The Production of Recombinant Factor VIII

Process Design Project – Final Report

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1. INTRODUCTION

1.1 Product, Intended Use, and Unit Value

Our model simulates the manufacturing process for recombinant Human Blood Factor VIII (rFVIII). The rFVIII protein is a large glycoprotein composed of identical 195,000-molecular-weight subunits jointed by disulfide bonds. We have designed our product to be similar to Bayer's version of rFVIII, which has the brand name Kogenate-FS (KG-FS). This is a "second-generation" recombinant Factor VIII protein, which means it is not formulated with any human blood derivatives, though human blood derivatives are used as intermediary components in its manufacture.

This product is indicated for use as a blood clotting agent replacement therapy for classic hemophiliacs (hemophilia A). Hemophilia afflicts approximately one male in 10,000, with a market of approximately 40,000 hemophiliacs in the US, EU, and Japan.[1]

The specific activity of our product is approximately 4000 IU/mg protein.[2] The product is typically administered at a potency of 1000 IU/dose. Patients should take one dose (one vial equals one dose) per week and upon any incidence requiring blood clotting, such as a cut or bruise. The unit value of the product is $1000/vial.

1.2 Objectives

The primary objective of the facility is to manufacture rFVIII in a safe, reproducible, and efficient manner to create a product with the highest levels of purity, safety, stability, and efficacy. The specific objectives for this project include the following:

1.2.1 Unit Cost Target

The market value of pure Factor VIII protein is $4000/mg. Assuming a profit margin of 85%, our target production cost will be $600/mg.

1.2.2 Production Volume

Currently, demand for recombinant Factor VIII exceeds supply. Companies manufacturing rFVIII are able to sell as much of the protein as they can produce. The estimated production volume for this facility is based on the current market share owned by Bayer (~10-20%). The target production volume for a given year is 1,000,000 vials of purified rFVIII (1 billion IU). This equates to approximately 250 grams of pure rFVIII protein annually.

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[1] Note that many hemophiliacs go without regular treatment due to the high cost of the drug.

[2] IU is the international potency unit for measuring Factor VIII. The potency (IU) is determined using the one-stage clotting assay against the FDA Mega standard which was calibrated against WHO standard in IU. One IU, as defined by the WHO standard for blood coagulation FVIII is approximately equal to the level of FVIII activity found in 1 mL of fresh pooled human plasma.

1.2.3 Product Purity

The key product characteristic that needs to be achieved is potency. In addition, the product should have minimal contaminants and meet all product sterility requirements. The product will meet the following purity standards:

Table 1 – Final Product Purity Standards

Contaminant	Kogenate (Bayer Data, Average)
Host cell proteins	4 ng/1000 IU
Host cell DNA	3 pg/1000 IU
Recombinant human insulin	0.50 pg/1000 IU
Murine IgG	1.6 ng/1000 IU

1.3 Product Definition

1.3.1 Product Composition

Our product has the same formulation and purification standards as Kogenate-FS. It is formulated with sucrose (0.9–1.3% w/w), glycine (21–25 mg/mL), and histidine (18–23 mM) as stabilizers in the final container. The final product also contains calcium chloride (2–3 mM), sodium (27–36 mEq/L), chloride (32–40 mEq/L), polysorbate 80 (not more than [NMT] 35 µg/mL), imidazole (NMT 20 µg/1000 IU), tri-n-butyl phosphate (NMT 5 µg/1000 IU), and copper (NMT 0.6 µg/1000 IU). The product contains no preservatives. The amount of sucrose in each vial is 28 mg.[3]

Because of the complexity of the Factor VIII protein, we require the use of human albumin as a stabilizing agent. The albumin is added as a stabilizing agent between the ultrafiltration and diafiltration process steps. This stabilizing agent is necessary to provide the product with an acceptable stability and shelf-life. The addition of albumin to the product increases the risk of viral contamination; therefore our product will be formulated using as little albumin as possible. The albumin is removed during the final product formulation.

Currently, almost all commercial forms of recombinant Factor VIII are manufactured using some human blood derivative as a stabilizing agent. Only Baxter has developed a third-generation recombinant Factor VIII manufacturing process which does not incorporate any human blood derivatives.

