To Analyze the Commercial Feasibility for

Establishing a Lactoferrin Production Plant from Cow Milk.

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BAEN 689

CONTENTS:

INTRODUCTION:

LACTOFERRIN:

Lactoferrin (LF) also known as Lactotransferrin (LTF) is a globular multifunctional protein with a molecular weight of 90,000Da. It is found in milk and many mucosal secretions such as tears, saliva. Human colostrum has the highest concentration of lactoferrin followed by human milk and then cow milk.

Lactoferrin is a multifunctional protein with a number of physiological roles. Lactoferrin is a multifunctional protein with a number of physiological possible roles. It is often referred to as an innate defense protein and frequently serves as the first line of defense in protection against pathogens. It has been shown to have the ability to bind iron, it is a natural anti-bacterial, anti-fungal and anti-viral, it is an antioxidant and it also has immunomodulatory properties. It has many beneficial properties, which make it a good candidate for a number of product applications. Considerable lactoferrin and glycoprotein research is currently going on to explain the various suggested biological functions of lactoferrin.

LACTOPEROXIDASE:

Lactoperoxidase is a glycoprotein consisting of a single peptide chain with a molecular weight of 78, 431 Da. The enzyme contains a haeme structure, with 1 iron molecule per mole of lactoperoxidase. The conformation of the protein is stabilized by a strongly chelated calcium ion.

Lactoperoxidase has been identified as an antimicrobial agent in milk, saliva and tears. It is a natural bacterial defence system through the oxidation of thiocyanate ions (SCN^-) by hydrogen peroxide. LPO has proven to be both bactericidal and bacteriostatic to a wide variety of microorgnisms, while having no effect on the proteins and enzymes of the organisms producing it (Ekstrand (1994.)

Milk Component	Percentage in milk
Ash	0.8110
BSA	0.0720
Casein Protein	2.9100
IgG	0.1090
LF	0.0145
LPO	0.0036
Lactose	5.0050
Water	91.0749

Table 1: Skim Milk composition

Since, the human milk cannot be used as a raw material to extract out lactoferrin and lactoperoxidase; it is extracted out of the bovine milk. Bovine milk is easily available in large quantities and is cheap. **Table 1** shows the composition of skim bovine milk. The current process report discusses the purification and production of LF and LPO at industrial scale.

Lately, a lot of attempts have been made to express the gene for human LF in plants such as rice (Somen Nandi, Dorice Yalda, Stephen Lu, Zivko Nikolov, Ryo Misaki, Kazuhito

Fujiyama & Ning Huang). These processes have shown success at the lab scale but no commercialization of such technology has been done yet.

SUPERPRO DESIGNER™:

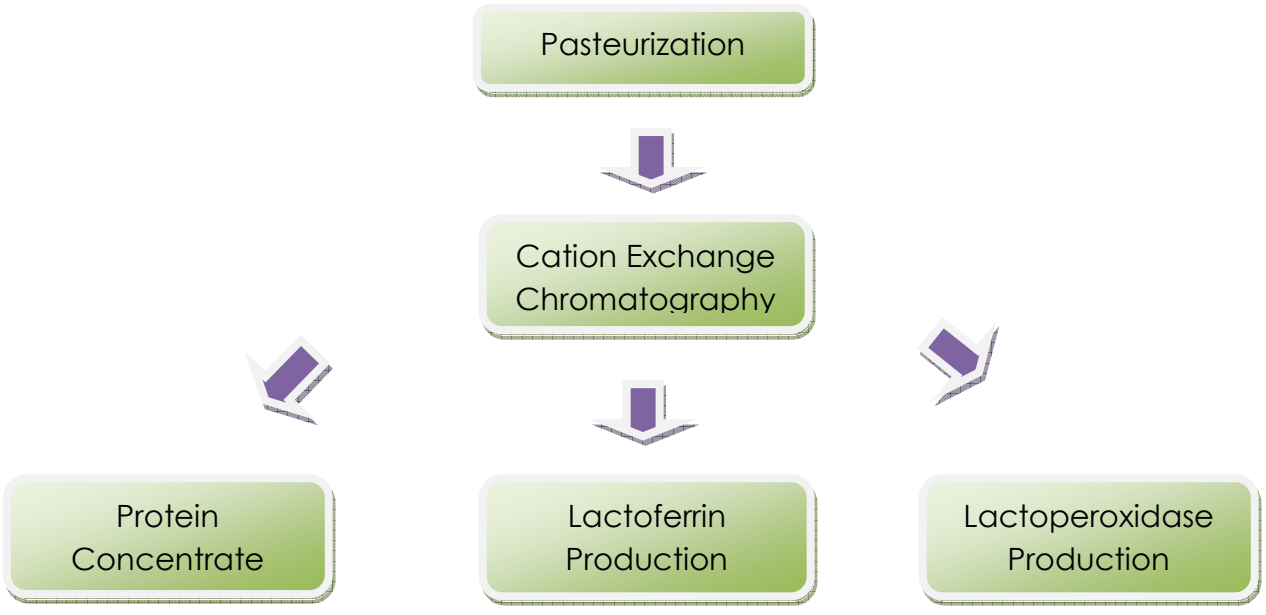
The software used for doing this project was SuperPro Designer™. This software is a process simulator that enables the user to readily analyze and represent integrated processes. SuperPro Designer™, an extention of BioPro, was created in to address the need of synthetic pharmaceutical, agro and food processing industries.it handles material balancing, equipment sizing and costing, economic evaluation, environmental impact assessment, process scheduling and debottlenecking of batch and continuous processes.

