DESIGN OF A TWO STAGE REACTOR SYSTEM FOR PROTEIN PRODUCTION USING PLASMID-ENCODED E. COLI AND WHEY PERMEATE AS A COMBINATION SUBSTRATE AND INDUCER

A Project Report
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Abstract

Whey disposal problems in the United States have increased over the past twenty years. Few economically attractive options are available for whey utilization.

The Cornell Excretion System (CES) uses a genetically engineered cell to produce plasmid-encoded proteins in a two stage continuous flow reactor system. The host cell has been E. coli RB791 with a pKN plasmid. The target protein gene is under control of the tac promoter, which is induced by lactose. Experimental systems have demonstrated the feasibility of continuous production of the ß-lactamase enzyme, at high levels (>0.5 g/L), with high levels of excretion (>90%), and high purity (>50%). Induction of the tac promoter leads to increasing plasmid copy number and eventual cell death. Mutants not responding to induction have a selective advantage. However, lactose (because it is both inducer and carbon/energy source) would prevent the formation of such mutants. The CES is very well suited to the utilization of whey permeate.

Enzymes have much higher economic value than products from previously proposed whey utilization schemes. A plant that produces commercial enzymes using the CES, and lactose from whey permeate was designed at six different scales (0.013 million pounds of milk permeate per day, and 0.50-1.03 million pounds of whey permeate per day). Based on molar production rates (0.5 fraction of molar rate), yield factors (150 units ß-lactamase/mg cell, 0.35 g cells/g lactose), and other assumptions, profitability and sensitivity analysis were performed. Under the hypothesis that the plant would produce Bacillus proteases, glucose isomerase, and calf rennet for 65, 30 and 5% of the total operating time, rates of return of 0 (milk permeate scale) to 26-35% (whey permeate scale), and total capital investments of 6.4 (milk permeate scale) to 56.6-90.9 (whey permeate scale) million dollars were obtained. When it was assumed that the milk permeate scale was producing calf rennet for 100% of its total operating time, a rate of return of 73%, and a total capital investment of 6.4 million dollars were calculated. It was concluded that there are economic incentives for the determination of the behavior of the CES when using lactose as an inducer to produce such enzymes.

Biographical Sketch

Fernando Portes was born in Sabana de la Mar (small town in eastern Dominican Republic). After his mother's immigration to the United States, he moved to Santo Domingo and completed his high school education in the Centro Masonico de Estudios. From January 1982 to October 1988, he attended the Universidad Autonoma de Santo Domingo (the oldest university in this continent) where he obtained degrees in Chemistry (B.S. "Magna Cum Laude"), and Chemical Engineering (B.Eng. "Magna Cum Laude. First in his class). His chemistry thesis (on Aloe Processing) was awarded as the best in the Dominican Republic in 1986. He supported his education, in part, by working as a high school teacher (chemistry), teaching assistant (organic chemistry), quality control chemist, quality control manager, and production manager. He also worked as a quality control supervisor.

He came to Cornell under the sponsorship of the United States Agency for International Development (USAID). After completion of his training, he will return to the Dominican Republic where he will be working in the Universidad Autonoma de Santo Domingo (in the Biochemical and Food Engineering areas).

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• The United States Agency for International Development (USAID) provided funding for my studies at Cornell, and other important activities of my training.

• The idea of this project comes from professor Michael Shuler.

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• Professor Dennis Miller, of the Food Science Department, was highly diligent and cooperative in the process of getting the M.P.S. approval.

• Jeffrey Fu and Paul Togna allowed me to use unpublished data from their research, and provided me initial guidelines when running the experimental part of this project.

• Cornell Information Technologies (CIT) (mostly Jose -IBM consultant-), provided me support when dealing with the spreadsheet (it got too big to be handled by the public computers available on campus), and with other unusual problems.

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Introduction, Scope, and Methodology

1.1 Introduction

Milk and whey permeate are 5% lactose solutions mostly discarded from cheese manufacturing plants (figure 1.1). Their pH and mineral concentration depend on the type of cheese that is produced. Sweet-type whey (pH 5.8 to 6.6)[1] is produced ten times in larger quantities than acid type whey (pH 4 to 5)[2].

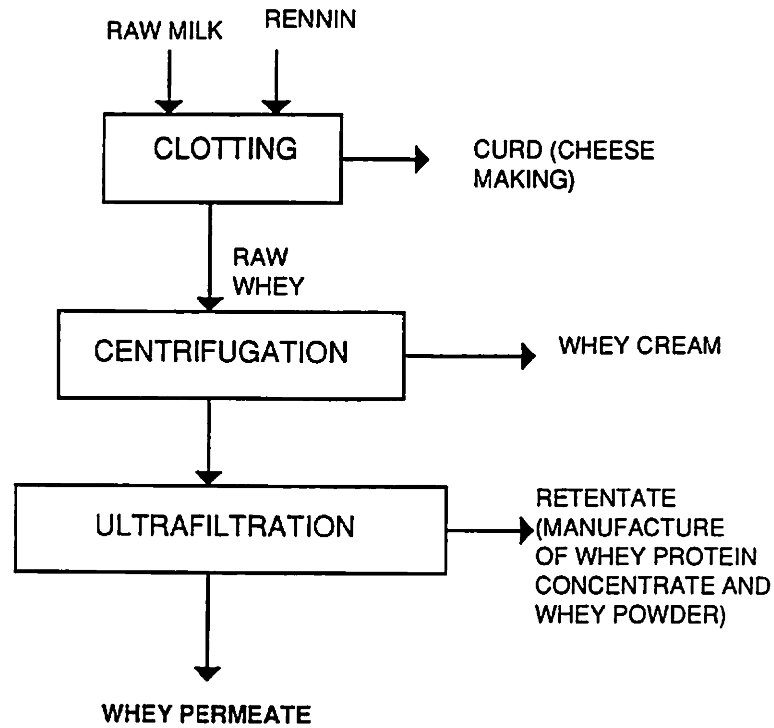
Over the last twenty years whey production in the United States has increased principally because of two trends:

> Increase in cheese consumption[3]. In 1989, total cheese shipments were 13.5% bigger over the previous year. Such increase is associated with higher pizza consumption which uses mozzarella cheese as principal ingredient[4].
>
> Decrease in milk consumption[3].

Roughly 45% of the whey produced is wasted[2]. The preferred disposal methods are open land discharge and sewage dumping. The first is limited by land availability within 20 miles of the cheese plant, which is regarded as the maximum hauling distance for keeping costs under control[5]. Any effluent with Biological Oxygen Demand (BOD) greater than 200 requires a surcharge in municipal sewage plants. Whey has a BOD greater than 30,000[1]. The problems with the sewage option are increasing surcharge (in some cases they have doubled or quadrupled), and the failing of treatment plants (most of them malfunction at least once a year and for 25% of the time are less than 75% efficient[5]).

Considerable effort has been devoted to the development of processes that convert whey or permeate into single cell protein, bakers yeast, ethanol[6], butanol/acetone, acetic acid, lactase, riboflavin, vitamin B12, hydrogen, methane, penicillin, etc.[7].

Whey Permeate[8]:



Milk Permeate[9]:

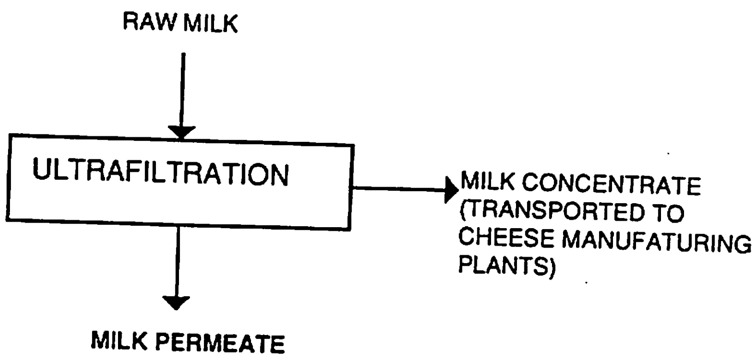


Figure 1.1 Permeate Sources Flowsheet

The Cornell Excretion System (CES) is considered a feasible way of producing proteins[10,11,12,13,14,15,16,17,18]. It consists of E. coli (RB791(pKN)) with the tac (hybrid trp-lac) promoter, which is induced by lactose or lactose analogs such as isopropyl ß-D-thiogalactoside (IPTG). After induction, E. coli can not normally synthesize outer membrane proteins and becomes leaky. That leads to high levels of protein excretion (table 1.1). The leaky phenotype results from increasing plasmid copy number and leads to eventual cell death. The formation of mutants that do not respond to the inducer is a problem, because these mutants grow normally and produce no product. Although the two stage system circumvent this problem, the potential use of lactose is attractive since it would act as both inducer and carbon/energy source, which would further suppress the formation of mutants.

Table 1.1 The Cornell Excretion System[15]

Basis: Induction of selected strains reduces the rate of outer membrane proteins synthesis, which leads to enzyme excretion.

Characteristics:

Extracellular Protein Concentration:	> 0.5 g/lit
Purity:	50%
Operation:	> 50 days
Expression Level:	25% of cellular protein
Excretion:	90% of ß-Lactamase
Reactors:	Two stage chemostat
Mutants:	< 0.1% in second fermentor
Strain:	RB791(pKN)
Inducer:	IPTG
Promoter:	tac
Medium:	glucose (2-4 g/lit) in Tanaka Me.
Cell Concentration:	< 4 g/lit

Experimental data of the CES are only available for IPTG as inducer under certain conditions. Given the lactose overproduction mentioned above, a plant that utilize it as inducer in the CES for the manufacturing of high volume proteins is very reasonable. It would convert part of the lactose into glucose and galactose to use them for cell growth. A simplified flowsheet of such plant is presented on figure 1.2.

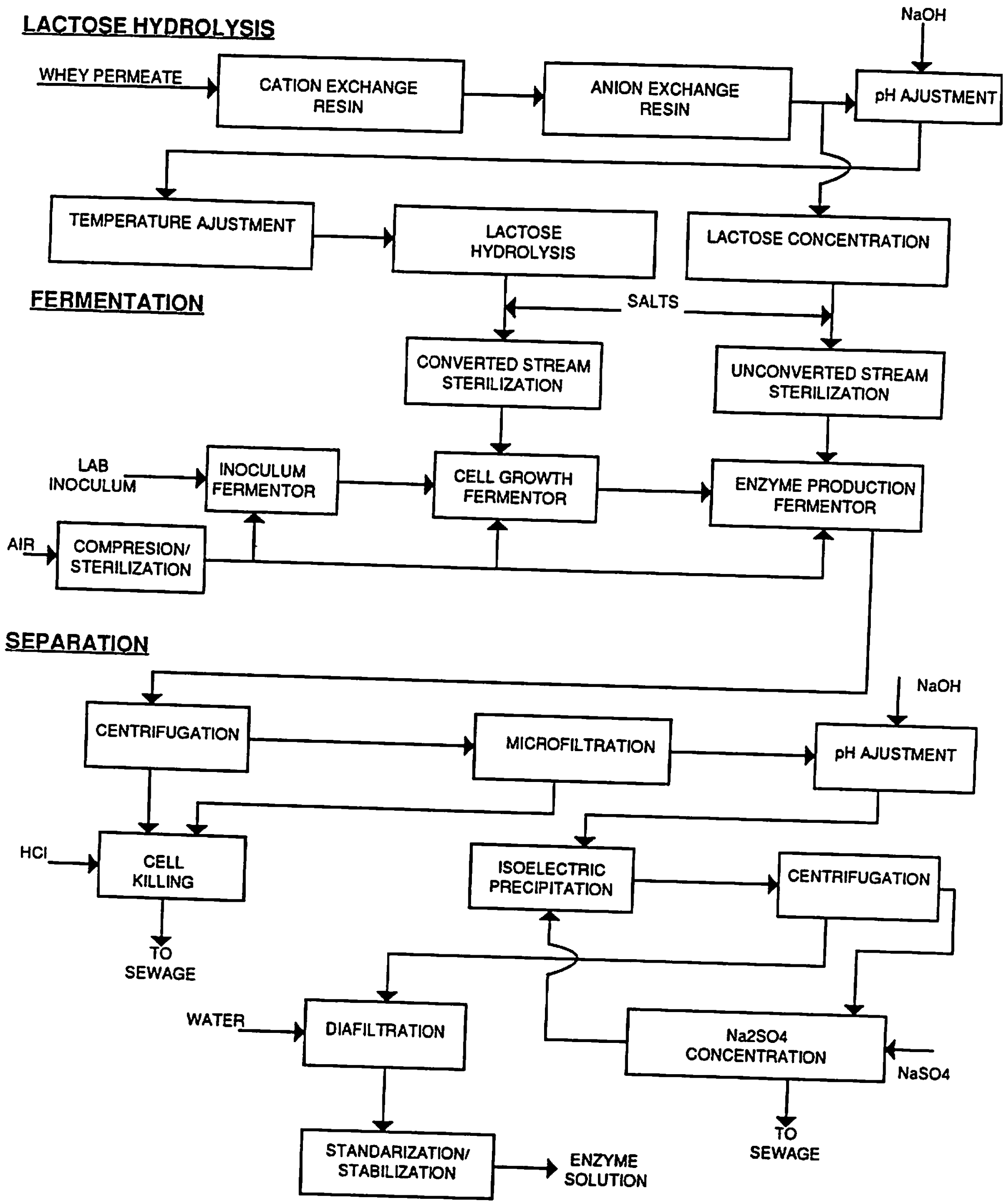


Figure 1.2 Enzymes' Production (Simplified Flowsheet)

1.2 Scope

The scope of this project is:

Design a plant that would produce commercial enzymes using the CES and whey permeate as feed.

Find out the profitability and perform sensitivity analysis on such plant.

Determine if this is a feasible alternative for dealing with the whey disposal problems in the United States.

1.3 Methodology

The plant was designed in six different scales. It would produce three enzymes: Bacillus proteases, glucose isomerase, and calf rennet. To allow flexibility, and to account for future experimental data, the calculations were performed on a spreadsheet (LOTUS), which has almost 1700 lines. The equipments and processes were designed using chemical[19,20,21,22,23,24,25,26,27], and biochemical and food engineering[28,29,30,31,32,33,34,35,36,37,38,39,40] approaches.

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Chapter 2

Lactose Hydrolysis

The Corning hydrolysis system was selected for the lactose hydrolysis. It was the first commercial process for such purpose available in the United States[1]. Its good performance has been demonstrated at pilot[2,3,4,5,6], and at industrial scale[7,8,9]. It consists of an immobilized lactase reactor operating from 35 to 50°C to maintain constant conversion. To increase its operating life, the whey is ultrafiltrated and demineralized. Since the feed of the plant is permeate, the UF step is unnecessary. The proposed flowsheet is shown on figure 2.1. The streams are detailed on table 2.1.

Other alternatives for lactose hydrolysis has been presented. They include lactase immobilization in cellulose triacetate, active carbon, acrylic beads, etc.[10].

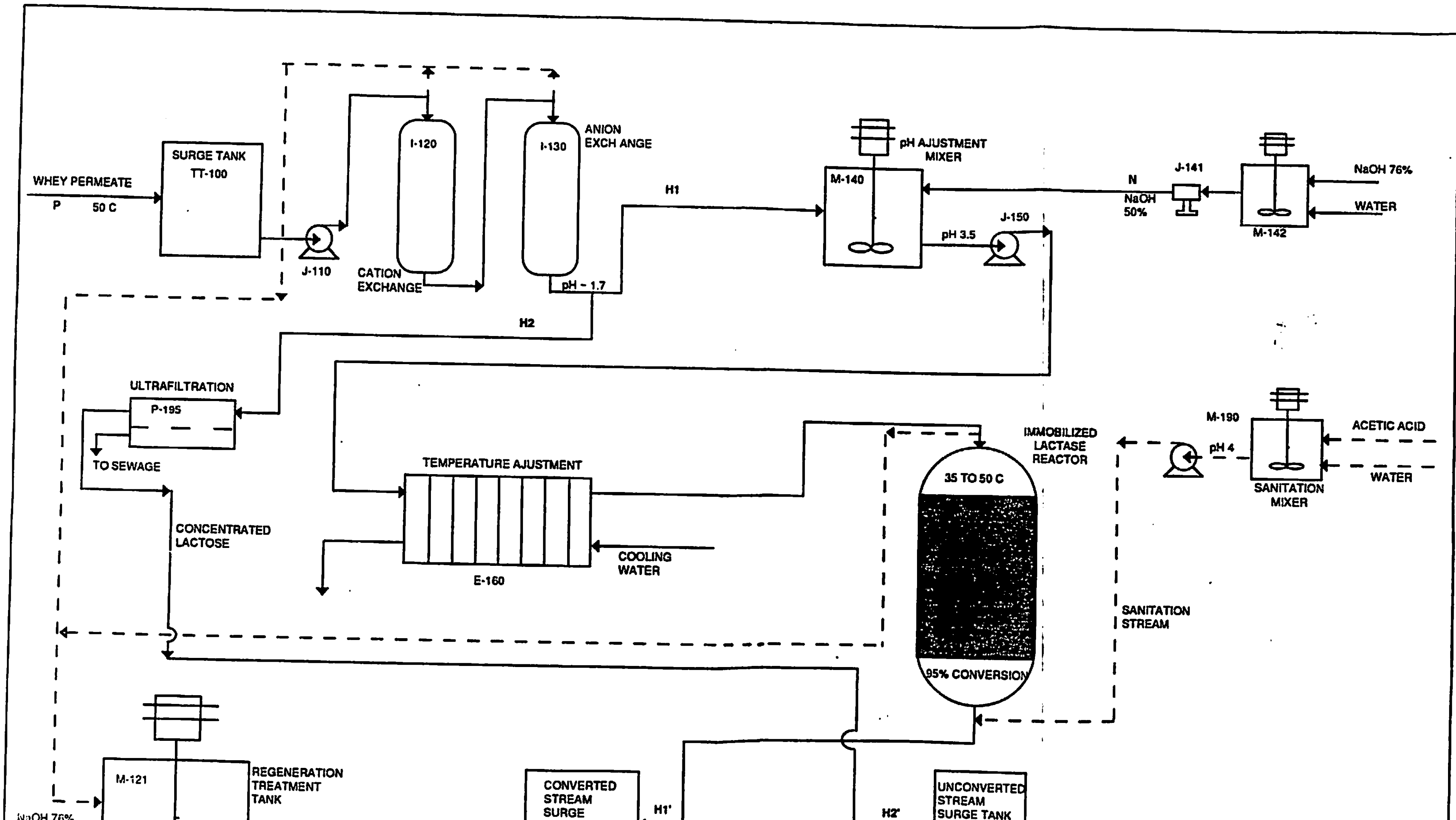
2.1 Whey Material Balances

In cheese manufacturing plants, for every 100 pounds of milk , 10 are converted into cheese and 90 are produced as whey, which is converted in part to Whey Protein Concentrated (WPC) and Whey Powder (WP). Economics and operating conditions of WPC and WP processes are presented on an study of the Cornell Program on Dairy Markets and Policy[11]. One of the assumptions of that study is the disposal of the permeate in the ultrafiltration step of the manufacturing. Another study[12] has indicated that to save transportation costs, it is profitable to concentrate the milk (process similar to the familiar orange juice) before shipping it to the cheese plants. The permeate originated after such concentration is also disposed. The feed of the proposed plant would be wasted permeate of the aforementioned cases.