1.3.2 Product Final Form/Image

The final product will be a lyophilized powder in vacuum-sealed, 5 mL vials. The product must be administered intravenously. The final product vials will be packaged with the following: sterile diluent (2.5 mL of WFI),[4] a sterile double-ended transfer needle, a sterile filter needle, and a sterile administration set.

[3] Composition information taken from Kogenate-FS product label.
[4] Sterile Water for Injection, USP.

1.3.3 Product Sensitivities

The Factor VIII protein is very sensitive to temperature, and must be kept cold throughout the manufacturing process. In accordance with the product license agreement for the production of Kogenate-FS, once the filtered fermentor intermediate (UFTCF) is removed from cold storage (-30°C), the purification process must be completed within 72 hours, after which time the purified product (UF/DF product) should again be frozen. Once the UF/DF product is removed from cold storage (-15°C), the product must be filled within 8 hours.

The final product should be stored under refrigeration (2-8°C; 36-46°F). Storage of the lyophilized powder at room temperature (up to 25°C or 77°F) for 2 months, such as in home treatment situations, may be done. The product should be protected from extreme exposure to light and freezing must be avoided.

2. PROCESS DESCRIPTION / EVALUATION

2.1 Process Overview

Our process uses a generic biotech production process, modeled specifically on Bayer's Factor VIII production facility in Berkeley, CA. Our overall process is divided into two main areas. The first is the continuous production area, which contains media production and fermentation. The downstream production is a batch processing area. The filtered fermentor intermediate (UFTCF) is purified by HPLC, and then sterile filtered. All purification, filtering, and formulating steps are performed at low temperatures due to the product's sensitivities. The purified product is then frozen and stored to await QA clearance. The product is then unfrozen, filled into vials, freeze-dried, and packaged.

2.2 Stoichiometry

The stoichiometry governing the cell culture growth, protein production, and product production was developed using the following assumptions and guidelines:

1. Glucose ($C_6H_{12}O_6$) and glutamine ($C_5H_{10}N_2O_3$) are the primary substrates. One mole of glucose fed is used as the basis for all of the stoichiometry calculations.
2. The amount of biomass produced per substrate used ($Y_{X/S}$) is set to 0.1 g biomass/g substrate.
3. The amount of total protein produced per biomass produced ($Y_{P/X}$) is set to 5 g total protein per 13 g biomass.

Based on these governing assumptions, the stoichiometry was determined by atom and electron balances to be as follows:

$$C_6H_{12}O_6 + 0.123\ C_5H_{10}N_2O_3 + 5.528\ O_2 \rightarrow$$

$$0.726\ CH_{1.77}O_{0.49}N_{0.24} + 5.601\ CO_2 + 5.709\ H_20 + 0.290\ CH_{1.83}O_{0.55}N_{0.25}$$

This calculated stoichiometry was modified slightly when modeled in SuperPro Designer. To achieve a mass balance, the stoichiometric coefficient of CO_2 was reduced to 5.561. The amount of actual product produced was accounted for in the SuperPro model by splitting the total protein

produced into Factor VIII (0.001 moles per mole of $C_6H_{12}O_6$) and non-product protein (0.289 moles per mole of $C_6H_{12}O_6$). This yields a $Y_{P/S}$ value of 0.04 g Factor VIII per gram substrate. The $Y_{P/S}$ value was determined to achieve a realistic yield for the fermentation process.

2.3 Continuous Processing

Please refer to Appendix 5.2 for the process flow diagram of this area.

2.3.1 Media Production

The baseline process uses the following media formulation:

Table 2 – Baseline Process Media Formulation

Ingredient Name	Flow Rate (kg/hr)	Mass Comp. (%)
Ammonium Sulfate	0.33200	0.3978
Calcium Chloride	0.08300	0.0995
Glucose	12.45000	14.9194
Glutamine	12.45000	14.9194
Recombinant Human Insulin	0.00001	0.00001
Potassium Chloride	0.08300	0.0995
Potassium Phosphate	0.20750	0.2487
Magnesium Sulfate	0.16600	0.1989
Murine IgG	0.03200	0.0383
Polysorbate	0.00064	0.0008
Sodium Citrate	0.08300	0.0995
Sodium Phosphate	0.12450	0.1492
WFI	57.43700	68.8292

This formulation was derived using a generic media formulation and adding specific components, such as Recombinant Human Insulin, that our research showed were necessary for the rFVIII production process. Murine IgG was added as part of the media, though in reality this material is a contaminant that is produced in very low amounts by the fermentation. It was added to the media (with no cost) to simplify the fermentation stoichiometry model.