This software is a registered trade mark and is owned by the Intelligen company. They are situated at 2326, Morse Avenue, Scotch Plains, NJ 07076, USA.

PROCESS DESCRIPTION:

The entire flow sheet for the production of LF (and LPO and Protein conc.) is shown in **figure 1**. It is divided into 5 sections:

1. **Pasteurization**: This step involves the unit operations of storage and blending, heat sterilization and cooling. The details of the process are given in the subsequent sections.
2. **Cation exchange chromatography:** This step involves the unit operation of cation exchange chromatography. Lactoferrin and Lactoperoxidase get bound to the resin due to their positive charge, the resin being a cation exchange resin. Details of the operation are given in the subsequent sections
3. **Lactoperoxidase production:** This step involves the unit operations of concentration, diafiltration and spray drying. The product stream is called the LPO production stream. Details of the operation are given in the subsequent sections.
4. **Lactoferrin production:** This step involves the unit operations of concentration, diafiltration and spray drying. The product stream is called the LF production stream. Details of the operation are given in the subsequent sections
5. **Protein concentrate production:** This step involves the unit operations of concentration, diafiltration and spray drying. The product stream is called the Protein conc. stream. Details of the operation are given in the subsequent sections.



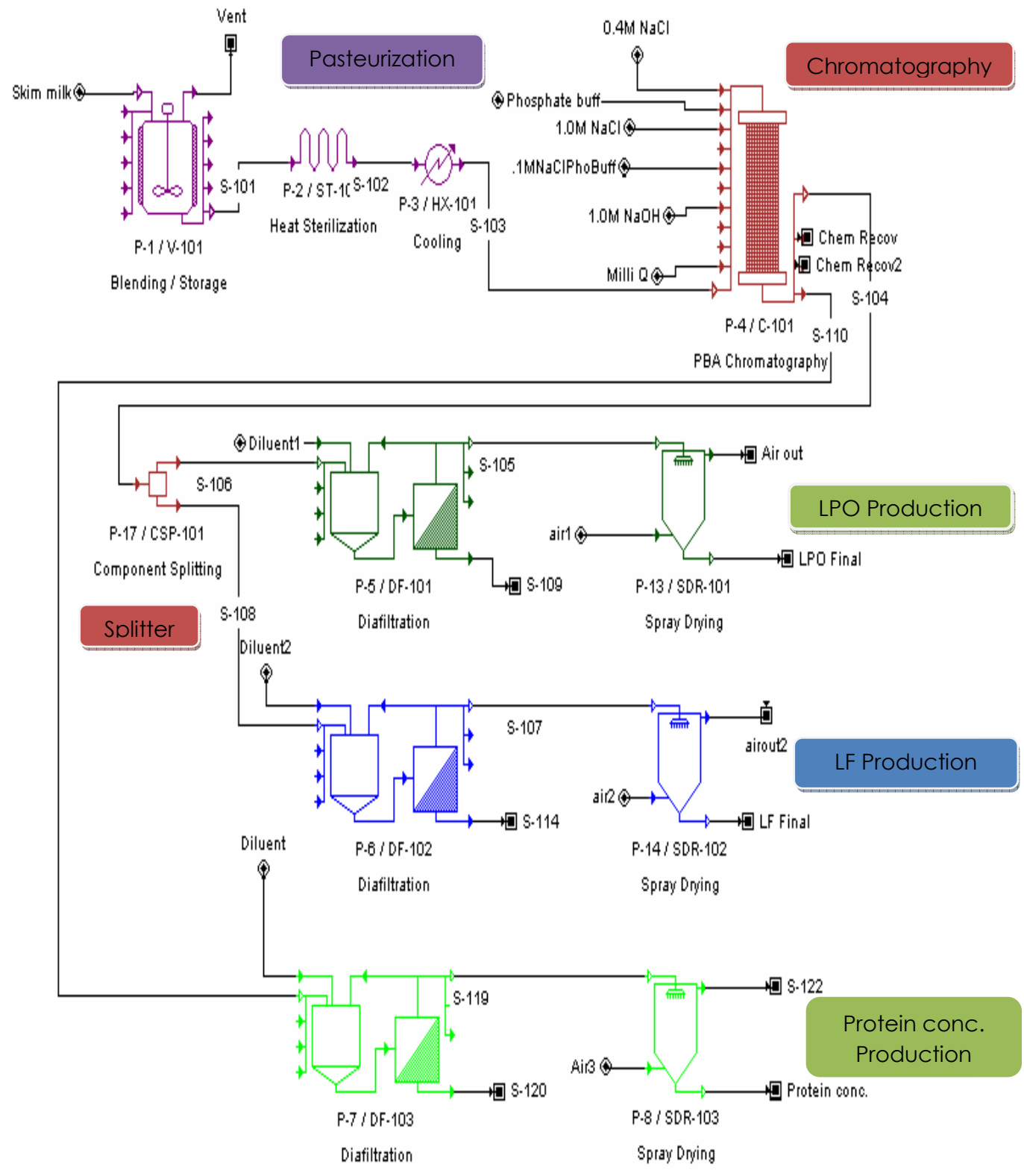


Figure 1: Overall process diagram

UNIT OPERATIONS:

STORAGE:

A blending and storage tank was selected for receiving and storing the milk.

- **Advantages:**
  - It prevents the settling down of milk components and keeps it homogenous.
  - Mixing also helps in keeping the temperature constant at a suitable level to keep the microbial growth at a minimal level.

In the process, only one blending tank is shown, but in reality we may require a stand by unit also. This will help in storing the milk if the downstream process is stopped because of some unforeseen circumstances.