The permeate was considered as Newtonian because whey shows that behavior below 20% of solids[13].

2.2 Demineralization

Demineralization is necessary to increase the operating life of the immobilized lactase. It can be performed by electrodialysis or by ion exchange. Electrodialysis was not considered because at the present time it is not economical for highly desalted solutions[14]. Besides, the demineralization requirement of the plant is 90% of the ions[2,5], and optimum conditions for electrodialysis are at 50% demineralization[15].



PRODUCTION OF PROTEINS USING PLASMID ENCODED GENES IN E. COLI AND WHEY PERMEATE AS COMBINATION SUBTRATE AND INDUCER

Figure 2.1 Lactose Hydrolysis

Table 2.1 Streams' Flows and Compositions (Lactose Hydrolysis System) (Scale: 0.5 Million Pounds Whey Permeate/Day)

Stream	pH	Temp. (°C)	Glucose (g/L)	Galactose (g/L)	Lactose (g/L)	NaOH (% -w/w-)	Flow (kg/hr)
P	~6	50	0	0	47	0	10732
H1	1.7	50	0	0	47	0	5366
H1'	3.5	35-50	23.48	23.48	2.35	-	5374
H1"	3.5	35-50	23.48	23.48	2.35	-	4729*
H2	1.7	50	0	0	47	0	5366
H2'	1.7	50	0	0	120	0	2100
H2"	1.7	50	0	0	120	0	1848*
N	-	20	0	0	0	50	8

* The lactose hydrolysis system would work 21 hr/day and the fermentation system 24 hr/day.
- Not calculated.

The total cation and anion contents in the permeate are roughly 80 and 60 meq/lit. Potassium, sodium, and calcium account for most of the cations, and chloride, diphosphate and lactate for most of the anions. Under such conditions, the use of strong acid and week basic resins produce the best results[16]. The selected resins are Dowex HCR2 (strong acid) and Dowex 66 (weak basic). They are both approved for food processing operations[17,18,19].

Sulfuric acid is sometimes used for resin regeneration. To avoid calcium sulfate precipitation, chloridic acid was selected. The weak basic resin would be regenerated with sodium hydroxide. The pH of the regenerant solution is adjusted to 7, before releasing it to the sewage. To avoid the discharge of salts (18 kg per m^3 of permeate treated), a new process was proposed. It uses ammonium and bicarbonate in the cation and anion exchanges. The ammonium bicarbonate produced is thermically decomposed into ammonia and carbon dioxide, which are recuperated and recycled as regenerants. For West German conditions (March 1983), the operating costs were estimated at 60% of the traditional ion exchange[15]. The plant of this project dispose enormous amounts of water which can be used to dilute the sodium chloride produced in the regeneration. If that is environmentally unacceptable, the aforementioned process is regarded as a feasible option.

2.3 Immobilized Lactase Reactor

Once demineralized, the permeate is split and part of it (H1') is converted into glucose and galactose. The hydrolysis is performed by lactase immobilized on porous glass. Characteristic of the reactor are presented on table 2.2.

Table 2.2 Immobilized Lactase Reactor Characteristics[2,3,4,5,6,20]

Rate expression:	V=kES / (S+Km(1+P/Ki))
Sanitation:	Acetic acid solution (pH 4)
Enzyme carrier:	Porous glass
Average pore diameter:	370 A
Pellet diameter:	0.46 mm
Void fraction:	0.35 (bench scale)
Carrier total area:	~ 82 m^2/g
Apparent activity:	300 lactase units/g
Optimum pH:	3.5
Operating temperatures:	35 to 50 °C
Deactivation energy:	12 kcal/mol
Half life (50 °C):	62 days
Enzyme operating life:	559 (predicted)
Enzyme operating life:	365 (experimental)
External mass transfer:	No observed
Internal mass transfer:	No observed
Axial dispersion:	Insignificant

The pH of the demineralized stream (H1) is approximately 1.7. It is raised to 3.5 (optimum pH of the reactor) with sodium hydroxide (figure 2.1). As regard to the temperature, the best operating strategy starts the reactor at 35 °C and increases the temperature to 50 °C to maintain constant conversion. Based on the half life at 50 °C, and the deactivation energy (table 2.1), the time for the 35-50 °C cycle was estimated as 559 days[2,5]. However, the times obtained in commercial operations were considerably lower (approximately one year)[8]. In spite of that, they are adequate for economic operation.

In the case of whey permeate, it enters the plant at 50 °C because it is sterilized before the UF step[11]. Although the milk permeate enters the plant at ambient temperature, its entering temperature was regarded as 50 °C. This does not affect the final results in a significant way.

Plate heat exchangers were selected for the temperature adjustment. They have advantages of ease of dismantling for sanitation purpose, high heat transfer coefficients, and are widely employed in the food industry. Their design procedures are given in the literature[21,22,23,24,25,26,27,28].

The kinetics of lactose hydrolysis is affected by galactose inhibition. The rate expression is presented on table 2.2. Values of the turnover number (k=6E-5 mol/units/hr), Michelis constant (Km=0.0528 mol/L), and inhibition constant (Ki=0.0054 mol/L), are given in the references[2,5]. One of the reason for the success of this technology is the immobilization of lactase in such a way that internal and external mass transfer effects be absent. Lactase was immobilized by the aqueous silane-glutaraldehyde method. For obvious reasons, only partial details of the immobilization procedure have been published[28].

The axial dispersion effects were considered insignificant by two different approaches:

> When the length of a packed bed reactor is more than 100 times the particle diameter, axial dispersion should not be important[29] . All the scales of this project comply with that consideration.
>
> In a packed bed, when the Reynolds number is less than 10, the Peclet number is close to 0.5. With the void fraction, particle diameter, and reactor length, the dispersion number (D/vL) can be calculated. Assuming first order kinetics, graphs[30] predict that the difference between the ideal plug flow and the real reactor is insignificant[2]. In fact, this is a conservative estimate because part of the reaction occurs at zero order (the order shifts from zero to one as the conversion increases), and zero order reactions are not affected by back mixing.

As regard to sanitation, it is achieved by back flushing the reactor with an acid solution brought to pH 4 by the addition of acetic acid[2,5]. This sanitation and the resin regeneration are the reasons why the lactose hydrolysis part of the plant would only work 21 hours per day.

2.4 Lactose Concentration

Lactose is concentrated to alter the nutrient ratio fed to both fermentors, and to lower the enzyme dilution in the second one. Since the stream has already been sterilized in the cheese plant[11], and passed through two ion exchange beds, the UF equipment would perform well under very favorable conditions. Besides, lactose is only concentrated up to ~120 g/L. This avoids concentration polarization problems. UF process design and economics have been given in the references[31,32].

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Chapter 3

Fermentation

3.1 Medium Formulation.

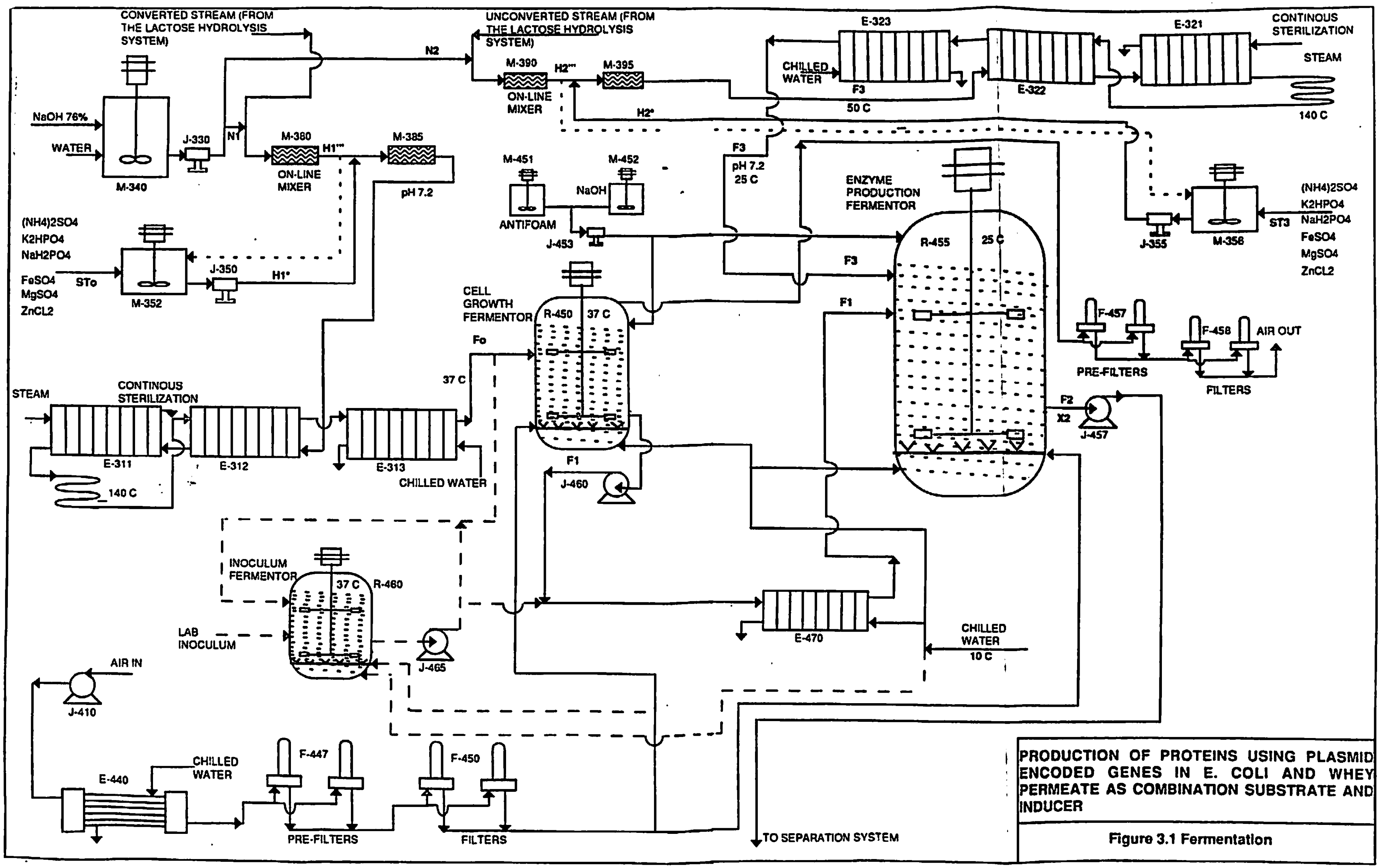
The pH of the converted (glucose+galactose) and unconverted streams (lactose) is adjusted to 7.2 with sodium hydroxide in on-line mixers (figure and table 3.1). Next, based on elements' material balances, salts necessary for cell metabolism and growth are added (ST_0 and ST_3 streams). It is known that some salts in the medium have limited solubilities, or may form insoluble compounds ($Ca_2(PO_4)_3$). In defining the concentrations, precautions were taken to avoid precipitation. Calcium ions are maintained at very low levels because the permeate stream has already being demineralized. Besides, the constant streams' movement assures that this problem will not be present.

3.2 Sterilization

Based on data of IPTG as inducer, the reactors are proposed to work continuously for 50 days (table 1.1). This is conservative, because lactose (by acting as inducer and as carbon/energy source) would prevent the formation of mutants. For the whey permeate, there is the advantage of low microbial load in the incoming stream because it has already being sterilized in the cheese plant. In spite of this favorable situation, the sterilization system was designed assuming worst case scenarios. Sterilization design procedures and data are given in the references[1,2,3,4,5]. Plate heat exchangers were selected for the heat transfer operations. Fast sterilizations will be achieved by the use of high temperature (140 °C). Heat integration among the streams was included (figure 3.1).

3.3 Fermentors

The basis of the fermentation part of the plant is the Cornell Excretion System (CES) (table 1.1). It has been tested with glucose as cell growth nutrient. In the proposed plant, it is assumed that E. coli will use glucose and galactose for cell growth. Experimental basis for this assumption have appeared in the literature[6]. Batch and continuous fermentations were performed with E. coli (B/r strain) using glucose and galactose as nutrients. In batch, galactose was only used after all the glucose was depleted. However, in continuous operations both carbohydrates were consumed at the same rate. This was attributed to the small steady state glucose concentrations which were not sufficient to cause galactose inhibition[6]. Design data for the converted stream (Fo) were assumed to be the same as for glucose alone (see spreadsheet on appendix 1).



PRODUCTION OF PROTEINS USING PLASMID ENCODED GENES IN E. COLI AND WHEY PERMEATE AS COMBINATION SUBSTRATE AND INDUCER

Figure 3.1 Fermentation

Table 3.1 Streams' Flows and Compositions (Fermentation System) (Scale: 0.50 Million Pounds of Whey Permeate/Day)

Stream	Cell C. (g/L)	Glucose (g/L)	Galactose (g/L)	Lactose (g/L)	ß-Lactamase (g/L)	Flow (kg/hr)
Fo	0	23.28	23.48	2.35	0	4811
F1	18.69	0.0025	0.0025	2.35	0	4811
F2	25.31	~0	~0	0.057	4.74	6759
F3	0	0	0	120	0	1948
H1'''	0	23.48	23.48	2.35	0	4729
H1*	0	23.48	23.48	2.35	0	13
H2'''	0	0	0	120	0	1848
H2*	0	0	0	120	0	12

The unconverted stream (F3) is fed to the enzyme production fermentor and the lactose acts as inducer and as nutrient for cell growth and enzyme production. Once induced, the cells are directed to produce the desired enzymes. In doing so, they alter the synthesis of outer membrane proteins and become leaky. That leads to enzyme excretion. Since most of the cells die in this process, new ones are fed from the cell growth fermentor to sustain steady state operation[7,8,9,10,11,12,13,14,15]. The CES has been tested for the production of ß-Lactamase and Human Epidermal Growth Factor (hEGF). The molar production rate of hEGF was 0.6 of the molar rate[11]. This means that

Broth Conc. of hEGF= Broth Conc. of ß-Lactamase*(MW hEGF / MW ß-Lactamase)*0.6

As a conservative estimate, a molar production rate of 0.5 for all the enzymes was assumed. It has been predicted that this value may be higher for enzymes with molecular weights greater than the molecular weight of ß-Lactamase (29,000). It is on the basis that the greater the molecular weight of the enzyme to be produced, the less resources that E. coli will have available for outer membrane protein synthesis. So, the cell would become more leaky, which would lead to higher levels of enzyme excretion[11].

It is assumed that the small amounts of lactose (~2.35 g/L) fed to the cell growth fermentor will not induce the cells. This concentration appears because the immobilized lactase reactor is operated at 95% conversion. Higher conversions increase disproportionately the size of such reactor. Future optimizations may be required to find out the best point between higher lactose conversions, and induction in the cell growth fermentor (if there is any).

Mathematical optimizations performed on this system have shown that optimum conditions (with glucose in both feeds) are achieved when the nutrient concentration of the streams to the reactors are equal[13]. This condition can be easily simulated in the spreadsheet (appendix 1) by changing the ratio H1/H2 (1 value used), or by changing the lactose concentration out of the UF step (120 g/L value used). Optimum conditions were not employed in the calculations. They may be different with lactose and glucose/galactose in the feeds.

The start-up of new operations is proposed to be by transferring the culture from the laboratory to the inoculum fermentor, and from there to the cell growth fermentor. The enzyme production fermentor would be started with cells from the cell growth fermentor. Because the time of these transients are very small compared to the steady state operations, they were disregarded in the calculations.

Antifoam and sodium hydroxide consumption are based on experimental values obtained in the CES[16].

High volume enzymes are produced in 20 to 200 m^3 fermentors[17]. Stirred tank reactors up to 400 m^3 are employed in antibiotic production[18]. As a compromise, fermentor volumes were limited to approximately 300 m^3. Fermentor design recommendations and were found in several references[19,20,21,22].

3.4 Air Compression and Sterilization.

Air would be compressed in centrifugal compressors and cooled in U-tube heat exchangers. Sterilization would be performed by membrane filters. To avoid the releasing of genetically modified microorganisms to the environment, exhausted air is pre-filtered and filtered. Heat sterilization of air was not considered because it is regarded too expensive at industrial scale[22]. Guidelines for air compression/sterilization are presented in the references[23,24,25,26,27,28].

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Chapter 4

Separation

The separation system is a compromise of different processes and limitations that have been described for individual enzymes[1,2,3,4]. It accounts for old and new trends in enzyme separation[5,6,7,8,9,10] (figure and table 4.1).

Some laboratory separations of the CES were performed using chromatography[11]. It was not included in the separation system because its cost at industrial scale has been estimated at $5 per gram of product protein[12], and most of the produced enzymes have prices between 100 and 250 $/kg[13].