The production process begins by dissolving the media mixture in WFI in an 880 L media tank (P-1-1). It is assumed that most of the components can be added in solid or concentrated liquid form. This process produces 83 L/hr of media.

The prepared media is then fed into a continuous sterilization process (P-1-2). In reality, this process would cause the degradation of some of our media components. The simulation does not allow for that, and a 100% yield of all media components from this sterilization process is assumed. This sterile media is continuously fed into two separate perfusion fermentors.

2.3.2 Perfusion Fermentation

Our recombinant technology relies on Baby Hamster Kidney (BHK) cells. The cell line is based on technology which was developed at Genentech and licensed to Bayer.

Sterile media is continuously fed into a pair of 127 L fermentors (P-1-5). The fermentors have a working volume of 100 L. We have also designed to allow a third fermentor to be available as a standby unit. Two fermentors are used as a way to hedge against the risk of contamination. Because of the continuous fermentation system, we expect an increase contamination risk and therefore believe that having more than one fermentor is prudent.

Based on Bayer's fermentor design, we assume that the optimal product production occurs when the system is processing 10 perfusion volumes/day. In total, we have a daily harvest of 1000 L from each fermentor. The harvest has a product titer of 0.957 mg/L.[5]

O_2 is supplied to the system by sparging pure oxygen gas into the bottom of the fermentor. This oxygen is first compressed (P-1-3), increasing its pressure to 6 bar. This high pressure O_2 is pumped through a sterile air filter (P-1-4) before it enters the fermentor. The fermentors have an aeration rate of 0.69 VVM. From the top of the fermentor, we collect the vent gas, which is 6.7% CO_2 and 93.3% O_2. The vent gas also goes through a sterile air filter (P-1-6) before being emitted to the environment. The oxygen uptake rate of the system is 0.54 kg-O_2/hr.

Based on Bayer's fermentor design, we assume that we can harvest 10 perfusion volumes/day. The units are continuous perfusion fermentors with cell recycle. The pre-harvest is pumped into a vibrating plate settler (P-1-7). The settler is used instead of a filter due to concerns about damaging cells. The flow from the bottom of the settler is fed back into the fermentor, while the top is harvested for filtration. The settler is designed so that 90% of the BHK cells are returned to the fermentor.

The stoichiometry for the fermentor reaction is given above in Section 2.2. In addition, a secondary reaction taking place in the fermentor to simulate cell death was added. This reaction converts BHK cells into host cell DNA and non-product protein. The reaction extent is set to 20% of the limiting component, which are the BHK cells.

Because the fermentation area is designed as a continuous process, we were unable to design the inoculation scale-up process. We simulated this by adding a small stream to feed BHK cells to the fermentor at a rate of 10 g-cell/hr. This allows us to estimate a cost for the inoculation process. With a purchase price of $29,166/kg, $2.129 million is spent annually to inoculate the fermentors.

After the ultrafiltration process, the processed harvest, called Ultrafiltered Clarified Tissue Culture Fluid (UFTCF), has the following composition:

[5] This titer is actually very high compared with the numbers in the literature. We expect a titer of 91 μg, but SuperPro is unable to handle concentrations that low.