PASTEURIZATION:

Owing to its composition, milk is a nutritious media for microbes to grow. Pasteurization step is required to sterilize the milk to prevent its spoiling. Pasteurization, now a days is a High Temperature Short Time (HTST) process. Since the heating is for a few seconds only, the proteins do not get denatured. **Table 2** shows the conditions for sterilization.

- **Advantages:**
  a. It prevents the spoiling of milk.
  b. It keeps the microbial load in product low, so that the instruments in subsequent operations do not get clogged with microbes.

Sterilization temperature	140C
Preheat outlet temperature	110C
Final exit temperature	35C
Heating agent	Steam
Cooling agent	Cooling water

Table 2: Pasteurization conditions

Pasteurization is often used as an energy efficient process. The milk at lower temperature coming into the pasteurizer is cross flowed with the pasteurized milk in a heat exchanger. This results in raising the temperature of the incoming milk and also decreasing the temperature of the outgoing milk. This saves money both ways.

COOLING:

The equipment used for cooing down the milk to 35C was a Shell& Tube type heat exchanger. This can also be used as an energy efficient step by cross-flowing the milk to unpasteurized milk. The cooling agent used was chilled water. The inlet and outlet temperatures of the milk were set to be 35C & 25C respectively.

CATION EXCHANGE CHROMATOGRAPHY:

The ion exchange chromatography is based on the principle of electrostatic attraction. Opposite charges attract each other. The LF and LPO are cationic proteins. So when milk is passed through an anionic column at a pH below the iso-electric points of LF and LPO, they bind to the column while rest of the proteins and other constituents pass through the column. The bound proteins are eluted out by using suitable solvent which has a higher concentration of cations that the proteins. The eluted proteins go into the product stream while rest of the constituents of the milk goes to other stream to be treated for the purification of milk protein concentrate.