4.1 Cell Recovery

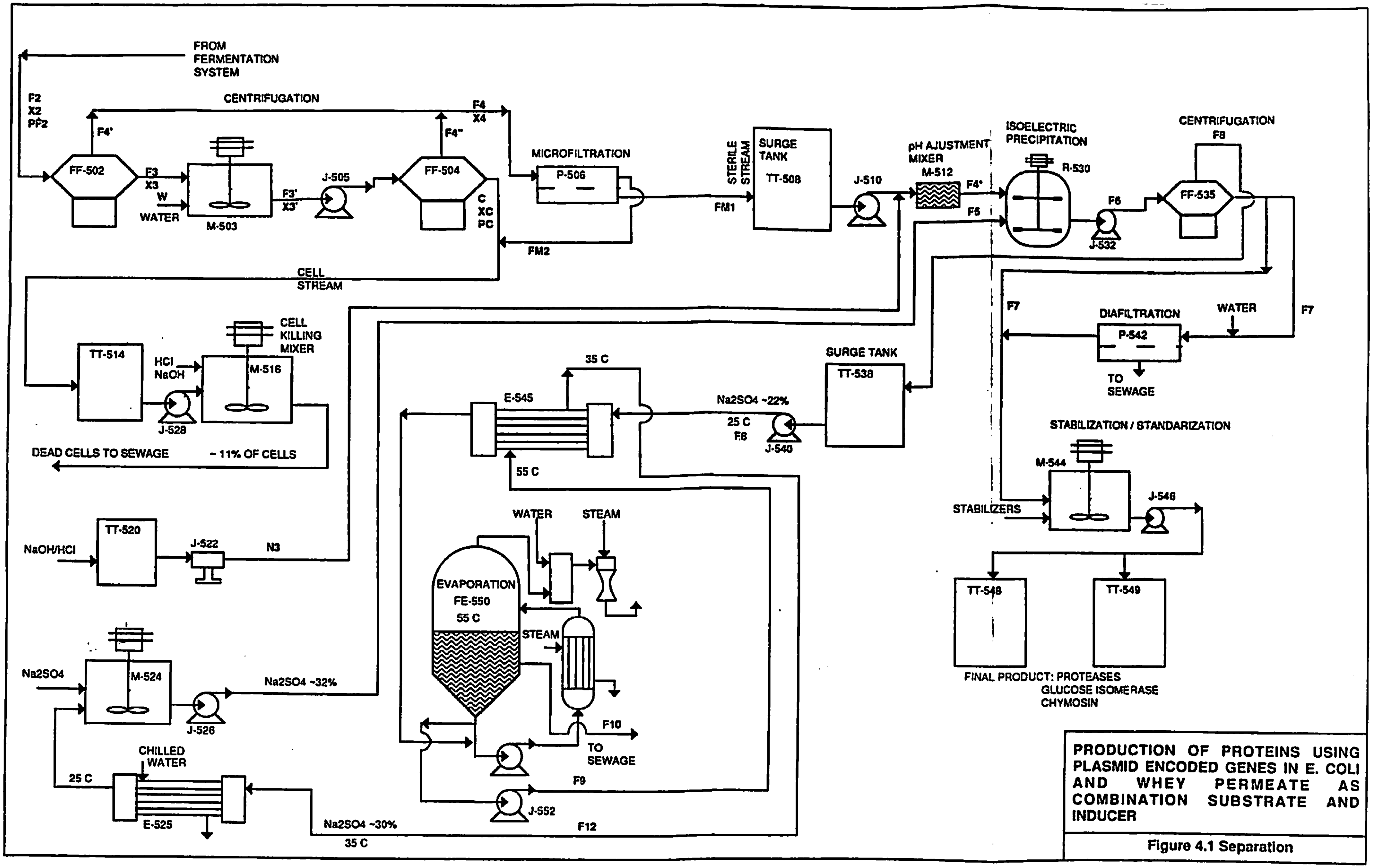
The cells are separated from the broth (F2) by centrifugation. Cell concentrations of 14% (dw) are achieved in both centrifuges (F3 and C). Ranges of 10 to 15% have been recommend for this variable[10]. The spreadsheet (appendix 1) allows different specifications of the centrifugation yield by designating it as independent variable. As it increases, the amount of water fed to the mixer (figure 4.1) increases.

The stream is sterilized on microfiltration membranes. A low solids loading ultrafiltration model was employed in this operation[14].

4.2 Precipitation/Centrifugation

Once the stream is sterilized (FM1), the pH is adjusted to the isoelectric point of the enzyme that is being produced. Enzyme denaturalization is avoided by employing very dilute solutions (N3) of NaOH and HCl.

Precipitation is performed continuously by adding a saturated solution (F5) (32% -w/w-) of Na_2SO_4. This lowers the Na_2SO_4 concentration to 22% (F6). Compared to Na_2SO_4, $(NH_4)_2SO_4$ has the advantage that it can be used as a fertilizer by spreading the discarded portion into open land. However, it is not compatible with Proteases[6,15]. "Ageing" of the precipitated protein is recommended to increase its recuperation efficiency[16,17]. It is achieved by proper selection of the residence time and agitation (shear) in the reactor. At this point precipitation theories does not allow proper prediction of those quantities. Rules of thumb from water treatment application are recommended for such purpose[18,15,16].



PRODUCTION OF PROTEINS USING PLASMID ENCODED GENES IN E. COLI AND WHEY PERMEATE AS COMBINATION SUBSTRATE AND INDUCER

Figure 4.1 Separation

Table 4.1 Streams' Flows and Compositions (Separation System) (Scale: 0.50 Million Pounds of Permeate/Day)

Stream	ß-lactamase (g/L)	Cell Concen. (g/L)	Na_2SO_4 (% -w/w-)	Temp. (° C)	Flow (kg/hr)
C	~0	141	0	25	1221
C+FM2	~0	111	0	25	1535
F2	4.74	25.31	0	25	6759
F3	-	141	0	25	1221
F3'	-	87	0	25	1974
F4	4.44	-	0	25	6291
F4*	4.44	0	0	25	5977
F5	0	0	32.0	25	12592
F6	1.61	0	21.7	25	18569
F7	1115	0	-	25	28
F8	0	0	21.7	25	18541
F9	0	0	30.0	55	12232
F10	0	0	30	55	5949
F12	0	0	30	35	12232
FM1	4.44	0	0	25	5977
FM2	-	-	0	25	315
N3	0	0	0	25	0.076
W	0	0	0	0	754

- Not calculated.

Centrifugation produces an enzyme sludge with Na_2SO_4 as impurity (F7). For proteases, (because their major application is as detergent ingredients), it does not represent any problem. However, for food processing enzymes (glucose isomerase and calf rennet) the salts must be taken out. This is one of the applications for which diafiltration has been recommended[19].

4.3 Cell Killing

FDA regulations prevent the release of recombinant DNA microorganisms to the environment. Cell killing is performed by keeping E. coli at pH 3 for four hours. Data presented in the references[20] indicates that such conditions are more than sufficient.

It is assumed that dead cells are discarted into the sewage. If that is environmentally unacceptable, digestion to biogas is regarded as an alternative. The design of that process is out of the scope of this project.

4.4 Sodium Sulfate Supply and Recuperation.

To save money and to prevent the release of large quantities of Na_2SO_4 to the environment , recycling is used. Reverse osmosis or electrodialysis could not be used because of the high concentrations of sodium sulfate, and the presence of small amounts of proteins. Evaporation with force circulation evaporators was selected. They can perform well under high scaling conditions. Heat integration was included in the design. The saturated stream (F5) (32% Na_2SO_4) is obtained by adding new Na_2SO_4. To avoid scaling problems, it was not produced in the evaporator

The material balance equations of the Na_2SO_4 recycle loop were specified in such a way that LOTUS could perform trial and error calculations on them.

4.5 Final Product

To stabilize the solution, sodium benzoate is employed. It is among some of the compounds that has been suggested for that purpose[15]. Glucose isomerase is sold principally in immobilized pellets, and part of the proteases is commercialized in dust-free powder. Information regarding the procedures utilized for converting the liquid enzymes to those commercial forms is scarce. For such reasons, liquid was assumed as the final commercial preparation of all the enzymes produced in the plant. Great amounts of enzymes are commercialized in that form[21].

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Chapter 5

Profitability and Sensitivity Analysis

5.1 Profitability

Table 5.1 presents the assumptions made for the profitability analysis:

Table 5.1 Profitability Analysis Assumptions

World Market*[1,2]			
	Bacillus Proteases	2	Millions kg/year
	Glucose Isomerase	0.125	"
	Calf Rennet	0.0138	"
Selling Prices*[1]			
	Bacillus Proteases	100	$/kg of pure enzyme
	Glucose Isomerase	250	"
	Calf Rennet	5000	"
Production Time*			
	Bacillus Proteases	65	% of total operating time
	Glucose Isomerase	30	"
	Calf Rennet	5	"
	Contamination Losses*	2	% of enzymes produced
	Plant Operating Life	10	Years

* Independent variables in the spreadsheet. Any of them can be changed to obtain new results.

The enzymes' world markets are given for 1991. Although they increase at a rate of 10 to 15% per year[1,2,3], they were assumed constant for the whole operating life of the plant. This is very conservative, but to a certain extent it accounts for unpredictable events that could affect negatively future operations of the plant (recession, fuel increases, etc.).

The hypothesis presented above and other parameters (shown in the spreadsheet in appendix 1) leads to the results given on figure 5.1 and table 5.2.

Under the above assumptions, the milk permeate scale (0.013 millions pounds of permeate per day) does not produce profits. However, if the plant manufacture calf rennet 100% of its operating time, it becomes profitable (73% rate of return, 22% world market share, and 6.4 milion dollars total capital investment).

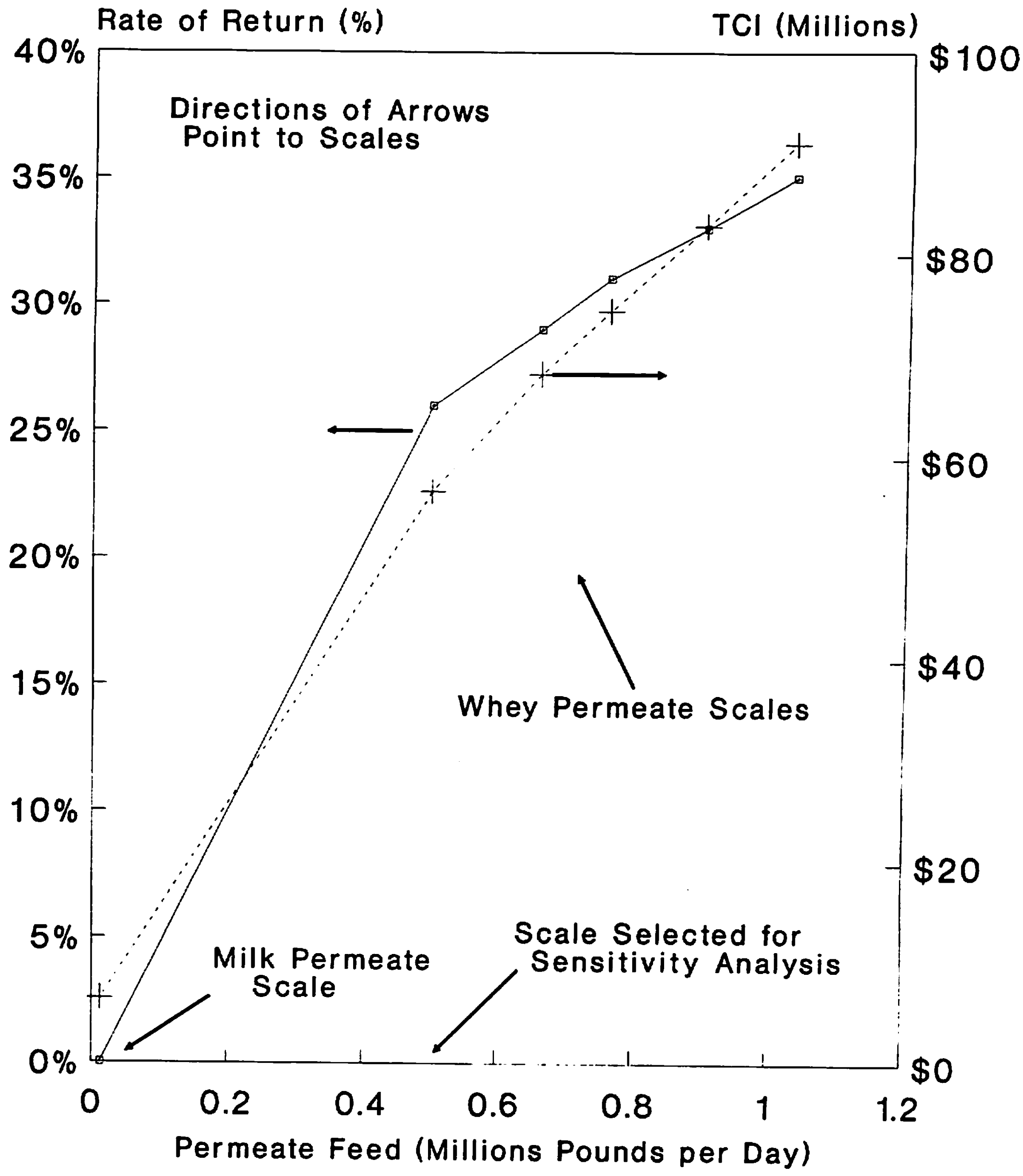


Figure 5.1 Enzymes' Production Profitability Analysis

The scales in the whey permeate show rates of returns from 26 to 35%, which are considered very good. Figure 5.1 shows that the Total Capital Investment (TCI) is almost a linear function of the permeate feed. Such behavior was expected. The payout time (time for the plant to pays for the TCI), was very low in all the whey permeate scales.

Table 5.2 Profitability Results

	Scale (Millions Lb Permeate/Day)					
	0.013	0.50	0.66	0.76	0.90	1.03
Total Production Cost (Millions $/yr)	1.8	22.4	28.1	31.3	35.7	40.0
Disposal Revenues (M$/yr)	1	56	74	85	101	116
Total Revenues (Millions $/yr)	1.2	47.5	63.4	72.6	85.8	99.1
Annual Profits (Millions $/yr)	-0.6	25.1	35.3	41.4	50.2	59.1
Rate of Return (%)	0	26	29	31	33	35
Return on Investment (%)	0	44	52	56	61	65
Payout Time (Yr)	-	2.0	1.7	1.6	1.5	1.4
Total Capital Investment (Millions $)	6.4	56.6	68.1	74.3	82.8	90.9
World Market Share (%)						
Bacillus Proteases	0.07	3	4	4	5	6
Glucose Isomerase	1	30	40	45	54	62
Calf Rennet	1	45	60	68	81	93

When the world market share is analyzed (table 5.2), a limitation of the plant shows up: the larger scales are not reasonable because the assumption that a plant would gain more than 50% of the world market of an enzyme, is very weak. Based on that, the sensitivity analysis was only performed on the smallest profitable scale (0.5 millions pounds of permeate per day). It should be recognized that the results obtained in any scale apply equally to other scales.

The disposal revenues represent the difference between the savings obtained by not putting permeate into land or sewage (\$3/1000 gal)[5], and the costs of disposing brackish water (\$2/1000 gal)[5] (from the resins' regeneration), dead cells (\$5/1000 gal)[5], and sodium sulfate (\$3/1000 gal)[5].

The total world whey production in 1987 was estimated at 100 millions metric tons[4]. It grows at 2% per year[5]. Based on that, the 1991 world whey production is close to 108 millions metric tons. In 1982, the US share of such production was 17% and 45% of that was discarded[6]. Assuming the same percentages for 1982 and 1991, the US would be wasting close to 8 millions metric tons of whey per year. The largest plant scale is 1.03 millions pounds per day. It represents less than 2% of the total whey disposed in the US, and would be satisfying close to 62 and 93% of the world market of glucose isomerase and calf rennet. This is not reasonable. Consequently, under the present conditions, the CES can not be claimed as a viable solution to solve the whey disposal problems in the US. However, this situation may change if markets for high volume proteins were found.

5.2 Sensitivity

The sensitivity analysis was performed very easily in the spreadsheet by changing the values of the analyzed variable. It is presented on figures 5.2 through 5.7. All the figures show that the TCI is only sensitive to the permeate fed to the plant. It was expected because the magnitudes of the streams are the major factors in the sizes of the equipments. As regard to the rate of return, it is most sensitive to the molar production rate and to the product yield coefficient (Yp/x) (figures 5.2 and 5.3). These two variables are strongly associated to how well the CES will excrete the enzymes. So, they may be regarded as only one parameter.

Because the risks associated with protein denaturalization, the most important yield in the separation system is correlated with the precipitation/centrifugation step. The rate of return is very sensitive to it (figure 5.5).

The facts that even quadruplicating the price of the immobilized lactase (figure 5.6) does not affect at all the economics of the plant, and that the lactose hydrolysis is not an important factor in the installed and operating costs (figure 5.7), imply that future developments efforts should be oriented toward the fermentation and the separation parts of the process.

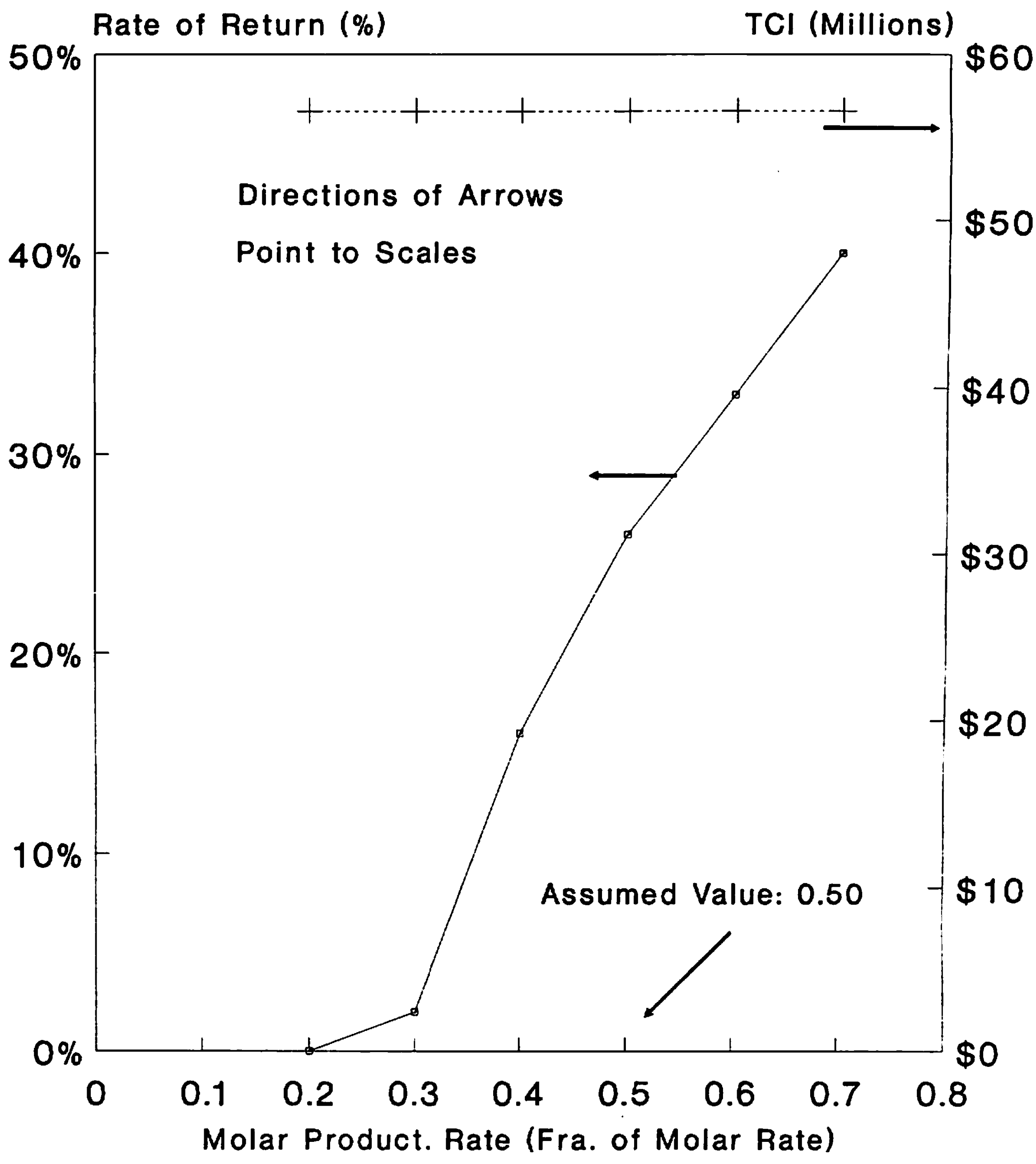


Figure 5.1 Enzymes' Production Sensitivity Analysis (Scale: 0.50 Millions Lb of Permeate/Day)