Table 3 – Baseline Process Harvest Steam Composition

Ingredient Name	Flow Rate (kg/hr)	Mass Comp. (%)
Ammonium Sulfate	0.30286	0.4511
Calcium Chloride	0.07567	0.1128
Factor VIII	0.00007	0.0001
Glucose	10.84989	16.1703
Glutamine	11.30047	16.8418
Host Cell DNA	0.02282	0.0340
Recombinant Human Insulin	0.00001	0.000015
Potassium Chloride	0.07567	0.1128
Potassium Phosphate	0.18917	0.2819
Magnesium Sulfate	0.15134	0.2255
Murine IgG	0.02917	0.0435
Non-Product Protein	0.04464	0.0665
Polysorbate	0.00058	0.0009
Sodium Citrate	0.07567	0.1128
Sodium Phosphate	0.11350	0.1692
Water (including WFI)	43.86642	65.3768

The UFTCF is then frozen and stored to await clearance from Quality Assurance for sterility, activity, and additional testing. This process step is not included in the SuperPro model.

2.4 Batch Processing

Please refer to Appendix 5.3 for the process flow diagram of this area.

When the UFTCF is obtained from the continuous fermentation, it is frozen, and must be heated before further processing can take place. The first unit in the batch process flow diagram (P-2-1) serves to heat 2000 kg/batch of the UFTCF. This quantity is equivalent to approximately 30 hour's worth of the continuous fermentation product. At the exit of this unit, 25% of the UFTCF is sent to a waste stream. This represents the percentage of UFTCF that becomes contaminated and must be disposed of. This is a conservative estimate of the actual amount of UFTCF lost to contamination in a large scale continuous fermentation (based on knowledge of the Bayer process). The other 75% of the UFTCF (1500 kg/batch) is sent on to the purification section of the batch process. This area processes 245 batches/year.

2.4.1 Purification

The two main types of unit operations used in this section are chromatography columns and dead-end filters.

After the heating, the UFTCF is loaded into an Ion-Exchange Chromatography column (P-2-2), which uses diethylaminoethyl (DEAE) as the ion-exchange group. DEAE is a form of weak anion-exchange, and has a maximum capacity over the range pH 1-6. The purpose of this column is to remove the majority of contaminates in the UFTCF. To achieve a realistic loading time, the column size was set to 110 L with a binding efficiency of 95% and overall yield of 85% for the active protein. The resin was assumed to cost $400/L. In this step, the product stream is reduced

from 1500 kg/batch to 230 kg/batch with 19.3% loss of active protein. This step represents the most significant loss of active protein during the purification process.

A dead end filter (P-2-3) is used to simulate a viral inactivation step, which would filter out viruses if any were present in the UFTCF. For the purposes of this model, no virus entities were added to the system.

Next, the product stream is loaded into a Copper Immobilized Metal Ion Affinity Chromatography (CuIMAC) column (P-2-4). This column has a high affinity for the active protein (100% binding, 98% yield), and allows for further separation of the desired product from contaminates. The resin used was assumed to cost $6000/L and the column is 196 L in size. In this step, the product stream is decreased from 230 kg/batch to 196 kg/batch with 1.7% loss of active protein. A second affinity chromatography column (P-2-5) is used to concentrate the product further. The resin was assumed to cost $6000/L and the column is 32 L in size. In this step, the product stream is reduced from 196 kg/batch to 64 kg/batch with 2.5% loss of active protein.

The next step in the purification process is to pass the product stream through an ultrafiltration unit (P-2-6) to prepare the product stream for formulation.

2.4.2 Formulation

After protein purification, a variety of raw materials are required to be added to the active protein mixture to obtain the desired final formulation. This process is achieved through a variety of mixing, storage, and buffer exchange (diafiltration) steps. The primary objective of the following process steps is to obtain the appropriate product composition prior to filling and freeze-drying.

Albumin is added to the product stream in process step P-2-7 to stabilize the product prior to heat and chemical treatment. The addition of albumin creates a risk of viral contamination, but is considered necessary to maintain an acceptable product yield through the viral inactivation step. The second viral inactivation step (P-2-8), using the dead-end filtration method, represents the heat/chemical treatment of the product. The next step in the formulation process involves a buffer exchange in a diafiltration unit (P-2-9) with CaCl and NaCl to adjust the pH and to remove all albumin.

Next, the product is frozen in a blast freezer to -15˚C (P-2-10). This step is simulated using a storage tank, which cools the product. Theoretically, the product is held at this step to allow for Quality Assurance (QA) testing and release. Once the product receives QA approval, it is thawed and transferred to the next production area.