The details of the column used are as follows:

a. Type of resin: dft PBA chrom resin
b. Binding capacity: 48.6 mg/ml
c. Material used: SS316. This material is costly but durable and hence saves cost in long run. Also, being stainless steel it is GMP compliant too.

Different steps involved in the chromatography step are as follows:

a. **Equilibration:** The column used is equilibrated before using it. This gives a proper pH and charge to the matrix to activate it. The buffer used for equilibration was phosphate buffer. 2 bed volumes (BV) of buffer were passed through the column at a linear velocity of 300cm/hr.

b. **Load:** The milk coming from the cooling step is passed loaded on the chromatography column. The conditions used for loading the sample are shown in table 3 :

Linear velocity	300 cm/hr
Resin binding capacity	95% (LF, LPO)
Yield Percent	90%

Table 3: Load conditions

c. **Wash:** The washing step is done to remove any unbound impurities which are still there in the column. The conditions used for wash step are shown in table 4:

Conditions	**Wash 1**	**Wash 2**
Buffer	Phosphate buffer	0.1M NaCl phosphate buffer
Linear Velocity	300cm/hr	300cm/hr
Wash volume	2	2

Table 4: Wash conditions

d. **Elution:** As previously mentioned, elution is a step to separate the bound proteins from the matrix. In the procedure, isocratic elution was used. Two different eluents were used because LPO and LF have different binding strength and different pI. LPO was eluted using 0.4M NaCl soln. and LF was eluted using 1.0M NaCl soln. The conditions for elution are shown in table 5:

Conditions	LPO	LF
Buffer	0.4M NaCl solution	1.0M NaCl solution
Linear Velocity	300cm/hr	300cm/hr
Wash volume	20	20

Table 5: Elution conditions for LF & LPO

e. **Column regeneration:** This step is done to regenerate the column and bring it back to the original state to reuse it. Ideally, this step should any bound unbound impurities form the column but over a period of time the quality of the column deteriorates because of the adsorption of impurities from the previous purifications which could not be removed by the regeneration step.

Regeneration involves passing a series of solutions from the matrix. The conditions for regeneration are shown in table 6:

Conditions	Regeneration 1	Regeneration 2	Regeneration 3	Regeneration 4
Buffer/Soln.	1.0M NaCl soln.	1.0M NaOH soln.	Deionized water	Phosphate buffer
Linear Velocity	300 cm/hr	300 cm/hr	300 cm/hr	300 cm/hr
Wash volume	5	2	10	5

Table 6: Column Regeneration conditions

SPLITTER:

This is an imaginary unit operation which represents those processes which can not be simulated by the SuperPro software. In the procedure, LPO eluted out first and then LF and there was no mixing of these two. Since there was only one product outlet and the software could not simulate time based separation, a splitter was used. This splitter represents that all the LPO had been separated into the upper stream and all the LF into the lower one, while water and salts were divided equally into the two streams.

CONCENTRATION & DIAFILTRATION:

This step was used for the concentration of product and removal of salts from the stream. The membrane used for this step was dft- membrane. The conditions for diafiltration and concentration are shown in table 7:

Property	LPO stream	LF stream	Protein conc. stream
Membrane area (m^2)	7.63	10.476	79.71
Concentration stages	5	5	2
Concentration factor	2	1.7	1.12
Active product	LPO	LF	IgG
Rejection coeff.	0.9	0.9	0.9

Table 7: Conditions for Concentration & Diafiltration in the LPO, LF & Protein conc. stream

SPRAY DRYING:

This step was used to remove the water content of the product stream. Spray drying is a cheaper way of reducing the water content of products if the products are not highly temperature sensitive. The conditions for spray drying are shown in table 8:

Property	LPO stream	LF stream	Protein conc. stream
Drying capacity (kg/hr)	100	100	26424
Water removal (%)	99.9	99.9	99.7
Water content (% wb) (product)	5.15	3.67	8.46

Table 8: Spray Dryer conditions

MATERIAL BALANCE:

According to the mass balance laws, what goes into the process should come out in one or another form. In normal conditions, mass can neither be created nor destroyed. The following table (table 9) shows the overall mass balance for all the components going and coming out of the system in different streams.