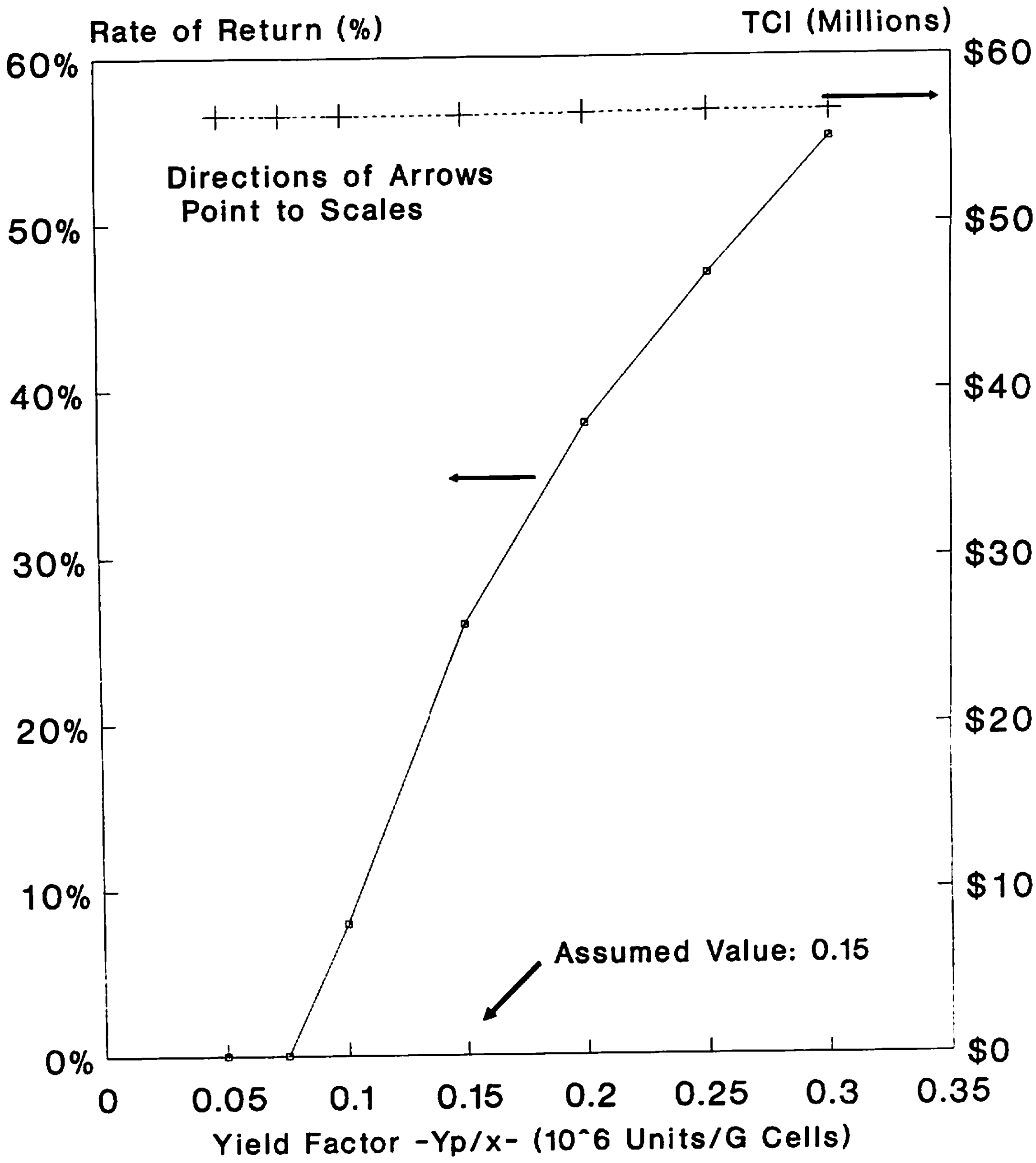


Figure 5.3 Enzymes' Production Sensitivity Analysis (Scale: 0.50 Millions Lb of Permeate/Day)

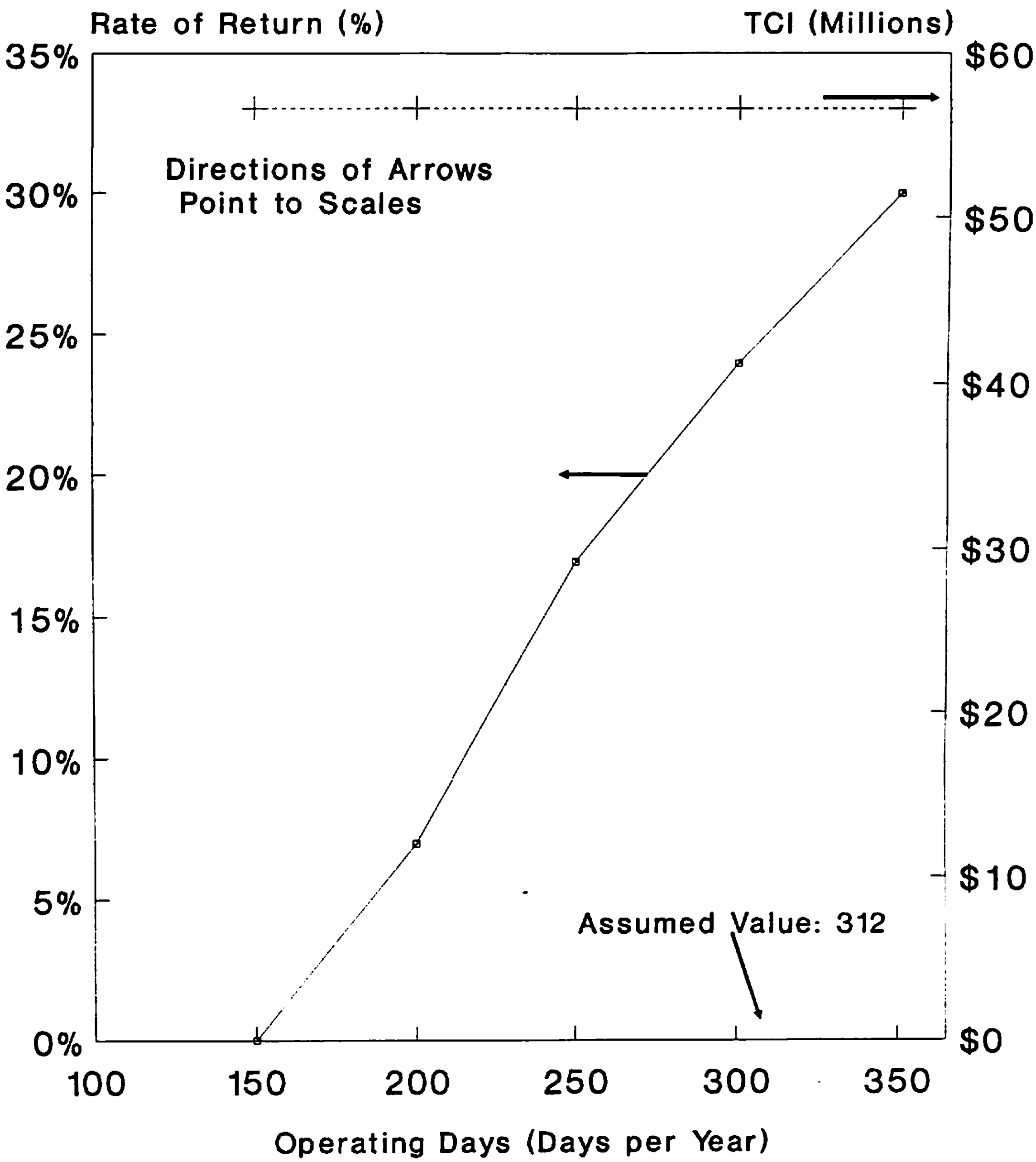


Figure 5.4 Enzymes' Production Sensitivity Analysis (Scale: 0.50 Millions Lb of Permeate/Day)

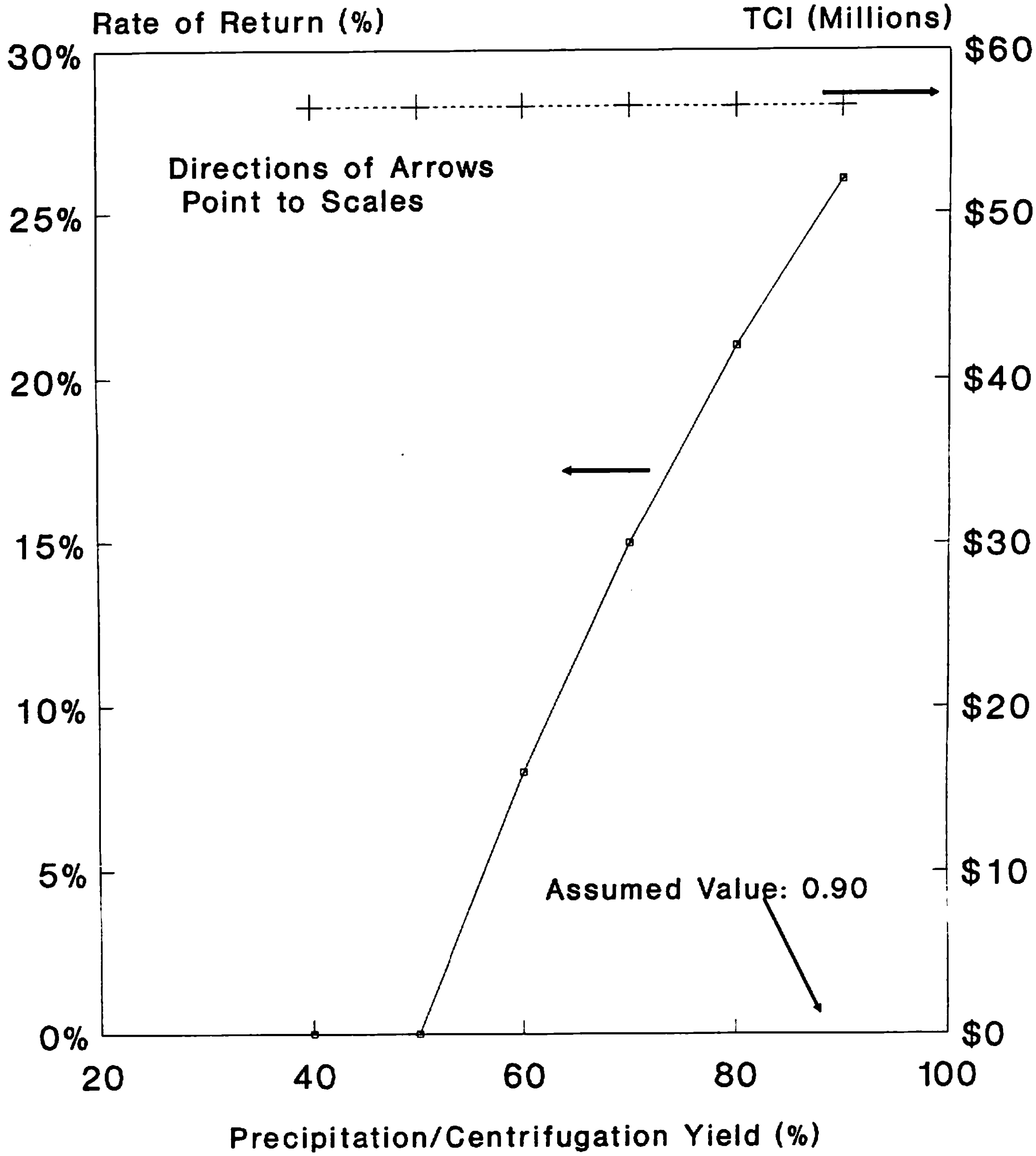


Figure 5.5 Enzymes' Production Sensitivity Analysis (Scale: 0.50 Millions Lb of Permeate/Day)

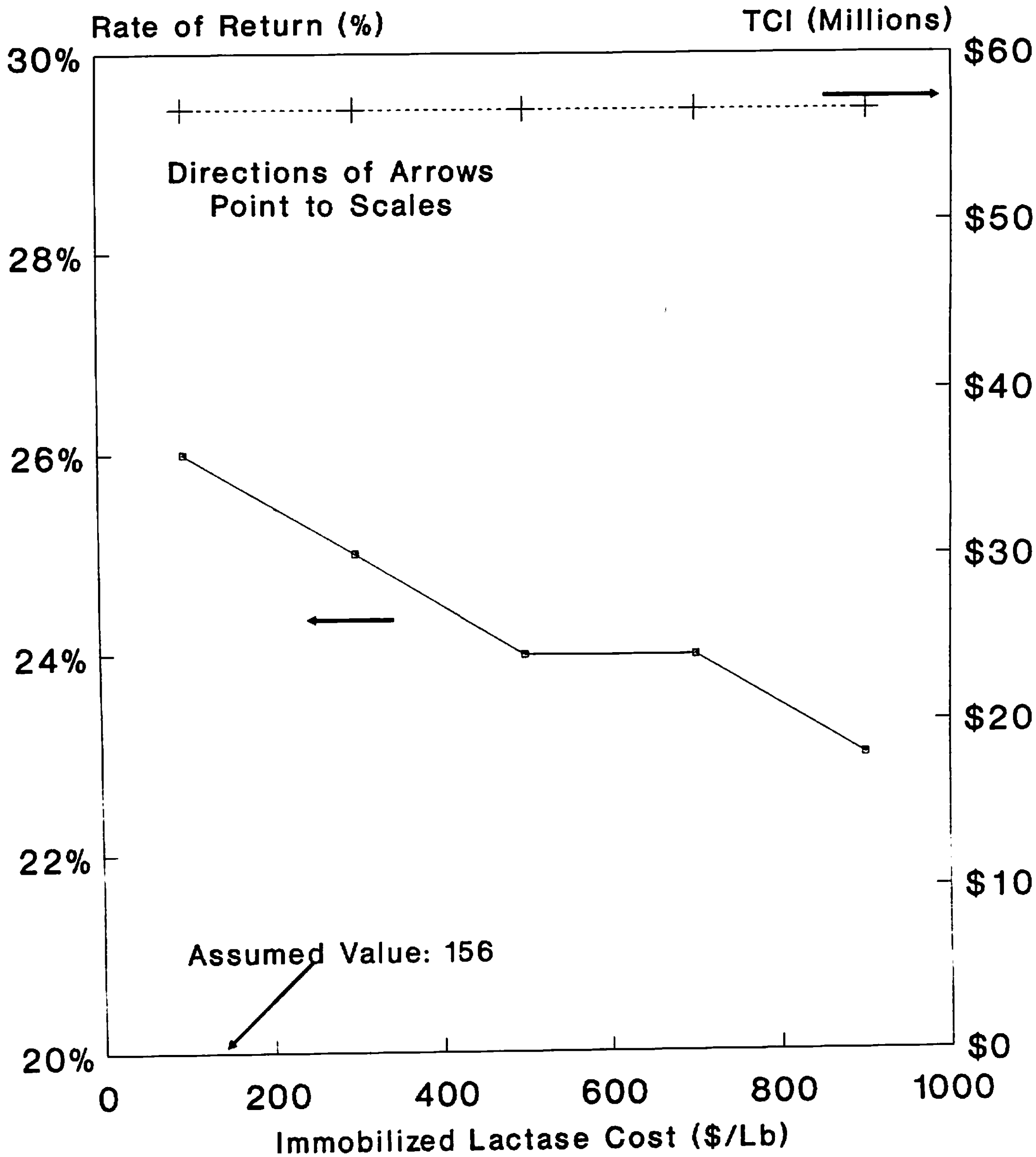
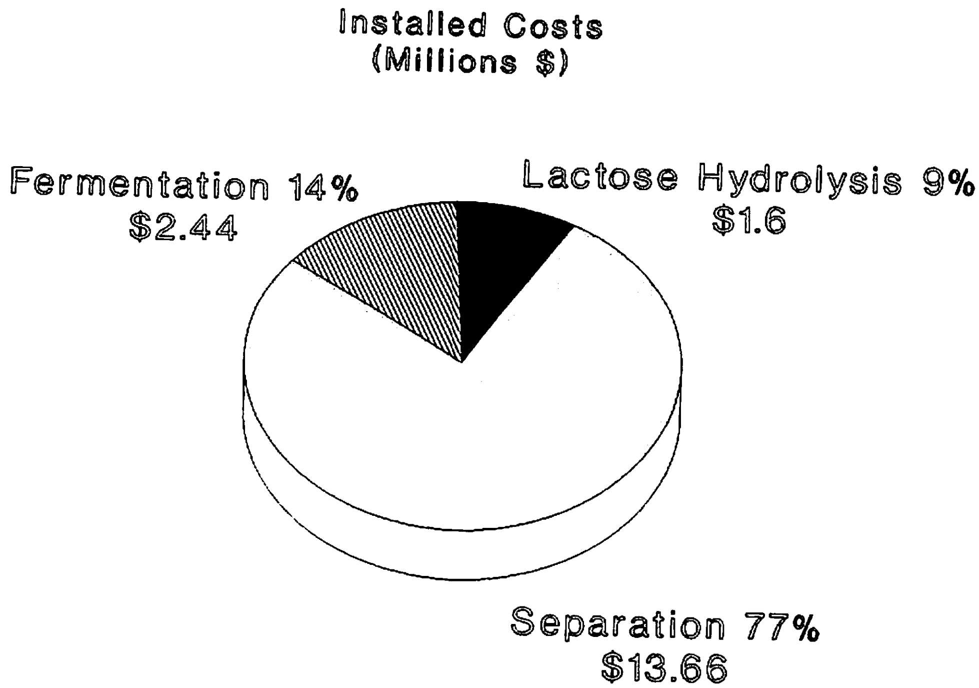


Figure 5.6 Enzymes' Production
Sensitivity Analysis
(Scale: 0.50 Millions Lb of Permeate/Day)



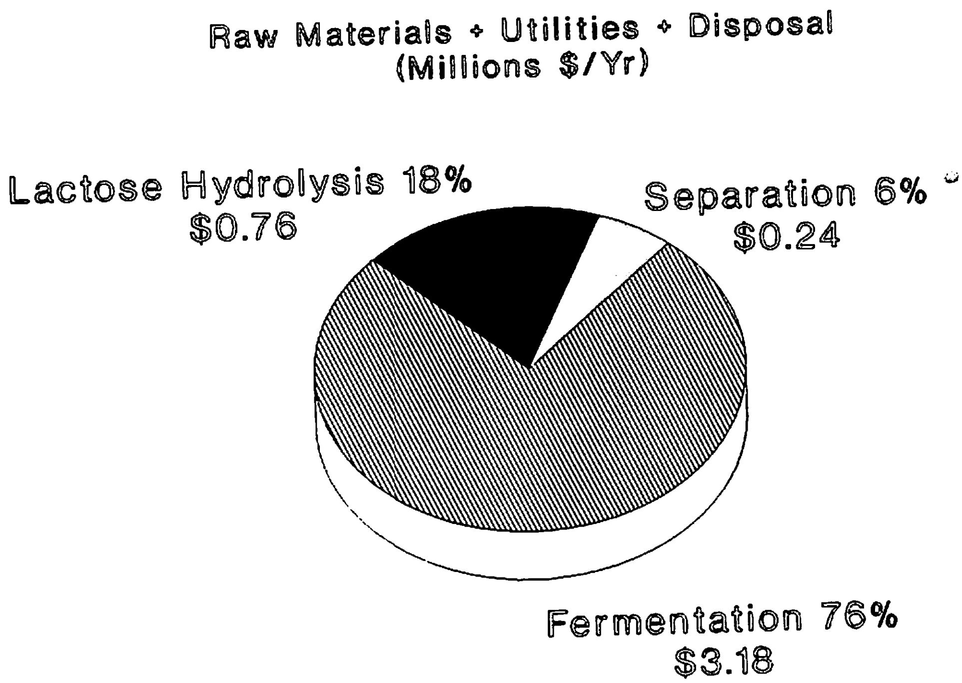


Figure 5.7 Enzymes' Production Cost Comparison (Scale: 0.50 Millions Lb of Permeate/Day)

5.3 References

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Chapter 6

Conclusions and Recommendations

6.1 Conclusions

• Commercial enzyme production utilizing whey permeate and the Cornell Excretion System (CES) is profitable for processing plants with scales greater than 0.5 million pounds of permeate per day, and for smaller operations (0.013 million pounds of permeate per day) producing calf rennet only.