The frozen product is thawed and the final formulation is simulated by doing a buffer exchange in a diafiltration unit (P-2-11). Glycine, histidine, and sucrose are introduced in the formulation feed stream in the appropriate amounts to obtain the proper freeze-dried product composition. Now the product is in the correct concentration for dosage.

2.4.3 Filling/Freeze-Drying/Final Packaging

In reality, the filling of vials would take place after this final formulation step. Each vial would be filled with 2.75 mL of product, and then freeze dried and packaged. In the simulation, however, freeze drying (P-2-12) must come before the filling (P-2-13). In the freeze drying, 97%

of the water is evaporated from the product. The freeze-drying process has a total cycle time of 17 hours. We recognize that this cycle time is unrealistic and that a normal freeze drying cycle would be between 48 and 72 hours. The final form of the product consists of: one vial with 1000 IU rFVIII, one vial of sterile diluent, and one syringe. The Factor VIII vials are labeled, assembled with the above required items, and boxed. At the end of the batch, we have produced 3705 boxed entities. Each boxed entity has a market value of $1000.

3. PROCESS MODIFICATIONS / OPTIMIZATION

The baseline process was evaluated for opportunities for increasing productivity. The primary focus was to improve product quality, reduce raw material and capital costs, and optimize equipment usage. The productivity analysis was based on the cost breakdown, which is discussed in detail in Section 4.

In the continuous processing area, more than 70% of the operating costs ($27.6 million/year out of $38.9 million/year) are associated with the raw materials.[6] Glutamine, which is part of the media feed, is the major component for this cost (90% of the total raw material costs). Two optimization scenarios were evaluated to reduce the raw material costs. First, an evaluation of the savings associated with optimizing the media feed composition was performed. Second, an assessment of the impact of improving the cell productivity (i.e. increasing $Y_{P/S}$) on the overall costs was performed.

3.2 Media Reformulation

For this modified process, please refer to the SuperPro files, "250A_Batch_8_New Media.spf" and "250A_Continuous_8_New Media.spf."

An economic analysis of the continuous production area showed that the unit production cost of the UFTCF was $79.43/kg. Almost 71% of this cost is captured in raw material costs, of which glutamine was the highest contributor. The baseline model uses media that is 14.9% (w/w) glucose and 14.9% (w/w) glutamine. Very little of these components were actually consumed in the fermentation process. Therefore, the media formulation was optimized by reducing the concentration of glucose to 1.74% (w/w) and the concentration of glutamine to 1.20% (w/w). This modification reduced the unit production cost of UFTCF to $32.25/kg, for a savings of $47.18/kg.

The revised UFTCF was inputted into the batch production model to evaluate the impact on the final product cost. The unit production cost was reduced from $60.9 per boxed entity to $35.4 per boxed entity.

3.3 Modified Product Yield from Fermentation

For this modified process, please refer to the SuperPro files, "250A_Batch_9_New Yield.spf" and "250A_Continuous_9_New Yield.spf."

[6] It should be noted that the upstream operating costs ($38.9 mil) for this process far exceed the capital costs ($12.1 mil) of that area. This justifies the approach of focusing on reducing the primary contributor to the operating costs.

Another option to optimize the process is to alter the product yield from the fermentors. In this scenario, it is assumed that the product titer and the amount of total protein produced would remain constant, but that there would be a 10-fold increase in the specificity of the cells to produce rFVIII over non-product proteins. This alteration of the reaction stoichiometry caused the concentration of non-product protein in the UFTCF to decrease from 0.0665% (w/w) to 0.0111% (w/w), an 83% decrease in concentration. This change had a negligible effect on the unit production cost of UFTCF, with a savings of only $1.02/kg, but the increase purity of the UFTCF had a significant effect on downstream processing.

The impact of this change on the downstream process was evaluated. To account for the reduced non-product protein levels, the Factor VIII yield of the DEAE affinity was increased from 85% to 95% and one of the CuIMAC columns was removed. This resulted in reduced capital costs and an increased in active protein yield. With these changes, the final batch size increased from 3705 vials to 4153 vials and the unit production cost decreased from $60.9 per boxed entity to $52.2 per boxed entity. It should be noted that this process yield slightly over 1,000,000 boxed entities per year, which matches the project volume objective.