Component	In	Out	Out-In
Ash	811	811	0
Air	889448.28	889448.28	0
BSA	72	72	0
Casein	2910	2910	0
IgG	109	109	0
Lactoferrin	14.5	14.5	0
Lactoperoxidase	3.6	3.6	0
Lactose	5005	5005	0
NaCl	1464.71	1464.71	0
Na2HPO4	9.48	9.48	0
NaH2PO4	7.14	7.14	0
NaOH	65.61	65.61	0
Water	427275.69	427275.69	0

Table 9: Overall material balance for the process

YIELD:

It is the ratio of amount of product produced to the amount of product present in the feed. It is a good criterion to check the efficiency of the process.

Product	Amount in feed	Amount in product	Yield%
LF	14.50	8.60	59.33
LPO	3.60	1.96	54.70

Table 10: Yield of the overall process for LF &LPO

SCHEDULING:

The overall process is taking ~ 33hr per batch. Minimum cycle time is 29.09 hrs. The total number of batches possible in a year is 268. The scheduling description of operations, equipments is given in the attached tables at the end of the report.

Chromatography was the longest step in the overall procedure and is acting as a bottleneck. Using two or three chromatography columns in parallel may help in reducing the time but will have an effect on the overall cost of the procedure. Since, the number of batches and hence the production can be increased by using more columns in parallel, this can offset the expenditure of having more than one column.

PRODUCTION:

The following table shows the production level of the plant and the purity of the product obtained. At this purity level, LF and LPO can be used in the supplementary baby foods.

Product	Amount of product (kg/batch)	Amount of product (kg/yr)	Purity (%)
LF	8.60360	2,602.00	88.63
LPO	1.97	652.00	80.92
Protein conc.	5,635.5	1,679,869.00	90.05

Table 11: Overall production level per batch, per year and the purity percentage

ECONOMIC EVALUATION:

The economic evaluation of the overall process was done for 100,000kg/batch feed rate. A summary of the entire is given here with each section discussed in detail later.

Total capital investment	**59,188,000.00 $**
Total capital investment charged to this project	59,188,000.00 $
Operating cost	56,943,000.00 $/yr
Production rate	2601.90 kg MP/yr
Unit production cost	21,887.95 $/kg MP
Total revenues	88,806,000 $/yr
Gross margin	34.40 %
Return on investment	38.80 %
Payback time	2.58 yr

Table 12: Summary of Economic evaluation

Equipment costs:

It was assumed that all the equipments were purchased new and there was no purchasing cost depreciation on the equipments when they were purchased. Please see the attached table for equipments costs. A total cost of $8,847,000 needs to be spent on the equipments. The breakdown of the equipment cost is given in figure 2.

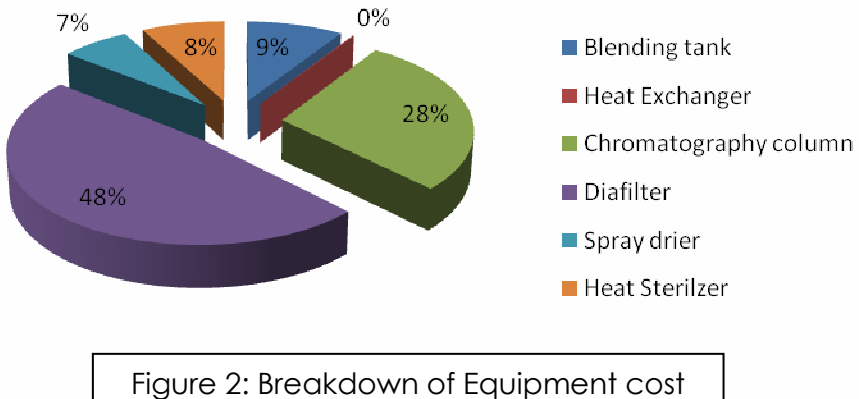


Figure 2: Breakdown of Equipment cost

Fixed capital estimate summary: Please see the attached table in the appendix for the summary of fixed capital estimate. A breakdown of the total plant cost is represented pictorially in figure 3. A total of $ 46,200,000 was estimated by the SuperPro.