• Under current conditions, large scale operations (0.66 to 1.03 million pounds of permeate per day) are not feasible, because there is not enough enzyme market for their production.

• If new markets for high volume proteins does not appear in the future, the CES can not be claimed as a partial solution to the whey disposal problems in the United States.

6.2 Recommendations

• Because of academic and economic incentives, it is advised that the CES be experimentally tested with lactose from whey permeate as inducer.

• The speadsheet must be kept to perform future economic analysis (when experimental data become available, or when new assumptions be desired).

Appendix 1: DESIGN OF A TWO STAGE REACTOR SYSTEM FOR PRODUCTION OF PROTEINS USING PLASMID-ENCODED GENES IN E. COLI AND WHEY PERMEATE AS COMBINATION SUBSTRATE AND INDUCER

INSTRUCTIONS: SELECT DESIRED VALUES OF THE VARIABLES IN SECTION I OR IN INDIVIDUAL SECTIONS (ONLY THE VARIABLES MARKED WITH AN ASTERIC AT THE RIGHT) AND THEN PRESS --F9-- FOR RUNING THE CALCULATIONS

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PAGE

I. SELECTED DATA AND MAJOR ASSUMPTIONS

I.1. LACTOSE HYDROLYSIS

LACTOSE CONTENT IN PERMEATE (2)	4.7 *	KG/100 KG PERMEATE
PERMEATE TEMPERATURE (1)	50	C
CATION RESIN CAPACITY (68)	3.85 *	EQUIV/LIT
ANION RESIN CAPACITY (69)	3.14 *	EQUIV/LIT
CATION RESIN REGENERANT LEVEL (68)	77 *	G HCL/LIT RESIN
ANION RESIN REGENERANT LEVEL (3)	94 *	G NaOH/LIT RESIN
CATION RESIN PRICE (106)	104.50 *	$/FT3 OF RESIN
ANION RESIN PRICE (106)	200.00 *	$/FT3 OF RESIN
IMMOBILIZED LACTOSE REACTOR		
LACTOSE CONVERSION	0.95 *	-
MICHAELIS CONSTANT -Km- (7)	0.0528 *	MOL-G/LIT
INHIBITION CONSTANT-Ki- (7)	0.0054 *	MOL-G/LIT
TURNOVER NUMBER -K- (7)	0.00006 *	MOL-G/(UNITS*HR)
CATALIST ACTIVITY (7)	300 *	UNITS/G
CATALIST DENSITY (14)	1.32 *	G/CM3
IMMO LACTASE COST (7)	156 *	$/LB
IMMO LACTASE OPER TIME (78)	312 *	DAYS/OPERATING CYCLE
VOID FRACTION (7)	0.35 *	-

I.2. FERMETATION

CELL GROWTH FERMENTOR		
-T1- TEMPERATURE (18)	37	C
pH (18)	7.2	-
- - MAX SP GR RATE (18)	0.7 *	1/HR
-Km- MONOD CONTANT (19)	0.001 *	G GLUCOSE/LIT
-m- MAINTENANCE COEFF (20)	0.2 *	G GLU/(G CELLS*HR)
-Yx/s- YIELD FACTOR (20)	0.48 *	G CELLS/G GLUCOSE
-D1- DILUTION RATE (18)	0.47 *	1/HR
PROTEIN PRODUCTION FERMENTOR		
-T2- TEMPERATURE (18)	25	C
pH (18)	7.2	-
- max- MAX SP GROWTH RATE (18)	0.3 *	1/HR

-Km- MONOD CONSTANT	0.0005 *	G LACTOSE/LIT
-m- MAINTENANCE COEFFICIENT	0.15 *	G LAC/(G CELLS*HR)
-Yp/s- YIELD FACTOR (19)	400 *	UNITS B-LAC/MG LAC
-Yx/s- YIELD FACTOR (21)	0.35 *	G CELLS/G LACTOSE
-Yp/x- YIELD FACTOR	150000 *	UNITS/G CELLS
B-LACTAMASE SPEC ACT (18)	3500 *	UNITS/MG ENZYME
B-LACTAMASE M.W. (18)	29000 *	MG/mMOL
-D2- DILUTION RATE (18)	0.056 *	1/HR
MOLAR PRODUCTION RATE (79)	0.5 *	FRACTION OF MOLAR RATE
INOCULUM SIZE	8 *	% VOLUME OF CELL GROWTH FERMENTOR
ENZYMES' MOLECULAR WEIGHTS		
B. PROTEASES (55)	27500 *	G/MOL-G
CALF CHYMOSIN (56)	40777 *	G/MOL-G
GLUCOSE ISOMERASE (58)	49740 *	G/MOL-G
E. COLI RESPIRATION RATE (22)	11 *	mMOL O2/(G CELLS*HR)

I.3. SEPARATION

CENTRIFUGATION YIELD (47,80)	0.95 *	-
ULTRAFILTRATION YIELD (47,80)	0.95 *	-
PREC/CENTR YIELD (47,80)	0.90 *	-
DIAFILTRATION YIELD (47,80)	0.95 *	-

I.4. UTILITIES

STEAM (150 PSIg) COST (10)	3.4 *	$/1000 LB
COOLING WATER (80 F) COST (10)	0.03 *	$/1000 GAL
CHILLED WATER (50 F) COST (39)	1.35 *	$/1000 GAL
PROCESS WATER COST (DEIONIZED) (74)	0.02 *	$/1000 GAL
ELECTRICITY COST (10)	0.04 *	$/KW-HR

I.5. RAW MATERIALS

HCL 20 Be COST (15)	65 *	$/2000 LB
NaOH 76% COST (5)	560 *	$/2000 LB
ACETIC ACID (5)	0.29 *	$/LB
AMMONIUM SULFATE (5)	122 *	$/2000 LB
SODIUM PHOSPHATE (DIBASIC) (5)	61.25 *	$/1000 LB
POTASSIUM PHOS (MONOBASIC)	70 *	$/1000 LB
ZINC CLORIDE (5)	4.44 *	$/LB
IRON SULFATE	1 *	$/LB
MAGNESIUM SULFATE (5)	0.16 *	$/LB
ANTIFOAM	12 *	$/GAL
SODIUM SULFATE (5)	0.23 *	$/LB
SODIUM BENZOATE (5)	0.89 *	$/LB

I.6. VARIOUS

M & S COST INDEX (104)	925 *	-
CE PLANT COST INDEX (104)	360 *	-
OPERATING DAYS	312 *	DAYS/YEAR
BRACKISH WATER (~ 0.6% NaCL) DISP COST (74)	2 *	$/1000 GAL
Na2SO4 DISPOSAL COST (74)	3 *	$/1000 GAL
CELL STREAM (~ 12%) DISPOSAL COST (74)	5 *	$/1000 GAL

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II. LACTOSE HYDROLYSIS

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II.1. WHEY MATERIAL BALANCES (1,2,24)

	WHEY PERMEATE (1)					MILK PERMEATE (2)	
RAW MILK	720000	960000	1100000	1300000	1500000	25000 *	LB/DAY
RAW WHEY	648000	864000	990000	1170000	1350000	N/A	LB/DAY
WHEY CREAM	5123	6830	7827	9250	10673	N/A	LB/DAY
UNCREAMED WHEY	642877	857170	982174	1160751	1339328	N/A	LB/DAY
RETENTATE	146475	195300	223781	264468	305156	12500	LB/DAY
WHEY/MILK PERMEATE	496403	661870	758393	896282	1034172	12500	LB/DAY

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II.2. PERMEATE COMPOSITION (2,9)

LACTOSE (2)	4.7	4.7	4.7	4.7	4.7	4.7 *	KG/100 KG PERMEATE
POTASSIUM (2)	0.155	0.155	0.155	0.155	0.155	0.155 *	KG/100 KG PERMEATE
MAGNESIUM (2)	0.00771	0.00771	0.00771	0.00771	0.00771	0.00771 *	KG/100 KG PERMEATE
SODIUM (2)	0.0414	0.0414	0.0414	0.0414	0.0414	0.0414 *	KG/100 KG PERMEATE
CALCIUM (2)	0.0305	0.0305	0.0305	0.0305	0.0305	0.0305 *	KG/100 KG PERMEATE
PHOSPHORUS (2)	0.0442	0.0442	0.0442	0.0442	0.0442	0.0442 *	KG/100 KG PERMEATE
LACTIC ACID (118)	0.08	0.08	0.08	0.08	0.08	0.08 *	KG/100 KG PERMEATE
CLORIDE (118)	0.11	0.11	0.11	0.11	0.11	0.11 *	KG/100 KG PERMEATE
IRON (2)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001 *	KG/100 KG PERMEATE
ZINC (2)	0.000025	0.000025	0.000025	0.000025	0.000025	0.000025 *	KG/100 KG PERMEATE

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II.3. DEMINERALIZATION

II.3.1. CATION EXCHANGE (I-120)

-P- PERMEATE FEED	10.73	14.31	16.40	19.38	22.36	0.27	M3/HR
	10732	14309	16396	19377	22358	270	KG/HR
	496403	661870	758393	896282	1034172	12500	LB/DAY
EQUIVALENTS							
POTASSIUM	39.68	39.68	39.68	39.68	39.68	39.68	MEQ/LIT
MAGNESIUM	6.36	6.36	6.36	6.36	6.36	6.36	MEQ/LIT
SODIUM	18.05	18.05	18.05	18.05	18.05	18.05	MEQ/LIT
CALCIUM	15.25	15.25	15.25	15.25	15.25	15.25	MEQ/LIT
IRON	0.04	0.04	0.04	0.04	0.04	0.04	MEQ/LIT
ZINC	0.01	0.01	0.01	0.01	0.01	0.01	MEQ/LIT
TOTAL EQUIVALENTS	79.38	79.38	79.38	79.38	79.38	79.38	MEQ/LIT
RESIN CAPACITY (68)	3.85	3.85	3.85	3.85	3.85	3.85	EQ/LIT
RESIN VOLUME	4.6	6.2	7.1	8.4	9.7	0.1	M3 OF RESIN
M & S COST INDEX	925	925	925	925	925	925	-
INSTALLED COST (4)	167	197	212	232	251	49	M$
RESIN COST	17	23	26	31	36	0	M$
REGENERANT LEVEL (68)	92.1	92.1	92.1	92.1	92.1	92.1	G HCL/LIT RESIN
AMOUNT OF HCL 8% PER REG	5350.0	7133.4	8173.6	9659.7	11145.9	134.7	KG/REG

II.3.2. ANION EXCHANGE (I-130)

EQUIVALNETS							
DIPHOSPHATE	28.60	28.60	28.60	28.60	28.60	28.60	MEQ/LIT
CLORIDE	31.02	31.02	31.02	31.02	31.02	31.02	MEQ/LIT
LACTATE	10.8	10.8	10.8	10.8	10.8	10.8	MEQ/LIT
TOTAL EQUIVALENTS	59.62	59.62	59.62	59.62	59.62	59.62	MEQ/LIT
RESIN CAPACITY (69)	3.14	3.14	3.14	3.14	3.14	3.14	EQ/LIT RESIN
RESIN VOLUME	4.3	5.7	6.5	7.7	8.9	0.1	M3
INSTALLED COST (4)	218	247	261	280	302	53	M$
RESIN COST	30	40	46	55	63	1	M$
REGENERANT LEVEL (3)	94	94	94	94	94	94	G NaOH/LIT RESIN
AMOUNT OF NaOH 4% PER REG	10056	13408	15363	18156	20950	253	Kg/REG

II.3.3. UTILITIES

WORKING DAYS	312	312	312	312	312	312	DAYS/YR
PROCESS WATER (DEIONIZED) COST (74)	0.02	0.02	0.02	0.02	0.02	0.02	$/1000 GAL
ANNUAL WATER (DEIONIZED) COST (9)	0.03	0.04	0.04	0.05	0.06	0.00	M$/YR

HCL 20 Be COST (5)	65	65	65	65	65	65	$/2000 LB
ANNUAL HCL COST (FOR RESIN REG)	30	40	46	54	62	1	M$/YR
NaOH 76% COST (76)	560	560	560	560	560	560	$/2000 LB
ANNUAL NaOH COST (FOR RESIN REG)	104	138	158	187	216	3	M$/YR

II.3.4. SEWAGE

NaOH 76% FOR NEUTRALIZATION	9	12	13	16	18	0	KG/REG
BRACKISH WATER WASTED	28456	37941	43474	51379	59283	717	GAL/REG
NACL IN WASTED WATER	0.6	0.6	0.6	0.6	0.6	0.6	%
SODIUM ACETATE IN WASTED WATER	0.0003	0.0003	0.0003	0.0003	0.0003	0.0003	%
BRACKISH WATER DISP COST (74)	2	2	2	2	2	2	$/1000 GAL
ANNUAL BRACKISH WATER DISP COST	18	24	27	32	37	0.4	M$/YR
ANNUAL NAOH 76% COST (FOR NEUT.)	1.68	2.24	2.57	3.04	3.50	0.04	M$/YR

II.4. STREAMS' DISTRIBUTION

-H1/H2-	1	1	1	1	1	1 *	-
-H2- UNCONVERTED STRM TO UF	5366	7155	8198	9688	11179	135	KG/HR
-H2- UNCONVERTED STRM UF	5.4	7.2	8.2	9.7	11.2	0.1	M3/HR
-H1- STRM TO pH AJUSTMENT TANK	5366	7155	8198	9688	11179	135	KG/HR
-H1- STRM TO pH AJUSTMENT TANK	5.4	7.2	8.2	9.7	11.2	0.1	M3/HR
-N- NaOH 50% REQUIRED	8	11	13	15	17	0	KG/HR
-H1'- STRM OUT OF IMMO LACTOSE REACT	5374	7166	8211	9703	11196	135	KG/HR
-H1'- STRM OUT OF IMMO LACTOSE REACT	5.4	7.2	8.2	9.7	11.2	0.1	M3/HR
-H2'- STRM OUT OF UF	2100	2799	3208	3791	4374	53	KG/HR
-H2'- STRM OUT OF UF	2.1	2.8	3.2	3.8	4.4	0.1	M3/HR

II.5. pH AJUSTMENT

pH IN P STREAM	6.0	6.0	6.0	6.0	6.0	6.0 *	-
pH IN H1 STREAM	1.7	1.7	1.7	1.7	1.7	1.7	-
pH IN H1' STREAM	3.5	3.5	3.5	3.5	3.5	3.5	-
NaOH 50% REQUIRED	175	234	268	317	365	4	KG/DAY
-N- NaOH 50% REQUIRED	8	11	13	15	17	0	KG/HR
ANNUAL NaOH COST	22	30	34	40	46	1	M$

II.6. TEMPERATURE AJUSTMENT

HEAT EXCHANGER (E-160)

PERMEATE INLET TEMP	50	50	50	50	50	50	C
HX DUTY	0.32	0.43	0.49	0.58	0.67	0.01	MILLIONS BTU/HR
OVERALL HEAT TRANSFER COE (10),(25)	650	650	650	650	650	650 *	BTU/HR*F*FT2
LOG MEAN TEMP	16	16	16	16	16	16	F
HX AREA	29.88	39.84	45.65	53.95	62.26	0.75	FT2
CE PLANT COST INDEX	360	360	360	360	360	360	-
HX INSTALLED COST (13)	70	79	84	90	96	15	M$

HX PRESSURE DROP

-A- PLATE AREA (14)	1.11	1.29	1.38	1.51	1.62	0.16	FT2
-n- NUMBER OF THERMAL PLATES (12)	27	31	33	36	38	5	-
-Nc- NUMBER OF CHANNELS	14	16	17	18	20	3	-
-Mc- CHANNEL FLOW RATE	0.24	0.27	0.30	0.32	0.35	0.03	LB/S
-Sc- FLOW AREA PER CHANNEL	0.003	0.003	0.003	0.004	0.004	0.000	FT2
-m- MASS VELOCITY	84.9	85.3	85.5	85.7	85.8	72.3	LB/(FT2*S)
-Re- REYNOLD NUMBER	3279	3294	3301	3308	3314	2791	-
-f- FRICTION FACTOR	0.13	0.13	0.13	0.13	0.13	0.14	-
- Pc- CHANNEL PRESSURE DROP (12)	1	1	1	1	1	1	PSI
-V- PORT VELOCITY (26)	7.5	8	8.5	8.7	9	6 *	FT/S
- Pp- PORT PRESSURE DROP (23)	1	1	1	1	1	0.4	PSI
- P- TOTAL PRESSURE DROP	2	2	2	2	2	1	PSI

