-1 (Pull Out)	1,113.23	0.00	6.55
P-13	CIP-1 (In-Place-Cleaning)	1,115.06	0.00	6.55

DE-103

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-14	START	822.39	0.00	0.00
P-14	HOLD-1 (Holding)	822.89	0.00	0.00
P-14	FILTER-1 (Dead-End Filtration)	823.89	0.00	0.00

DE-104

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-15	START	824.73	0.00	0.00
P-15	HOLD-1 (Holding)	825.23	0.00	0.00
P-15	FILTER-1 (Dead-End Filtration)	1,113.23	0.00	0.00

V-105

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-16	START	1,113.23	0.00	25.56
P-16	SIP-1 (In-Place-Steaming)	1,114.06	0.00	25.56
P-16	TRANSFER-IN-1 (Transfer In)	1,114.56	19,506.42	2.46
P-16	TRANSFER-OUT-1 (Transfer Out)	1,123.00	0.00	24.57
P-16	CIP-1 (In-Place-Cleaning)	1,124.83	0.00	24.57

DE-105

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-18	START	1,114.56	0.00	0.00
P-18	HOLD-1 (Holding)	1,115.06	0.00	0.00
P-18	FILTER-1 (Dead-End Filtration)	1,123.00	36.46	0.00
P-18	TRANSFER-OUT-1 (Transfer Out)	1,123.00	0.00	0.00

V-106

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-19	START	1,110.73	0.00	17.32
P-19	SIP-1 (In-Place-Steaming)	1,111.56	0.00	17.32
P-19	HOLD-1 (Holding)	1,114.56	0.00	17.32
P-19	TRANSFER-IN-1 (Transfer In)	1,123.00	13,219.60	1.64
P-19	TRANSFER-OUT-1 (Transfer Out)	1,133.59	0.00	16.37
P-19	CIP-1 (In-Place-Cleaning)	1,135.43	0.00	16.37

C-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-20	START	1,122.58	0.00	0.00
P-20	EQUILIBRATE-1 (Column Equilibration (Simplified))	1,133.41	0.00	0.00
P-20	HOLD-1 (Holding)	1,135.41	3,304.90	0.00
P-20	LOAD-1 (PBA Column Loading (Simplified))	1,133.59	0.00	0.00
P-20	WASH-1 (Column Wash (Simplified))	1,134.34	0.00	0.00
P-20	ELUTE-1 (Column Elution (Simplified))	1,134.97	0.00	0.00
P-20	REGENERATE-1 (Column Regeneration (Simplified))	1,135.22	0.00	0.00
P-20	CIP-1 (In-Place-Cleaning)	1,136.47	0.00	0.00

DE-106

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-21	START	1,123.43	0.00	0.00
P-21	HOLD-1 (Holding)	1,123.93	0.00	0.00
P-21	FILTER-1 (Dead-End Filtration)	1,134.97	0.00	0.00

V-107

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-22	START	1,123.93	0.00	24.63
P-22	SIP-1 (In-Place-Steaming)	1,124.76	0.00	24.63
P-22	HOLD-1 (Holding)	1,126.93	0.00	24.63
P-22	TRANSFER-IN-1 (Transfer In)	1,134.97	18,796.72	2.46
P-22	TRANSFER-OUT-1 (Transfer Out)	1,139.48	0.00	24.62
P-22	TRANSFER-IN-2 (Transfer In)	1,139.48	3,759.85	20.17
P-22	TRANSFER-OUT-2 (Transfer Out)	1,154.65	0.00	24.60
P-22	CIP-1 (In-Place-Cleaning)	1,156.48	0.00	24.60

DF-101

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-23	START	1,133.14	0.00	0.00
	AFTER AUTO-INIT	1,133.14	18,796.72	0.00
P-23	SIP-1 (In-Place-Steaming)	1,133.97	18,796.72	0.00
P-23	FLUSH-1 (Flush)	1,134.97	18,796.72	0.00
P-23	DIAFILTER-1 (Diafiltration)	1,139.48	3,759.84	0.00
P-23	CIP-1 (In-Place-Cleaning)	1,141.06	3,759.84	0.00
P-23	END	1,141.06	0.00	0.00

V-108

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-24	START	1,138.64	0.00	4.93
P-24	SIP-1 (In-Place-Steaming)	1,139.48	0.00	4.93
P-24	TRANSFER-IN-1 (Transfer In)	1,140.73	3,759.84	0.49
P-24	PULL-IN-1 (Pull In)	1,141.06	3,760.59	0.49
P-24	HOLD-1 (Holding)	1,142.56	3,760.59	0.49
P-24	TRANSFER-OUT-1 (Transfer Out)	1,157.98	0.00	0.49
P-24	CIP-1 (In-Place-Cleaning)	1,159.82	0.00	0.49

DE-107

Procedure	Operation	Time (in h)	Volume (in L)	Vapor (in kg)
P-25	START	1,142.06	0.00	0.00
P-25	HOLD-1 (Holding)	1,142.56	0.00	0.00
P-25	FILTER-1 (Dead-End Filtration)	1,157.98	0.00	0.00