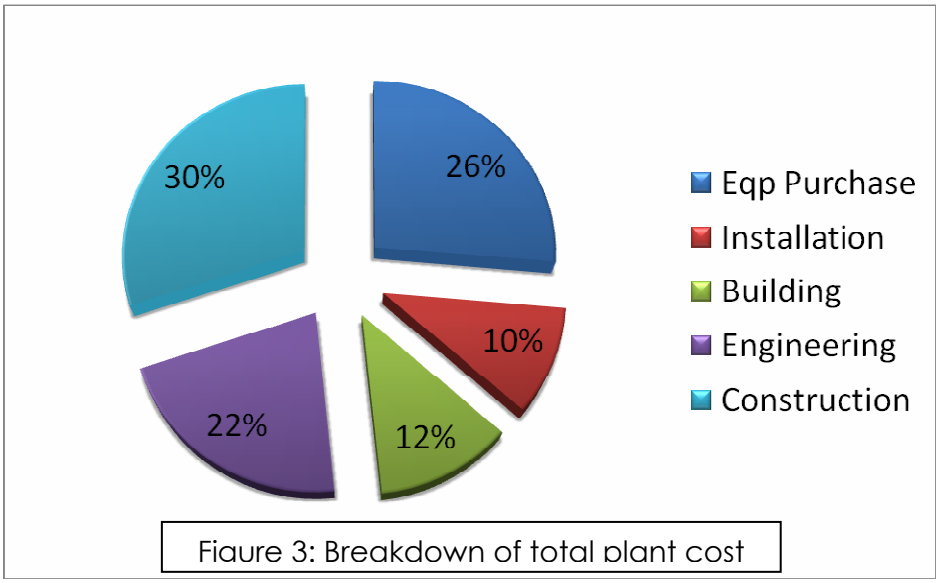


Figure 3: Breakdown of total plant cost

Chemicals & Raw Materials:

Purchasing cost for chemical and raw materials was fed in the system. The purchasing costs of chemicals were taken from the Sigma-Aldrich website and were scaled down. Please see the table 13 & figure 4 for more details.

Components	Purchasing price ($/kg)
HCl	0.26
Na2HPO4	1.80
NaH2PO4	2.15
NaCl	0.30
NaOH	0.45
Milk	0.75
Water	0.10
Dft-membrane (unit)	400.00
PBA resin	1,500.00

Table 13: Percentage expenses on raw materials based on an annual expense of $32,552,271.

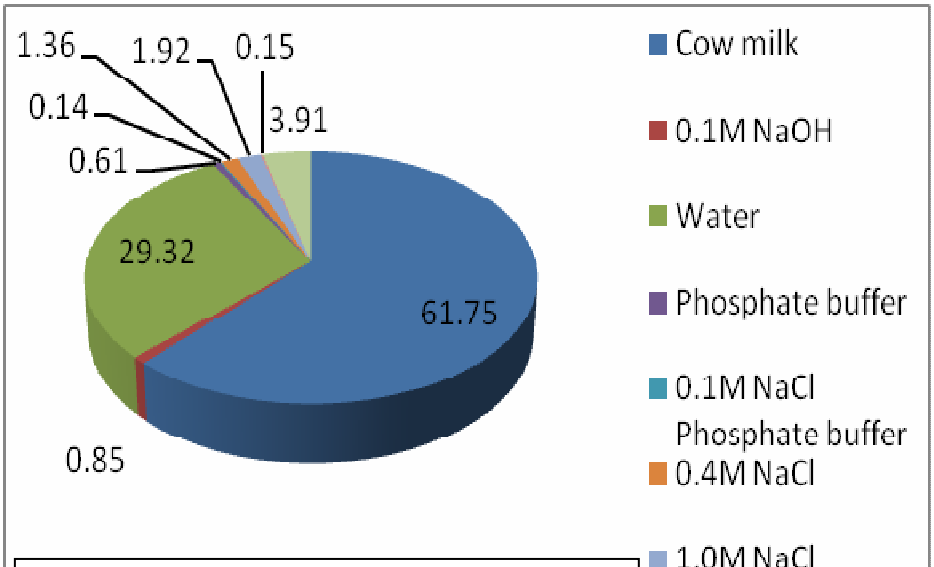


Figure4: Breakdown of raw material cost

Labor cost:

An average value of $69/hr for labor over the entire procedure was already present in the system and was used in the process.

Product selling price:

The selling price for the three product streams was determined by consulting with the advisor and looking in the literature for product cost for the purity level at which the product was. Table 14 summarizes the selling price of the products obtained.

Components	Selling price ($/kg)
LF	1,000.00
LPO	1,000.00
Protein conc.	50.00

Table 14: Product selling price

Utilities cost:

A total cost of $1,384,953 was incurred on the utilities. The breakdown of the utilities is as shown in figure 5. As seen in the pie chart, steam generation takes the highest fraction of the utilities cost.