3.4 Alternative Productivity Ideas

The following productivity ideas were developed during the final review of the model. The economic feasibility of these ideas has not been fully explored and must be examined prior to implementation.

- Reduce Factor VIII product waste: A significant portion of Factor VIII is lost during the downstream processing. Most noticeable is that 25% of the incoming fermentation product is rejected prior to the start of purification. This is a considerable opportunity to improve product yields by determining the cause of these failures and implementing appropriate corrective actions. In addition, Factor VIII is lost in relatively high amounts during the first purification step due to the 85% yield for this column. This is another potential area for improving product yield.

- Optimize the batch size for downstream processing: In the current model, it was assumed that 30 hours worth of continuous fermentation product would be purified, filled, and packaged as one batch. However, using this assumption, the final batch size for the product is rather small (approximately 3705 vials or final units). While this batch size is appropriate for purification processes, the small batch size results in inefficient utilization of the filling, freeze-drying, and packaging unit operations as the actual line running time is a minuscule when compared to the time spent cleaning, sterilizing, and setting up the equipment. It may be feasible to increase the batch size for the post-purification processing areas to ensure better equipment utilization.

4. CONCLUSION

4.1 Economic Viability

The baseline process was evaluated for economic viability. The following changes were made to the SuperPro Designer given assumptions to reflect better the actual process costs:

1. The basic labor rate was increased from $25/hour to $40/hour.

2. The Laboratory/Quality Assurance/Quality Control costs were increased from 15% of total labor to 100% of total labor costs.

3. The electrical cost for unlisted equipment was increased from 5% to 10% in the batch processing area to account for the fact that the area is being kept at 5ºC.

4. The operating hours per year for the continuous process was set at 7300 hrs. The operating hours for the batch process was used to process all of the fermentation product within a given year. Since the continuous process is rate limiting, the batch process was only required to operate for 4200 hrs/yr.

Table 4 summarizes the key economic figures for the baseline process. The data demonstrates that the process is extremely financially viable as the revenue is nearly 10 times that of the capital investment and operating costs and the profit margin is 94%. This exceeds the initial target objective of 85% profit margin despite not achieving the target production volume of 1,000,000 entities per year.

Table 4 – Baseline Process Cost Breakdown

Category	Value
Capital Investment	$52.9 mil
Operating Cost	$55.2 mil / year
Revenue	$907.8 mil / year
Total Production Volume	907,848 boxed entities
Unit Production Cost	$60.9 / boxed entity

4.2 Optimal Process Configuration

From Section 3, it was demonstrated that the baseline process can be significantly improved. One major improvement was optimizing the media formulation to reduce the raw material costs, especially by reducing glutamine consumption. Another process improvement was to increase the specific productivity of the fermentation to produce more Factor VIII protein for a given substrate amount. This improvement reduced the unit production cost by approximately 15%, simplified the downstream purification process, and increased the overall facility output to above the target of 1,000,000 entities per year. The optimal process configuration would be to combine these two process improvements into one manufacturing process.

4.3 Critical Process Parameters

The critical process parameters are primarily associated with the continuous fermentation process. It is critical that the appropriate fermentation yield (with the proper Factor VIII concentration) is achieved on a consistent basis. This is critical as all of the downstream processing steps have been established based on this particular fermentation yield and concentration. Other critical process parameters are those associated with equipment and product sterility in the continuous processing area (such as SIP processing parameters). These parameters are critical as the level of contamination of the product from the fermentation was assumed to be 25%. These parameters need to be tightly controlled to hopefully reduce this amount of rejected product.

The processing parameters for the downstream equipment are important, but have less overall impact than that of the continuous process. This is primarily because the continuous process is the rate-limiting step and the batch processes are only being used for approximately 50% of the year.