HX UTILITIES

COOLING WATER COST (80 F) (10)	0.15	0.15	0.15	0.15	0.15	0.15	$/1000 LB
COOLING WATER ANNUAL COST	2	2	2	3	3	0.0	M$/YR

II.7. IMMOBILIZED LACTASE REACTOR (R-170)

INITIAL LACTOSE CONC	0.1372	0.1372	0.1372	0.1372	0.1372	0.1372	MOL-G/LIT
CONVERSION	0.95	0.95	0.95	0.95	0.95	0.95	-
FINAL LACTOSE CONC	0.0069	0.0069	0.0069	0.0069	0.0069	0.0069	MOL-G/LIT
MICHAELIS CONSTANT -Km- (7)	0.0528	0.0528	0.0528	0.0528	0.0528	0.0528	MOL-G/LIT
INHIBITION CONSTANT-Ki- (7)	0.0054	0.0054	0.0054	0.0054	0.0054	0.0054	MOL-G/LIT
TURNOVER NUMBER -K- (7)	0.00006	0.00006	0.00006	0.00006	0.00006	0.00006	MOL-G/(UNITS*HR)
REQ IMMO LACTASE ACTIVITY	2.72E+08	3.62E+08	4.15E+08	4.91E+08	5.66E+08	6.84E+06	UNITS
CATALIST ACTIVITY (7)	300	300	300	300	300	300	UNITS/G
CATALIST DENSITY (6)	1.32	1.32	1.32	1.32	1.32	1.32	G/CM3
IMMO LACTASE VOLUME	0.69	0.91	1.05	1.24	1.43	0.02	M3
IMMO LACTASE COST	156	156	156	156	156	156	$/LB
IMMO LACTASE OPER TIME	312	312	312	312	312	312	DAYS/OPERATING CYC
ANNUAL LACTASE COST	311	415	475	562	648	8	M$/YR
FREE REACTOR SPACE	20	20	20	20	20	20	%
REACTOR VOLUME	0.8	1.1	1.3	1.5	1.7	0.02	M3
H/D (10)	10	10	10	10	10	10 *	-
REACTOR DIAMETER	1.55	1.70	1.78	1.88	1.98	0.45	FT
REACTOR HEIGHT	15.48	17.03	17.82	18.84	19.76	4.54	FT
REACTOR COST (10)	29	35	38	42	46	3	M$
REACTOR BACK-FLUSHING							
CONCENTRATION ACETIC ACID USED	99.5	99.5	99.5	99.5	99.5	99.5	%
TIME FOR BACK-FLUSHING (86)	0.75	0.75	0.75	0.75	0.75	0.75 *	HR
EXCESS FLOW FOR BACK-FLUSHING	30	30	30	30	30	30 *	%
KG ACETIC ACID 99.5% USED (86)	0.21	0.28	0.32	0.38	0.44	0.01	KG/REGENERRATION
ACETIC ACID PRICE (5)	0.29	0.29	0.29	0.29	0.29	0.29	$/LB
ACETIC ACID ANNUAL COST	0.04	0.06	0.06	0.08	0.09	0.001	M$/YR
SOL FOR REACTOR BACKFLUSHING	9	12	13	16	18	0	M3/REG
REACTOR PRESSURE DROP							
DEPTH OF BED	13	14	15	16	16	4	FT
CATALYST DIAMETER (7)	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015 *	FT
VOID FRACTION (7)	0.4	0.4	0.4	0.4	0.4	0.4	-
SUPERFICIAL MASS VEL	2	2	2	2	2	1	LB/S*FT2
REYNOLDS NUMBER	3.90	4.30	4.50	4.75	4.99	1.14	-
FRICTION FACTOR (6)	26	23	22	21	20	88	-
PRESSURE DROP	0.04	0.05	0.06	0.06	0.07	0.00	PSI

II.8. LACTOSE CONCENTRATION

MATERIAL BALANCES							
MAXIMUM LACTOSE CONCENTRATION	140	140	140	140	140	140	G/LIT
-LH2- LACT CONC IN H2	47	47	47	47	47	47	G/LIT
-LH2'- LACT CONC IN H2'	120	120	120	120	120	120 *	G/LIT
-H2- STREAM TO UF	5366	7155	8198	9688	11179	135	KG/HR
-H2'- STREAM OUT OF UF	2100	2799	3208	3791	4374	53	KG/HR
-W- WATER SEPARATED	3266	4355	4990	5898	6805	82	KG/HR
ULTRAFILTRATION (P-195)							
UF OPERATING COST (38)	260	300	321	349	375	41	M$/YR
UF INSTALLED COST (38)	454	516	548	591	630	88	M$

II.9. PUMPS (16,13,9)

ION EXCHANGE PUMP (J-110)

TOTAL PRES DROP IN BEDS (9)	35	35	35	35	35	35 *	PSI
CAPACITY	47	63	72	85	98	1	GPM
HEAD	81	81	81	81	81	81	FT
INSTALLED COST (93,94)	3	3	3	3	3	2	M$
POWER CONSUMTION (16,13)	3	3	3	4	4	0.05	HP
pH AJUSTMENT PUMP (J-141)							
CAPACITY	0.0240	0.0320	0.0367	0.0434	0.0500	0.0006	GPM
INSTALLED COST (16)	145	168	181	197	212	21	M$
HX AND COLUMN PUMP (J-150)							
TOTAL PRESSURE DROP	2	2	2	2	2	1	PSI
CAPACITY	24	32	36	43	49	1	GPM
HEAD	4	5	5	5	5	3	FT
INSTALLED COST (93,94)	2	2	2	2	2	2	M$
POWER CONSUMTION (16,13)	0	0	0	0	0	0.00	HP
REACTOR BACK-FLUSHING PUMP (J-180)							
EXCESS FLOW FOR BACK-FLUSHING	30	30	30	30	30	30	%
TOTAL PRESSURE DROP	0.04	0.05	0.06	0.06	0.07	0.00	PSI
CAPACITY	31	41	47	56	64	1	GPM
HEAD	0	0	0	0	0	0	FT
INSTALLED COST (93,94)	2	2	2	2	2	2	M$
POWER CONSUMTION (16,13)	0	0	0	0	0	0.00	HP
CONVERTED STEAM PUMP (J-192)							
ONLINE MIXERS PRESS DROP	9	9	9	9	9	9	PSI
HEAT EXCHANGERS PRESS DROP	10	11	11	11	11	6	PSI
TOTAL PRESSURE DROP	19	20	20	20	20	15	PSI
CAPACITY	21	28	32	38	43	1	GPM
HEAD	44	45	46	46	47	34	FT
INSTALLED COST (93,94)	2	2	2	2	2	2	M$
POWER CONSUMTION (16,13)	1	1	1	1	1	0.01	HP
UNCONVERTED STREAM PUMP (J-193)							
ONLINE MIXERS PRESS DROP	9	9	9	9	9	9	PSI
HEAT EXCHANGERS PRESS DROP	2	2	3	3	3	1	PSI
TOTAL PRESSURE DROP	11	11	12	12	12	10	PSI
CAPACITY	8	11	12	15	17	0	GPM
HEAD	26	26	27	27	27	24	FT
INSTALLED COST (93,94)	2	2	2	2	2	2	M$
POWER CONSUMTION (16,13)	0.07	0.09	0.11	0.13	0.15	0.00	HP

II.10. MIXERS AND TANKS (13,28,16)

NaOH DILUTION MIXER (M-142)							
VOLUME	33	44	51	60	69	1	GAL
VOLUME	0.1	0.2	0.2	0.2	0.3	0.00	M3
MIXER COST (28)	5	6	6	7	7	1	M$
POWER CONSUMTION (13)	0.06	0.07	0.08	0.10	0.12	0.00	HP
pH AJUSTMENT MIXER (M-140)							
VOLUME	780	1040	1191	1408	1624	20	GAL
VOLUME	3	4	5	5	6	0.1	M3
MIXER COST (28)	27	31	34	37	40	4	M$
POWER CONSUMPTION (13)	1	2	2	2	3	0	HP
SEWAGE MIXER (M-121)							
VOLUME	31301	41735	47822	56516	65211	788	GAL
VOLUME	118	158	181	214	247	3	M3

MIXER COST (28)	197	231	248	272	294	27	M$
POWER CONSUMPTION (13)	52	70	80	94	109	1	HP
ACETIC ACID MIXER (M-190)							
VOLUME	3345	4460	5111	6040	6969	84	GAL
VOLUME	13	17	19	23	26	0	M3
MIXER COST (28)	59	69	74	81	87	8	M$
POWER CONSUMPTION (13)	6	7	9	10	12	0	HP
CONVERTED STREAM STORAGE TANK (TT-171)							
VOLUME	4686	6247	7159	8460	9762	118	GAL
VOLUME	18	24	27	32	37	0	M3
TANK COST (16)	72	78	81	84	87	10	M$
UNCONVERTED STREAM STORAGE TANK (TT-172)							
VOLUME	4678	6238	7147	8447	9746	118	GAL
VOLUME	18	24	27	32	37	0	M3
TANK COST (16)	72	78	81	84	87	10	M$
FEED TANK (TT-100)							
VOLUME	1559	2078	2381	2814	3247	39	GAL
VOLUME	6	8	9	11	12	0	M3
TANK COST (16)	48	54	57	61	64	4	M$

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II.11. POWER CONSUMTION

TOTAL POWER CONSUMTION	63	83	95	112	129	2	HP
ANNUAL POWER COST	14	19	21	25	29	0	M$

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III. FERMENTATION REACTORS' SYSTEM

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III.1. STREAMS' DEFINITION

-P- PERMEATE FEED	10732	14309	16396	19377	22358	270	KG/HR
-Fo/F3-	2.5	2.5	2.5	2.5	2.5	2.5	-
-LH2'=LF3- LACT IN UNCONVERTED FEED	120	120	120	120	120	120	G/LIT
-LFo=LF1- LACT IN CONVERTED FEED	2.35	2.35	2.35	2.35	2.35	2.35	G/LIT
-F3- SUP FEED TO SECOND REAC	1948	2597	2976	3517	4059	49	KG/HR
-F3- SUP FEED TO SECOND REAC	1.9	2.6	3.0	3.5	4.0	0.0	M3/HR
-F1=Fo- FEED TO FIRST REACTOR	4811	6414	7349	8686	10022	121	KG/HR
-F1=Fo- FEED TO FIRST REACTOR	4.8	6.4	7.3	8.7	10.0	0.1	M3/HR
-STo- SALTS ADDED TO Fo	81	108	123	146	168	2	KG/HR
-ST3- SALTS ADDED TO F3	100	133	153	181	209	3	KG/HR
-F2'=F1 + F3- DUMMY STREAM	6759	9012	10326	12203	14081	170	KG/HR
-LF2'- LACTOSE TO SECOND REACTOR	36	36	36	36	36	36	G/LIT
-F2- STRM LEAVING SECOND REACTOR	6759	9012	10326	12203	14081	170	KG/HR
-H1''- CONVERTED STREAM TO M-380	4729	6306	7225	8539	9853	119	KG/HR
-H1'''- CONVERTED STREAM TO M-385	4729	6306	7226	8539	9853	119	KG/HR
-H1*- CONVERTED STRM FOR MEDIUM	13	17	20	23	27	0	KG/HR
-H2''- UNCONVERTED STREAM TO M-390	1848	2463	2823	3336	3849	47	KG/HR
-H2'''- UNCONVERTED STREAM TO M-395	1848	2463	2823	3336	3849	47	KG/HR
-H2*- UNCONVERTED STRM FOR MEDIUM	12	15	18	21	24	0.29	KG/HR

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III.2. pH AJUSTMENT

pH IN CONVERTED STREAM	3.5	3.5	3.5	3.5	3.5	3.5	-
pH IN UNCONVERTED STREAM	1.7	1.7	1.7	1.7	1.7	1.7	-
pH IN FERMENTORS (27)	7.2	7.2	7.2	7.2	7.2	7.2	-

NaOH 50% REQUIRED FOR H1'' STREAM	3	4	4	5	6	0.1	KG/DAY
-N1- NaOH 50% REQUIRED FOR H1'' STRM	0.12	0.16	0.18	0.22	0.25	0.00	KG/HR
NaOH 50% REQUIRED FOR H2' STREAM	1.1	1.5	1.7	2.0	2.3	0.03	KG/DAY
-N2- NaOH 50% REQUIRED FOR H2' STRM	0.05	0.06	0.07	0.08	0.10	0.00	KG/HR
TOTAL NaOH 50% REQUIRED	4	5	6	7	8	0.1	KG/DAY
ANNUAL NaOH COST	0.5	0.7	0.8	0.9	1.1	0.01	M$/YR
CONVERTED STREAM PIPE DIAMETER (16)	1.7	1.9	2.0	2.2	2.3	0.3	IN
UNCONVERTED STREAM PIPE DIAMETER (16	1.1	1.2	1.3	1.4	1.5	0.2	IN
MIXERS COST (M-380 ˆ M-385) (13)	4	5	5	5	6	2	M$
MIXERS COST (M-390 ˆ M-395) (13)	3	3	4	4	4	2	M$

III.3. MEDIUM FORMULATION

AMMONIUM SULFATE IN Fo	0.1028	0.1028	0.1028	0.1028	0.1028	0.1028	MOL-G/LIT
SODIUM PHOSPHATE (DIBASIC) IN Fo	0.0146	0.0146	0.0146	0.0146	0.0146	0.0146	MOL-G/LIT
POTASSIUM PHOS (MONOBASIC) IN Fo	0.0053	0.0053	0.0053	0.0053	0.0053	0.0053	MOL-G/LIT
ZINC CLORIDE IN Fo	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014	MOL-G/LIT
IRON SULFATE IN Fo	0.0007	0.0007	0.0007	0.0007	0.0007	0.0007	MOL-G/LIT
MAGNESIUM SULFATE IN Fo	0.0042	0.0042	0.0042	0.0042	0.0042	0.0042	MOL-G/LIT
-To- TOTAL SALT CONC IN Fo	17	17	17	17	17	17	G/LIT
-Fo-	4811	6414	7349	8686	10022	121	KG/HR
-H1*-	13	17	20	23	27	0.32	KG/HR
AMMONIUM SULFATE IN F2'	0.0965	0.0965	0.0965	0.0965	0.0965	0.0965	MOL-G/LIT
SODIUM PHOSPHATE (DIBASIC) IN F2'	0.0094	0.0094	0.0094	0.0094	0.0094	0.0094	MOL-G/LIT
POTASSIUM PHOS (MONOBASIC) IN F2'	0.0034	0.0034	0.0034	0.0034	0.0034	0.0034	MOL-G/LIT
ZINC CLORIDE IN F2'	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	MOL-G/LIT
IRON SULFATE IN F2'	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	MOL-G/LIT
MAGNESIUM SULFATE IN F2'	0.0027	0.0027	0.0027	0.0027	0.0027	0.0027	MOL-G/LIT
AMMONIUM SULFATE IN F3	0.33	0.33	0.33	0.33	0.33	0.33	MOL-G/LIT
SODIUM PHOSPHATE (DIBASIC) IN F3	0.03	0.03	0.03	0.03	0.03	0.03	MOL-G/LIT
POTASSIUM PHOS (MONOBASIC) IN F3	0.01	0.01	0.01	0.01	0.01	0.01	MOL-G/LIT
ZINC CLORIDE IN F3	0.00	0.00	0.00	0.00	0.00	0.00	MOL-G/LIT
IRON SULFATE IN F3	0.00	0.00	0.00	0.00	0.00	0.00	MOL-G/LIT
MAGNESIUM SULFATE IN F3	0.01	0.01	0.01	0.01	0.01	0.01	MOL-G/LIT
-T3- TOTAL SALT CONC IN F3	52	52	52	52	52	52	G/LIT
-F3-	1948	2597	2976	3517	4059	49	KG/HR
-H2*-	12	15	18	21	24	0	KG/HR
AMMONIUM SULFATE IN H1*	38.46	38.46	38.46	38.46	38.46	38.46	MOL-G/LIT
SODIUM PHOSPHATE (DIBASIC) IN H1*	5.45	5.45	5.45	5.45	5.45	5.45	MOL-G/LIT
POTASSIUM PHOS (MONOBASIC) IN H1*	2.00	2.00	2.00	2.00	2.00	2.00	MOL-G/LIT
ZINC CLORIDE IN H1*	0.54	0.54	0.54	0.54	0.54	0.54	MOL-G/LIT
IRON SULFATE IN H1*	0.28	0.28	0.28	0.28	0.28	0.28	MOL-G/LIT
MAGNESIUM SULFATE IN H1*	1.58	1.58	1.58	1.58	1.58	1.58	MOL-G/LIT
AMMONIUM SULFATE IN H2*	56.23	56.23	56.23	56.23	56.23	56.23	MOL-G/LIT
SODIUM PHOSPHATE (DIBASIC) IN H2*	5.45	5.45	5.45	5.45	5.45	5.45	MOL-G/LIT
POTASSIUM PHOS (MONOBASIC) IN H2*	2.00	2.00	2.00	2.00	2.00	2.00	MOL-G/LIT
ZINC CLORIDE IN H2*	0.54	0.54	0.54	0.54	0.54	0.54	MOL-G/LIT
IRON SULFATE IN H2*	0.28	0.28	0.28	0.28	0.28	0.28	MOL-G/LIT
MAGNESIUM SULFATE IN H2*	1.58	1.58	1.58	1.58	1.58	1.58	MOL-G/LIT
ANNUAL COSTS							
AMMONIUM SULFATE	152	203	233	275	318	4	M$/YR
SODIUM PHOSPHATE (DIBASIC)	162	216	247	292	337	4	M$/YR
POTASSIUM PHOS (MONOBASIC)	77	102	117	139	160	2	M$/YR

ZINC CLORIDE	132	177	202	239	276	3	M$/YR
IRON SULFATE	17	23	26	30	35	0	M$/YR
MAGNESIUM SULFATE	12	16	19	22	26	0	M$/YR
TOTAL ANNUAL COST	553	737	844	998	1151	14	M$/YR