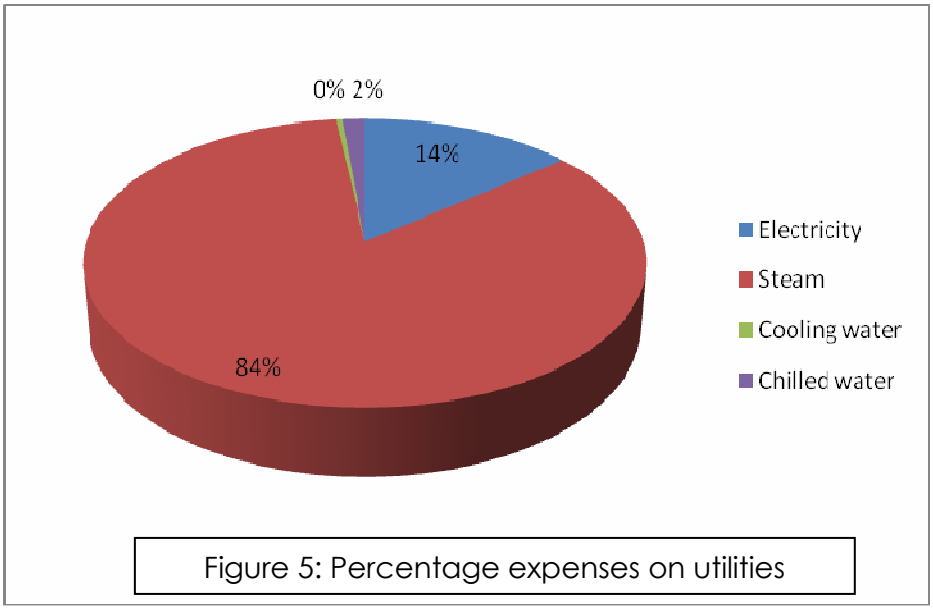


Figure 5: Percentage expenses on utilities

Annual operating cost:

Figure 6 shows the percentage contribution of different components in the annual operating cost of $56,943,000. Raw materials costs the highest fraction of the overall cost after which facility costs rank.

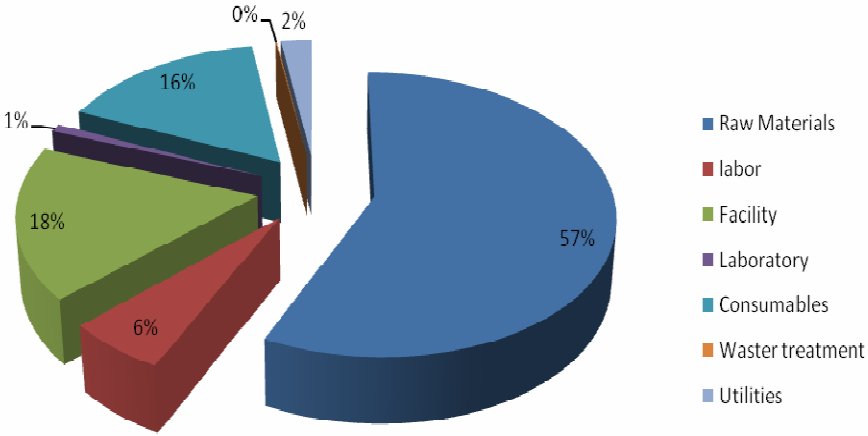


Figure 6: Breakdown of Annual operating cost

Section wise cost:

The cost analysis for each of the five sections was done by the software. Pasteurization was the costliest step, costing 46% of the overall process cost. Next to it was the Cation exchange chromatography step contributing 23% of the entire process cost. A pictorial representation of the contribution of different sections in the overall cost of $56, 943,989 is shown in figure 7.