4.4 Critical Costs

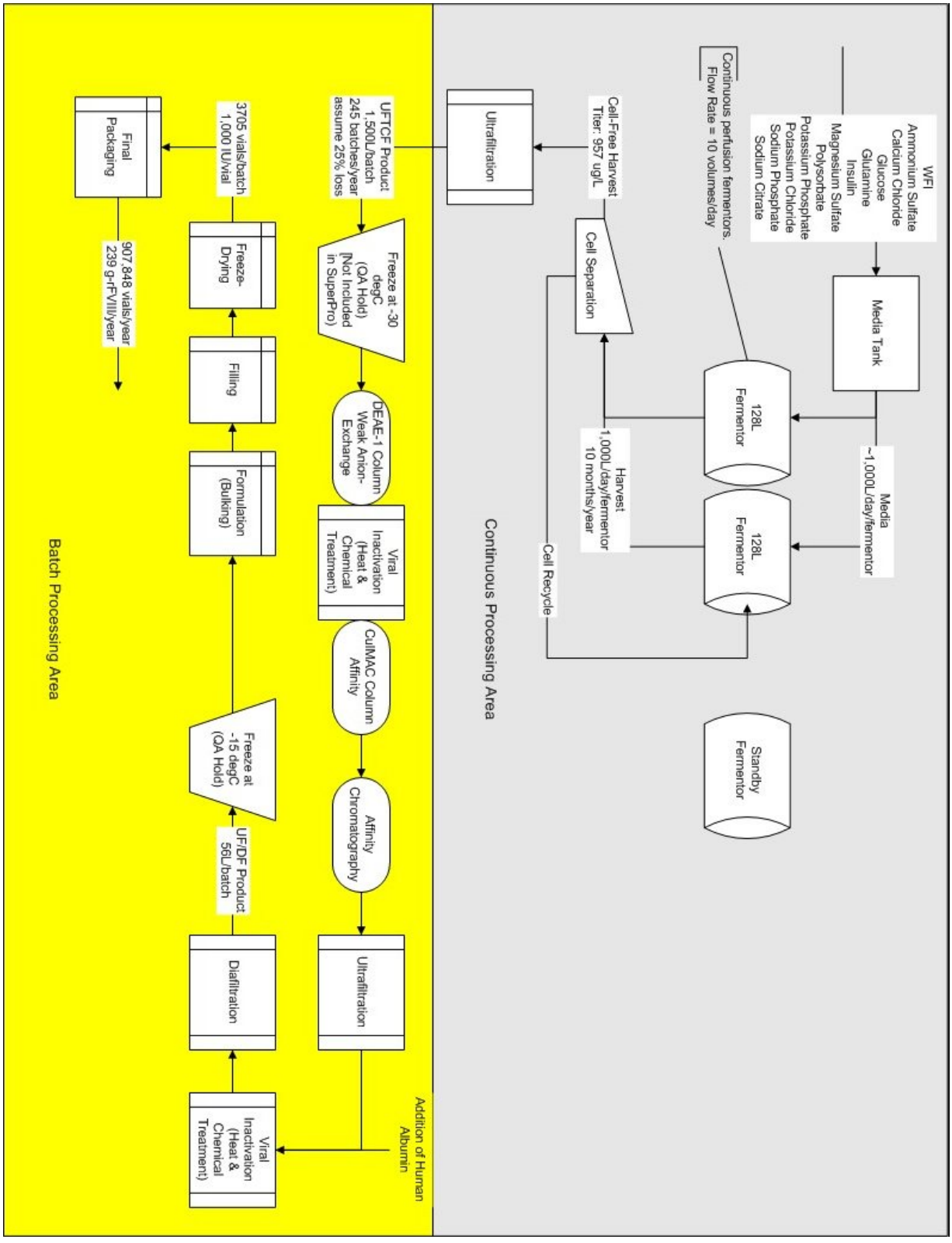
The critical costs for this manufacturing process are the raw material costs and the capital costs. The raw material costs consist of over 70% of the total operating costs ($55.2 mil/year). It has been demonstrated that these raw material costs can be drastically reduced by reformulating the media composition to reduce the glutamine level. The capital costs have been calculated to be approximately $52.9 million and primarily associated with the batch processing steps.