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III.4. STERILIZATION

III.4.1. CONVERTED STREAM

-Fo- STREAM TO STERILIZE	4811	6414	7349	8686	10022	121	KG/HR
-D- CONVERTED STRM PIPE DIAM (16)	1.7	1.9	2.0	2.2	2.3	0.3	IN
-Re- REYNOLD NUMBER	39989	46845	50487	55345	59877	5279	-
-V- LINEAR VELOCITY	0.94	0.97	0.98	1.00	1.01	0.65	M/S
-E/(V*D)- (35)	0.275	0.271	0.270	0.268	0.266	0.323	-
-E- AXIAL DISPERSION COEFFICIENT	0.007	0.007	0.007	0.007	0.007	0.005	M2/S
-La- ASSUMED STERILIZER LENGHT	0.25	0.26	0.27	0.27	0.27	0.2	M
-Pe- PECLET NUMBER	36	38	39	40	40	24	-
-N/No- STER CRITERIA (18, 34)	9.6E-20	7.2E-20	6.3E-20	5.3E-20	4.6E-20	3.8E-18	-
-Nr- REACTION NUMBER (34)	100	99	95	92	91	105	-
-Lc- CALCULATED STERILIZER LENGHT	0.25	0.25	0.25	0.24	0.24	0.18	M
-Ld- DESIGN STERILIZER LENGHT	0.3	0.3	0.3	0.3	0.3	0.3	M

HEAT EXCHANGERS

-Fo- FEED TO CELL GROWTH REACTOR	4811	6414	7349	8686	10022	121	KG/HR
-Fo- FEED TO CELL GROWTH REACTOR	4.8	6.4	7.3	8.7	10.0	0.1	M3/HR

E-312

-Td1- TEMP STER STRM OUT OF E-312	70	70	70	70	70	70 *	C
HX DUTY	1	2	2	2	3	0.03	MILLIONS BTU/HR
-Tb1- TEMP OF Fo OUT OF E-312	105	105	105	105	105	105	C
-U- OVERALL HEAT TRANSFER COEF	650	650	650	650	650	650 *	BTU/(HR*F*FT2)
- Tln- LOG MEAN TEMP	63	63	63	63	63	63	F
-A- AREA	33	43	50	59	68	1	FT2
INSTALLED COST	73	82	87	93	99	16	M$
PRESSURE DROP							
-A- PLATE AREA (14)	1.05	1.22	1.31	1.42	1.53	0.16	FT2
-n- NUMBER OF THERMAL PLATES (12)	31	36	38	41	44	5	-
-Nc- NUMBER OF CHANNELS	16	18	20	21	23	3	-
-Mc- CHANNEL FLOW RATE	0.18	0.21	0.23	0.25	0.27	0.02	LB/S
-Sc- FLOW AREA PER CHANNEL	0.003	0.003	0.003	0.004	0.004	0.000	FT2
-m- MASS VELOCITY	69.99	70.27	70.40	70.53	70.65	60.76	LB/(FT2*S)
-Re- REYNOLD NUMBER	2703	2714	2719	2724	2728	2347	-
-f- FRICTION FACTOR	0.01	0.01	0.01	0.01	0.01	0.01	-
- Pc- CHANNEL PRESSURE DROP (23)	0.08	0.08	0.08	0.08	0.08	0.07	PSI
-V- PORT VELOCITY (26)	7.5	8	8.5	8.7	9	6 *	FT/S
- Pp- PORT PRESSURE DROP (23)	1	1	1	1	1	0	PSI
- P- TOTAL PRESSURE DROP	1	1	1	1	1	0	PSI

E-311

HX DUTY	0.7	0.9	1.0	1.2	1.4	0.0	MILLIONS BTU/HR
-U- OVERALL HEAT TRANSFER COEF	650	650	650	650	650	650 *	BTU/(HR*F*FT2)
- Tln- LOG MEAN TEMP	111	111	111	111	111	111	F
-A- AREA	9	12	14	17	19	0	FT2
INSTALLED COST	43	48	51	55	58	9	M$
STEAM (150 PSIg) CONSUMPTION	18716	24955	28594	33793	38992	471	LB/DAY
STEAM (150 PSIg) COST	3.4	3.4	3.4	3.4	3.4	3.4	$/1000 LB
ANNUAL STEAM COST	20	26	30	36	41	0	M$/YR
PRESSURE DROP							
-A- PLATE AREA (14)	1.05	1.22	1.31	1.42	1.53	0.16	FT2
-n- NUMBER OF THERMAL PLATES (12)	9	10	11	12	13	1	-
-Nc- NUMBER OF CHANNELS	5	6	6	6	7	1	-

-Mc- CHANNEL FLOW RATE	0.60	0.71	0.76	0.84	0.91	0.06	LB/S
-Sc- FLOW AREA PER CHANNEL	0.003	0.003	0.003	0.004	0.004	0.000	FT2
-m- MASS VELOCITY	229	232	234	235	236	153	LB/(FT2*S)
-Re- REYNOLD NUMBER	8856	8974	9025	9085	9133	5914	-
-f- FRICTION FACTOR	0.10	0.10	0.10	0.10	0.10	0.11	-
- Pc- CHANNEL PRESSURE DROP (23)	7	8	8	8	8	4	PSI
-V- PORT VELOCITY (26)	7.5	8	8.5	8.7	9	6 *	FT/S
- Pp- PORT PRESSURE DROP (23)	1	1	1	1	1	0	PSI
- P- TOTAL PRESSURE DROP	8	8	8	8	9	4	PSI
E-313							
HX DUTY	0.63	0.84	0.96	1.14	1.31	0.02	MILLIONS BTU/HR
-U- OVERALL HEAT TRANSFER COEF	650	650	650	650	650	650 *	BTU/(HR*F*FT2)
- Tln- LOG MEAN TEMP	30	30	30	30	30	30	F
-A- AREA	32	42	49	57	66	1	FT2
INSTALLED COST	189	214	226	243	258	40	M$
CHILLED WATER (10 C) CONSUMTION	6993	9324	10684	12627	14569	176	LB/HR
PRESSURE DROP							
-A- PLATE AREA (14)	1.05	1.22	1.31	1.42	1.53	0.16	M2
-n- NUMBER OF THERMAL PLATES (12)	30	35	37	40	43	5	-
-Nc- NUMBER OF CHANNELS	16	18	19	21	22	3	-
-Mc- CHANNEL FLOW RATE	0.19	0.22	0.24	0.26	0.28	0.02	LB/S
-Sc- FLOW AREA PER CHANNEL	0.003	0.003	0.003	0.004	0.004	0.000	FT2
-m- MASS VELOCITY	72	72	72	72	72	62	LB/(FT2*S)
-Re- REYNOLD NUMBER	2772	2783	2788	2794	2798	2398	-
-f- FRICTION FACTOR	0.14	0.14	0.14	0.14	0.14	0.14	-
- Pc- CHANNEL PRESSURE DROP (23)	0.99	0.99	1.00	1.00	1.00	0.77	PSI
-V- PORT VELOCITY (26)	7.5	8	8.5	8.7	9	6 *	FT/S
- Pp- PORT PRESSURE DROP (23)	1	1	1	1	1	0	PSI
- P- TOTAL PRESSURE DROP	2	2	2	2	2	1	PSI

III.4.2. UNCONVERTED STREAM

-F3- STREAM TO STERILIZE	1948	2597	2976	3517	4059	49	KG/HR
-D- UNCON STRM PIPE DIAM (16)	0.88	1.00	1.06	1.15	1.22	0.17	IN
-Re- REYNOLD NUMBER	30900	36197	39012	42766	46268	4079	-
-V- LINEAR VELOCITY	1.39	1.43	1.45	1.47	1.50	0.96	M/S
-E/(V*D)- (35)	0.28	0.28	0.28	0.27	0.27	0.33	-
-E- AXIAL DISPERSION COEFFICIENT	0.010	0.010	0.010	0.010	0.010	0.008	M2/S
-La- ASSUMED STERILIZER LENGHT	0.3	0.3	0.3	0.3	0.3	0.25	M
-Pe- PECLET NUMBER	42	43	43	43	43	30	-
-N/No- STER CRITERIA (18, 34)	2.4E-19	1.8E-19	1.6E-19	1.3E-19	1.1E-19	9.4E-18	-
-Nr- REACTION NUMBER (34)	85	86	87	88	89		-
-Lc- CALCULATED STERILIZER LENGHT	0.3	0.3	0.3	0.3	0.4	0.0	M
-Ld- DESIGN STERILIZER LENGHT	0.3	0.3	0.3	0.3	0.3	0.3	M
HEAT EXCHANGERS							
-F3- FEED TO PROTEIN PROD FERMENTOR	1948	2597	2976	3517	4059	49	KG/HR
-F3- FEED TO PROTEIN PROD FERMENTOR	1.9	2.6	3.0	3.5	4.1	0.0	M3/HR
E-322							
-Td2- TEMP STER STRM OUT OF E-322	70	70	70	70	70	70	
HX DUTY	0.54	0.72	0.83	0.98	1.13	0.01	MILLIONS BTU/HR
-Tb2- TEMP OF F3 OUT OF E-322	120	120	120	120	120	120	C
-U- OVERALL HEAT TRANSFER COEF	650	650	650	650	650	650 *	BTU/(HR*F*FT2)
- Tln- LOG MEAN TEMP	36	36	36	36	36	36	F
-A- AREA	23	31	35	42	48	1	FT2
INSTALLED COST	63	71	75	81	86	13	M$
PRESSURE DROP							

-A- PLATE AREA (14)	0.7	0.8	0.8	0.9	1.0	0.1	FT2
-n- NUMBER OF THERMAL PLATES (12)	35	40	43	47	50	6	-
-Nc- NUMBER OF CHANNELS	18	21	22	24	26	3	-
-Mc- CHANNEL FLOW RATE	0.07	0.08	0.08	0.09	0.10	0.009	LB/S
-Sc- FLOW AREA PER CHANNEL	0.002	0.002	0.002	0.002	0.002	0.000	FT2
-m- MASS VELOCITY	40	40	40	40	40	35	LB/(FT2*S)
-Re- REYNOLD NUMBER	1550	1556	1558	1561	1563	1366	-
-f- FRICTION FACTOR	0.02	0.02	0.02	0.02	0.02	0.02	-
- Pc- CHANNEL PRESSURE DROP (23)	0.05	0.05	0.05	0.05	0.05	0.04	PSI
-V- PORT VELOCITY (26)	7.5	8	8.5	8.7	9	6 *	FT/S
- Pp- PORT PRESSURE DROP (23)	1	1	1	1	1	0	PSI
- P- TOTAL PRESSURE DROP	1	1	1	1	1	0	PSI

E-321

HX DUTY	0.15	0.21	0.24	0.28	0.32	0.00	MILLIONS BTU/HR
-U- OVERALL HEAT TRANSFER COEF	650	650	650	650	650	650 *	BTU/(HR*F*FT2)
- Tln- LOG MEAN TEMP	484	484	484	484	484	484	F
-A- AREA	0.49	0.65	0.75	0.89	1.02	0.01	FT2
INSTALLED COST	63	71	75	81	86	13	M$
STEAM (150 PSIg) CONSUMPTION	4331	5775	6617	7820	9023	109	LB/DAY
STEAM (150 PSIg) COST	3.4	3.4	3.4	3.4	3.4	3.4	$/1000 LB
ANNUAL STEAM COST	5	6	7	8	10	0	M$/YR
PRESSURE DROP							
-A- PLATE AREA (14)	0.7	0.8	0.8	0.9	1.0	0.1	FT2
-n- NUMBER OF THERMAL PLATES (12)	35	40	43	47	50	6	-
-Nc- NUMBER OF CHANNELS	18	21	22	24	26	3	-
-Mc- CHANNEL FLOW RATE	0.07	0.08	0.08	0.09	0.10	0.01	LB/S
-Sc- FLOW AREA PER CHANNEL	0.002	0.002	0.002	0.002	0.002	0.000	FT2
-m- MASS VELOCITY	40	40	40	40	40	35	LB/(FT2*S)
-Re- REYNOLD NUMBER	1550	1556	1558	1561	1563	1366	-
-f- FRICTION FACTOR	0.16	0.16	0.16	0.16	0.16	0.17	-
- Pc- CHANNEL PRESSURE DROP (23)	0.36	0.36	0.36	0.36	0.37	0.29	PSI
-V- PORT VELOCITY (26)	7.5	8	8.5	8.7	9	6 *	FT/S
- Pp- PORT PRESSURE DROP (23)	1	1	1	1	1	0	PSI
- P- TOTAL PRESSURE DROP	1	1	1	1	1	1	PSI

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HX DUTY	0.35	0.46	0.53	0.63	0.72	0.01	MILLIONS BTU/HR
-U- OVERALL HEAT TRANSFER COEF	650	650	650	650	650	650 *	BTU/(HR*F*FT2)
- Tln- LOG MEAN TEMP	22	22	22	22	22	22	F
-A- AREA	24	32	37	43	50	1	FT2
INSTALLED COST	64	72	77	82	87	14	M$
CHILLED WATER (10 C) CONSUMTION	3862	5149	5900	6973	8046	97	LB/HR
PRESSURE DROP							
-A- PLATE AREA (14)	0.66	0.76	0.82	0.89	0.96	0.10	FT2
-n- NUMBER OF THERMAL PLATES (12)	37	42	45	49	52	6	-
-Nc- NUMBER OF CHANNELS	19	22	23	25	27	4	-
-Mc- CHANNEL FLOW RATE	0.06	0.07	0.08	0.09	0.09	0.01	LB/S
-Sc- FLOW AREA PER CHANNEL	0.002	0.002	0.002	0.002	0.002	0.000	FT2
-m- MASS VELOCITY	39	39	39	39	39	34	LB/(FT2*S)
-Re- REYNOLD NUMBER	1489	1494	1496	1498	1500	1318	-
-f- FRICTION FACTOR	0.02	0.02	0.02	0.02	0.02	0.02	-
- Pc- CHANNEL PRESSURE DROP (23)	0.04	0.04	0.04	0.04	0.04	0.04	PSI
-V- PORT VELOCITY (26)	7.5	8	8.5	8.7	9	6 *	FT/S
- Pp- PORT PRESSURE DROP (23)	1	1	1	1	1	0	PSI
- P- TOTAL PRESSURE DROP	1	1	1	1	1	0	PSI

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III.5. CELL GROWTH FERMENTOR

-T1- TEMPERATURE (18)	37	37	37	37	37	37	C
pH (18)	7.2	7.2	7.2	7.2	7.2	7.2	-
- - MAX SP GR RATE (18)	0.7	0.7	0.7	0.7	0.7	0.7	1/HR
-Km- MONOD CONTANT (19)	0.001	0.001	0.001	0.001	0.001	0.001	G GLUCOSE/LIT
-m- MAINTENANCE COEFF (20)	0.2	0.2	0.2	0.2	0.2	0.2	G GLU/(G CELLS*HR)
-Yx/s- YIELD FACTOR (20)	0.48	0.48	0.48	0.48	0.48	0.48	G CELLS/G GLUCOSE
-So- GLU+GAL AT INLET STRM	46.95	46.95	46.95	46.95	46.95	46.95	G/LIT
-S1- GL+GA OUTLET STRM (18)	0.005	0.005	0.005	0.005	0.005	0.005 *	G/LIT
-DF1- DENS STRM LEAVING REAC	1001	1001	1001	1001	1001	1001	KG/M3
-D1- DILUTION RATE (27)	0.47	0.47	0.47	0.47	0.47	0.47	1/HR
-X1- CELL CONC IN F1	18.69	18.69	18.69	18.69	18.69	18.69	G CELLS/LIT
% CELL IN F1	2.0	2.0	2.0	2.0	2.0	2.0	%
LIQUID VOLUME	10	14	16	19	21	0.3	M3
FREE SPACE (22)	25	25	25	25	25	25	%
FERMENTOR VOLUME (30)	13	17	20	23	27	0	M3
FERMENTOR COST (39)	94	102	106	113	119	73	M$

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III.6. ENZYME PRODUCTION FERMENTOR

-T2- TEMPERATURE (18)	25	25	25	25	25	25	C
pH (18)	7.2	7.2	7.2	7.2	7.2	7.2	-
- max- MAX SP GROWTH RATE (18)	0.3	0.3	0.3	0.3	0.3	0.3	1/HR
-Km- MONOD CONSTANT	0.0005	0.0005	0.0005	0.0005	0.0005	0.0005	G LACTOSE/LIT
-m- MAINTENANCE COEFFICIENT	0.15	0.15	0.15	0.15	0.15	0.15	G LAC/(G CELLS*HR)
-LF2'- LACTOSE IN (F1+F3)	36	36	36	36	36	36	G/LIT
-LF3- LACTOSE IN F3	120	120	120	120	120	120	G/LIT
-LF1- LACTOSE IN F1	2.35	2.35	2.35	2.35	2.35	2.35	G/LIT
-S2- LACT LEAVING THE REAC	0.057	0.057	0.057	0.057	0.057	0.057 *	G/LIT
-Yp/s- YIELD FACTOR (19)	400	400	400	400	400	400	UNITS B-LAC/MG LAC
-Yx/s- YIELD FACTOR (21)	0.35	0.35	0.35	0.35	0.35	0.35	G CELLS/G LACTOSE
-Yp/x- YIELD FACTOR (18)	150000	150000	150000	150000	150000	150000	UNITS/G CELLS
B-LACTAMASE SPEC ACT (27)	3500	3500	3500	3500	3500	3500	UNITS/MG ENZYME
B-LACTAMASE M.W. (27)	29000	29000	29000	29000	29000	29000	MG/mMOL
-DF2- DENS STRM LEAVING REAC	1001	1001	1001	1001	1001	1001	KG/M3
-D2- DILUTION RATE	0.056	0.056	0.056	0.056	0.056	0.056	1/HR
-XF2'- CELL CONC STRM ENTERING REAC	13.30	13.30	13.30	13.30	13.30	13.30	G CELLS/LIT
- - GROWTH RATE	0.027	0.027	0.027	0.027	0.027	0.027	1/HR
LIQUID VOLUME	121	161	184	218	251	3	M3
-X2- CELL CONC STRM LEAVING REAC	25.31	25.31	25.31	25.31	25.31	25.31	G CELLS/LIT
% CELL IN F2	2.7	2.7	2.7	2.7	2.7	2.7	%
-P2- ENZYME CONC IN BROWTH	0.164	0.164	0.164	0.164	0.164	0.164	mMOL/LIT
B-LACTAMASE CONC IN BROWTH	4.74	4.74	4.74	4.74	4.74	4.74	G/LIT
GENE EXPRESSION	0.5	0.5	0.5	0.5	0.5	0.5	FRACTION OF MOLAR
MOLECULAR WEIGHTS							
B. PROTEASES (55)	27500	27500	27500	27500	27500	27500	MOL-G/LIT
CALF CHYMOSIN (56)	40777	40777	40777	40777	40777	40777	MOL-G/LIT
GLUCOSE ISOMERASE (58)	49740	49740	49740	49740	49740	49740	MOL-G/LIT
CONCENTRATIONS IN THE BROWTH							
B. PROTEASES	2.25	2.25	2.25	2.25	2.25	2.25	G/LIT
CALF CHYMOSIN	3.33	3.33	3.33	3.33	3.33	3.33	G/LIT
GLUCOSE ISOMERASE	4.07	4.07	4.07	4.07	4.07	4.07	G/LIT
FREE SPACE (22)	25	25	25	25	25	25	%
FERMENTOR VOLUME (30)	151	201	230	272	314	4	M3
FERMENTOR COST (39)	335	423	475	548	621	79	M$