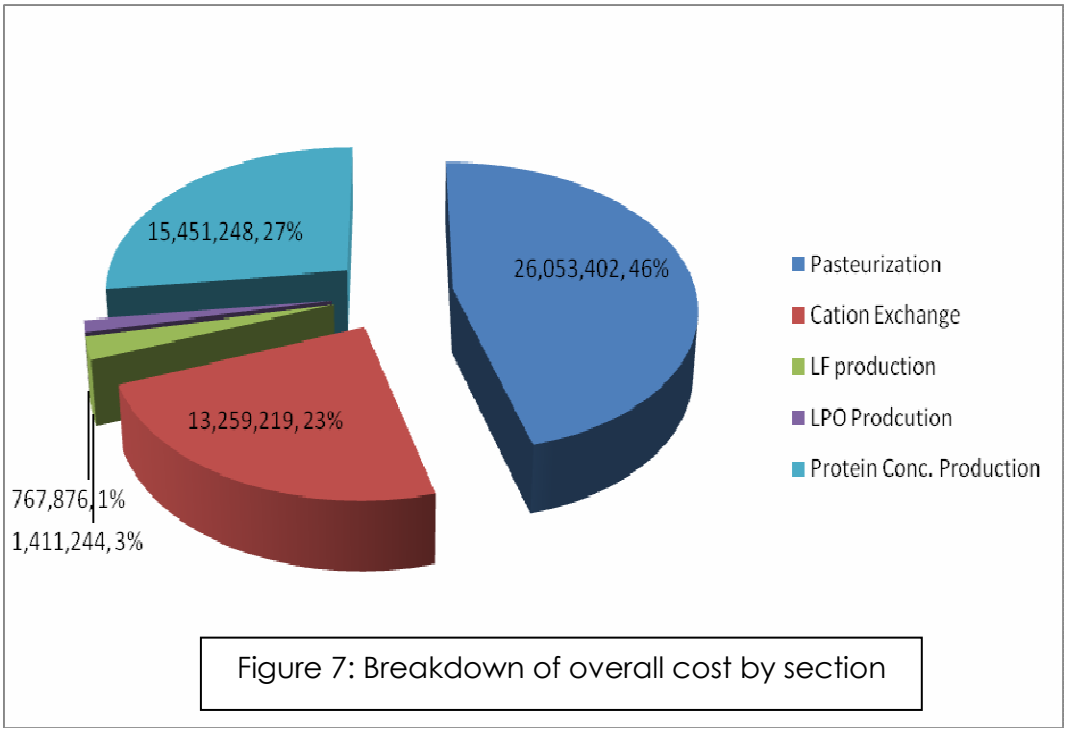


Figure 7: Breakdown of overall cost by section

Profitability analysis based on feed per batch:

A profitability analysis of the process was done by simulating different feed rates. As shown in table 15, 1000kg/batch and 10,000kg/batch are too small a rate and gives highly negative margin rates. The process is economically feasible at a level of 100,000kg/batch or above level. Though it is true that higher the production rate, higher is the margin but for a start up a feed level of 100,000kg/batch is safe. Higher production levels require higher investments also.

Processing rate (kg/batch)	Gross margin (%)	Unit production cost ($/kg)	Return of investment (%)
1,000	-675.89	88,792	-36.08
10,000	-180.76	59,278	-27.09
100,000	34.40	21,887	38.80
1,000,000	63.29	13,278	389.67

Table 15: Profitability analysis based on processing scale.

SUGGESTIONS TO REDUCE COST:

Following are some of the ways by which the production can be lowered and the profit margins can be increased. Some drawbacks associated with the suggestions are also discussed.

1. **Use of old equipments:** In the process, we assumed that all the equipments are new which increases their costs. Used equipments can be bought and used in the facility. This can reduce the costs substantially as these will be cheaper to buy. The drawback associated with this suggestion is that the equipments will not be advanced but these can be upgraded. Another disadvantage associated with this is the frequent breakdown of equipments which can raise the maintenance costs.

2. **Existing facility:** Buying and using an existing facility can make the process much faster as there will not be a construction and setup time involvement. Also, it saves on construction costs as the rates for construction always increase. But a factor to consider while buying or renting an existing facility is that there is sufficient space available for expansion.

3. **Strategic location:** Location plays a big role in the overall success of the venture. If the facility is located at such a place where raw material is readily available, this can save a lot of expenses on transportation of raw materials. Some of the important factor to consider while choosing a location can be:

a. proximity to the raw material source

b. proximity to the market for product

c. cheap labor availability

d. abundant and cheap water supply

e. low or no waste disposal costs

It is impossible to have all these factors in one location, but a wise combination can help a lot to maximize the margins. Lots of state governments give special discounts on land, taxes and waste disposals to set up industries in their states. A wise businessman would like to make use of these concessions.

REFERENCES:

The references used for making this project are as follows:

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