4.5 Future Development

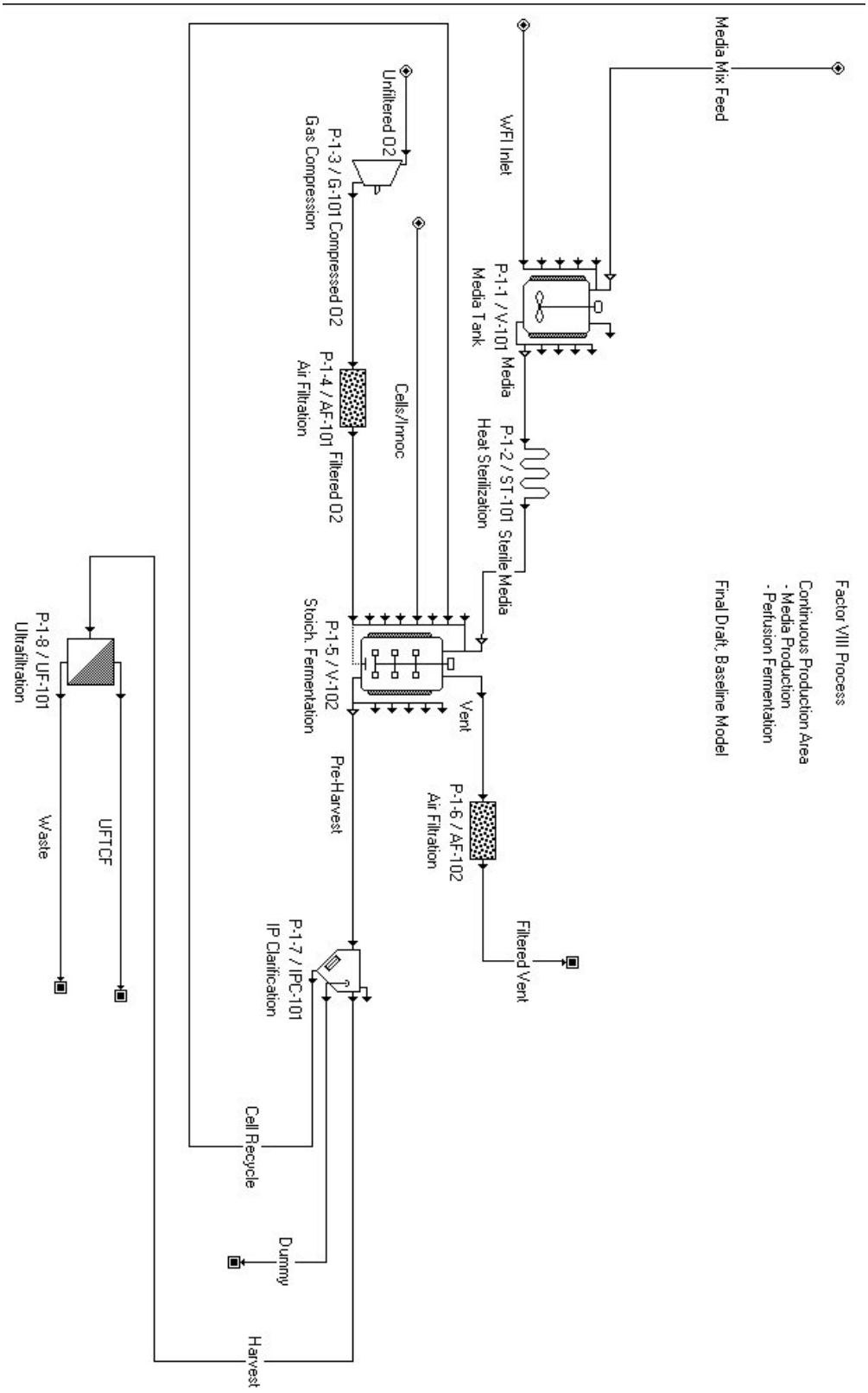
Two scenarios for future development have been evaluated. Both media reformulation and cell productivity optimization will reduce overall costs significantly. The media reformulation had a bigger overall impact and should be pursued first.

5. APPENDIX

5.1 Overall Process Flow Map



5.2 Continuous Processing Area, Baseline Process



5.3 Batch Processing Area, Baseline Process