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III.7. INOCULUM FERMENTOR

INOCULUM SIZE	8	8	8	8	8	8 *	% OF VOLUME IN FER
-Vin- LIQUID VOLUME	0.8	1.1	1.3	1.5	1.7	0.02	M3
-Xm- MAXIMUN CELL CONC	20	20	20	20	20	20	G/LIT

-tl-LOSS TIME(LAG P+HARV+PREP) (22)	10	10	10	10	10	10 *	HR
-Vlab-INC VOL TRANSFERED FROM THE LA	66	88	101	119	137	2	LIT
-Xi- INITIAL CELL CONC	2	2	2	2	2	2	G/LIT
- max- MAX SP GROWTH RATE (18)	1	1	1	1	1	1 *	1/HR
TOTAL GROWTH TIME	14	14	14	14	14	14	HR
FREE REACTOR SPACE (22)	25	25	25	25	25	25 *	%
FERMENTOR VOLUME (30)	1	1	2	2	2	0.03	M3
FERMENTOR COST (39)	2	2	3	3	4	0.05	M$

III.8. ANTIFOAM AND NaOH ADDITION

ANTIFOAM ADDITION (100)	4.17	4.17	4.17	4.17	4.17	4.17 *	uL/(LIT FERM*HR)
ANTIFOAM CONSUMTION	4	5	6	7	9	0	M3/YR
ANTIFOAM COST	13	17	20	23	27	0	M$/YR
NaOH (2 N) ADDITION (100)	0.50	0.50	0.50	0.50	0.50	0.50 *	ML/(LIT FERM*HR)
NaOH CONSUMPTION	490	653	749	885	1021	12	M3/YR
NaOH COST	32	43	49	58	67	1	M$/YR

III.9. OXYGEN SUPPLY AND POWER CONSUMTION

III.9.1. CELL GROWTH FERMENTOR

E. COLI RESP RATE (22)	11	11	11	11	11	11	mMOL O2/(G CELLS*H
OXYGEN TRANSFER COEFFICIENT (KLa)	0.24	0.24	0.24	0.24	0.24	0.24	1/S
FERMENTOR DIAMETER (27)	6.5	7.1	7.5	7.9	8.3	1.9	FT
FERMENTOR AREA	33.0	39.9	43.7	48.7	53.6	2.9	FT2
TOTAL HEIGHT	13.0	14.3	14.9	15.8	16.5	3.8	FT
LIQUID HEIGHT	9.7	10.7	11.2	11.8	12.4	2.9	FT
OXYGEN SUPPLY	28	37	43	50	58	1	SCFM
TOTAL PRESSURE (HYD+OVERPRES)	22	23	23	23	23	19	PSIa
AIR FLOW RATE (37 C)	200	262	297	347	396	6	FT3/MIN
AIR FLOW RATE (0 C)	133	178	203	240	277	3	SCFM
SUPEFICIAL GAS VELOCITY	0.10	0.11	0.11	0.12	0.12	0.03	FT/S
-Pg- REQUIRED POWER	23	30	34	40	46	1	HP
POWER BY ISOTH AIR EXPANSION (40)	-2	-3	-4	-5	-6	-0.0	HP
TOTAL POWER SUPPLIED BY AERATION	2	3	4	5	6	0	HP
TOTAL POWER BY MECHANICAL AGITATION	21	27	31	36	41	1	HP
POWER PER UNIT VOLUME	8	8	8	8	8	9	HP/1000 GAL
BRAKE EFFICIENCY (40)	92	92	92	92	92	92 *	%
BRAKE HORSE POWER	22	29	33	39	44	1	HP
ELECTRIC POWER	25	32	37	43	49	1	HP

III.9.2. ENZYME PRODUCTION FERMENTOR

E. COLI RESP RATE (22)	11	11	11	11	11	11	mMOL O2/(G CELLS*H
OXYGEN TRANSFER COEFFICIENT (KLa)	0.26	0.26	0.26	0.26	0.26	0.26	1/S
FERMENTOR DIAMETER (27)	14.6	16.1	16.8	17.7	18.6	4.3	FT
FERMENTOR AREA	167.4	202.4	221.4	247.2	271.7	14.7	FT2
TOTAL HEIGHT	29.2	32.1	33.6	35.5	37.2	8.7	FT
LIQUID HEIGHT	21.9	24.1	25.2	26.6	27.9	6.5	FT
OXYGEN SUPPLY	443	591	677	800	923	11	SCFM
TOTAL PRESSURE (HYD+OVERPRES)	27	28	29	30	30	21	PSIa
AIR FLOW RATE (25 C)	2464	3176	3578	4140	4689	82	FT3/MIN
AIR FLOW RATE (0 C)	2110	2814	3224	3810	4396	53	SCFM
SUPEFICIAL GAS VELOCITY	0.2	0.3	0.3	0.3	0.3	0.1	FT/S
-Pg- REQUIRED POWER	278	368	420	495	569	8	HP
POWER BY ISOTHERMAL AIR EXPANSION	-70	-101	-120	-148	-177	-1	HP
TOTAL POWER SUPPLIED BY AERATION	70	101	120	148	177	1	HP
TOTAL POWER BY MECHANICAL AGITATION	207	267	300	347	392	7	HP
POWER PER UNIT VOLUME	9	9	9	9	9	10	HP/1000 GAL
BRAKE EFFICIENCY (40)	92	92	92	92	92	92	%
BRAKE HORSE POWER	226	290	326	377	426	8	HP

ELECTRIC POWER	251	322	363	419	473	9	HP
III.9.3. INOCULUM FERMENTOR							
E. COLI RESP RATE (22)	11.00	11.00	11.00	11.00	11.00	11.00	mMOL O2/(G CELLS*H
OXYGEN TRANSFER COEFFICIENT (KLa)	0.26	0.26	0.26	0.26	0.26	0.26	1/S
FERMENTOR DIAMETER (27)	3	3	3	3	4	1	FT
FERMENTOR AREA	6	8	8	9	10	1	FT2
TOTAL HEIGHT	6	6	6	7	7	2	FT
LIQUID HEIGHT	4.2	4.6	4.9	5.1	5.4	1.3	FT
OXYGEN SUPPLY	2	3	4	4	5	0.1	SCFM
TOTAL PRESSURE (HYD+OVERPRES)	20	20	20	20	20	19	PSIa
AIR FLOW RATE (37 C)	19	26	29	34	40	1	FT3/MIN
AIR FLOW RATE (0 C)	12	15	18	21	24	0	SCFM
SUPEFICIAL GAS VELOCITY	0.1	0.1	0.1	0.1	0.1	0.02	FT/S
-Pg- REQUIRED POWER	2	3	3	4	4	0.06	HP
POWER BY ISOTHERMAL AIR EXPANSION	-0.1	-0.1	-0.2	-0.2	-0.2	-0.001	HP
TOTAL POWER SUPPLIED BY AERATION	0.1	0.1	0.2	0.2	0.2	0.0	HP
TOTAL POWER BY MECHANICAL AGITATION	2	3	3	4	4	0.06	HP
POWER PER UNIT VOLUME	10	10	10	10	10	11	HP/1000 GAL
BRAKE EFFICIENCY (40)	92	92	92	92	92	92	%
BRAKE HORSE POWER	2	3	3	4	5	0.1	HP

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III.10. AIR COMPRESSION AND STERILIZATION

ENZYME PRODUCTION FERMENTOR							
PRESSURE DROP HEAT EXCHANGER	3	3	3	3	3	3 *	PSI
PRESS DROP IN PRE-FILTERS (33)	3	3	3	3	3	3 *	PSI
PRESS DROP IN FILTERS (33)	3	3	3	3	3	3 *	PSI
PRESS DROP IN SPARGERS	2	2	2	2	2	2 *	PSI
OVERPRESSURE	18	18	18	18	18	18	PSI
HYDROSTATIC PRESSUSRE	9	10	11	12	12	3	PSI
TOTAL PRESSURE HEAD	38	39	40	41	41	32	PSI
TOTAL AIR FLOW (20 C, TPH) (Q1+Q2)	984	1280	1449	1687	1920	30	FT3/MIN
TOTAL AIR FLOW (0 C, 1 ATM) (Q1+Q2)	2243	2991	3427	4051	4674	56	SCFM
TOTAL AIR FLOW (Q1+Q2)	10881	14508	16623	19646	22668	274	LB/HR
COMPRESSOR (J-410)							
REQUIRED POWER	70	93	107	127	147	2	HP
COMPRESSION RATIO	2.6	2.7	2.7	2.8	2.8	2.2	-
BRAKE HORSE POWER	77	104	119	141	163	2	HP
ELECTRICAL POWER	86	115	132	157	181	2	HP
INSTALLED COST (10)	188	239	268	308	347	9	M$
AIR HX (E-440)							
-T1- IN AIR TEMP	238	243	245	248	251	200	F
HX DUTY	2	2	2	3	3	0	MILLIONS BTU/HR
OVERALL HEAT TRANSFER COEF (10)	20	20	20	20	20	20 *	BTU/(HR*FT2*F)
HEAT TRANSFER AREA	2350	3244	3781	4564	5365	43	FT2
INSTALLED COST (10)	243	299	331	374	415	18	M$
CHILLED WATER FLOW	9025	12104	13908	16493	19087	214	LB/HR
PREFILTERS (F-447)							
TOTAL AIR TO FILTERS	2243	2991	3427	4051	4674	56	SCFM
OVERCAPACITY	15	15	15	15	15	15 *	%
NUMBER OF CARTRIDGES	9	11	13	16	18	0	-
NUMBER OF HOUSINGS	2	2	3	3	4	0	-
TIME BETWEEN STERILIZATIONS	2	2	2	2	2	2 *	DAYS
CARTRIDGES' COST (102)	38	38	38	38	38	38 *	$/UNIT
HOUSINGS' COST (102)	1192	1192	1192	1192	1192	1192 *	$/UNIT
CARTRIDGES ANNUAL COST	43	58	66	78	90	1	M$/YR

HOUSING INSTALLED COST	3	4	4	5	6	0	M$
FILTERS (F-450)							
OVERCAPACITY	15	15	15	15	15	15 *	%
NUMBER OF CARTRIDGES	9	11	13	16	18	0	-
NUMBER OF HOUSINGS	2	2	3	3	4	0	-
TIME BETWEEN STERILIZATIONS	2	2	2	2	2	2 *	DAYS
CARTRIDGES' COST (102)	38	38	38	38	38	38 *	$/UNIT
HOUSINGS' COST (102)	1192	1192	1192	1192	1192	1192 *	$/UNIT
CARTRIDGES ANNUAL COST	43	58	66	78	90	1	M$/YR
HOUSING INSTALLED COST	3	4	4	5	6	0	M$
EXHAUSTED AIR PRE-FILTERS (F-457)							
TOTAL AIR TO FILTER	1772	2363	2708	3200	3692	45	SCFM
OVERCAPACITY	15	15	15	15	15	15 *	%
NUMBER OF CARTRIDGES	7	9	10	12	14	0	-
NUMBER OF HOUSINGS	1	2	2	2	3	0	-
TIME BETWEEN STERILIZATIONS	2	2	2	2	2	2 *	DAYS
CARTRIDGES' COST (102)	38	38	38	38	38	38 *	$/UNIT
HOUSINGS' COST (102)	1192	1192	1192	1192	1192	1192 *	$/UNIT
CARTRIDGES ANNUAL COST	34	46	52	62	71	1	M$/YR
HOUSING INSTALLED COST	2	3	3	4	4	0	M$
EXHAUSTED AIR FILTERS (F-458)							
TOTAL AIR TO FILTER	1772	2363	2708	3200	3692	45	SCFM
OVERCAPACITY	15	15	15	15	15	15 *	%
NUMBER OF CARTRIDGES	7	9	10	12	14	0	-
NUMBER OF HOUSINGS	1	2	2	2	3	0	-
TIME BETWEEN STERILIZATIONS	2	2	2	2	2	2 *	DAYS
CARTRIDGES' COST (102)	38	38	38	38	38	38 *	$/UNIT
HOUSINGS' COST (102)	1192	1192	1192	1192	1192	1192 *	$/UNIT
CARTRIDGES ANNUAL COST	34	46	52	62	71	1	M$/YR
HOUSING INSTALLED COST	2	3	3	4	4	0	M$

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III.11. HEAT BALANCES

III.11.1. CELL GROWTH FERMENTOR							
HEAT PRODUCED BY MICROBIAL GROWTH	1.01	1.34	1.54	1.82	2.10	0.03	MILLIONS BTU/HR
HEAT PRODUCED BY AGITATION	0.06	0.08	0.09	0.10	0.12	0.00	MILLIONS BTU/HR
TOTAL HEAT PRODUCED	1.07	1.42	1.63	1.92	2.22	0.03	MILLIONS BTU/HR
FERMENTOR TEMPERATURE (18)	37	37	37	37	37	37	C
AMBIENT TEMPERATURE	25	25	25	25	25	25 *	C
LIQUID HEIGHT	10	11	11	12	12	3	FT
FERMENTOR DIAMETER	6	7	7	8	8	2	FT
FERMENTOR SURFACE AREA	198	239	262	292	321	17	FT2
HEAT LOSS BY RAD AND CONV (40)	0.01	0.01	0.01	0.01	0.01	0.001	MILLIONS BTU/HR
HUMIDITY AIR IN (16)	0.035	0.035	0.035	0.035	0.035	0.035 *	LB WATER/LB DRY AI
HUMIDITY AIR OUT (16)	0.036	0.036	0.036	0.036	0.036	0.036 *	LB WATER/LB DRY AI
EVAPORATED WATER	2	2	3	3	4	0	LB/HR
HEAT LOSS BY EVAP	0.00	0.00	0.00	0.00	0.00	0.00	MILLIONS BTU/HR
HEAT TO BE REMOVED	1.06	1.41	1.62	1.91	2.20	0.03	MILLIONS BTU/HR
INPUT TEMP OF CHILLED WATER	10	10	10	10	10	10 *	C
OUTPUT TEMP OF CHILLED WATER	27	27	27	27	27	27 *	C
FLOW OF CHILLED WATER	34543	46068	52792	62397	72002	860	LB/HR
III.11.2. ENZYME PRODUCTION FERMENTOR							
HEAT PRODUCED BY MICROBIAL GROWTH	15.99	21.32	24.42	28.87	33.31	0.40	MILLIONS BTU/HR
HEAT PRODUCED BY AGITATION	0.71	0.94	1.07	1.26	1.45	0.02	MILLIONS BTU/HR
TOTAL HEAT PRODUCED	16.69	22.25	25.49	30.13	34.76	0.42	MILLIONS BTU/HR
FERMENTOR TEMPERATURE (18)	25	25	25	25	25	25	C

AMBIENT TEMPERATURE	25	25	25	25	25	25 *	C
LIQUID HEIGHT	22	24	25	27	28	6	FT
FERMENTOR DIAMETER	15	16	17	18	19	4	FT
FERMENTOR SURFACE AREA	1004	1214	1328	1483	1630	88	FT2
HEAT LOSS BY RAD AND CONV (40)	0	0	0	0	0	0	MILLIONS BTU/HR
HUMIDITY AIR IN (16)	0.035	0.035	0.035	0.035	0.035	0.035 *	LB WATER/LB DRY AI
HUMIDITY AIR OUT (16)	0.036	0.036	0.036	0.036	0.036	0.036 *	LB WATER/LB DRY AI
EVAPORATED WATER	25	34	39	46	53	1	LB/HR
HEAT LOSS BY EVAP	0.03	0.04	0.04	0.05	0.05	0.00	MILLIONS BTU/HR
HEAT TO BE REMOVED	16.67	22.22	25.45	30.08	34.70	0.42	MILLIONS BTU/HR
INPUT TEMP OF CHILLED WATER	10	10	10	10	10	10 *	C
OUTPUT TEMP OF CHILLED WATER	15	15	15	15	15	15 *	C
FLOW OF CHILLED WATER	1851976	2468642	2828305	3342050	3855736	46836	LB/HR

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III.11.3. BIOMASS COOLING HEAT EXCHANGER (E-470)

-F1- FEED OUT OF FIRST FERM	4811	6414	7349	8686	10022	121	KG/HR
-TF1- TEMPERATURE OF F1	37	37	37	37	37	37	C
-U- OVERALL HEAT TRANSFER COEF	650	650	650	650	650	650 *	BTU/(HR*FT*F)
- Tln- LOG MEAN TEMP	22	22	22	22	22	22	F
HX DUTY	0.23	0.31	0.35	0.41	0.48	0.01	MILLIONS BTU/HR
-A- AREA	16	21	24	29	33	0	FT2
INSTALLED COST	54	61	65	69	74	12	M$
CHILLED WATER FLOW	7488	9984	11440	13520	15599	189	LB/HR
PRESSURE DROP							
-A- PLATE AREA	2	2	2	2	2	2 *	FT2
-n- NUMBER OF THERMAL PLATES (12)	8	11	12	14	17	0	-
-Nc- NUMBER OF CHANNELS	5	6	7	8	9	1	-
-Mc- CHANNEL FLOW RATE	0.7	0.7	0.7	0.7	0.7	0.1	LB/S
-Sc- FLOW AREA PER CHANNEL	0.0	0.0	0.0	0.0	0.0	0.0	FT2
-m- MASS VELOCITY	131	134	136	138	139	25	LB/(FT2*S)
-Re- REYNOLD NUMBER	5050	5194	5251	5313	5359	953	-
-f- FRICTION FACTOR	0.12	0.12	0.12	0.12	0.12	0.18	-
- Pc- CHANNEL PRESSURE DROP (12)	3	3	3	3	3	0	PSI
-V- PORT VELOCITY (26)	7.5	8	8.5	8.7	9	6	FT/S
- Pp- PORT PRESSURE DROP (23)	1	1	1	1	1	0	PSI
- P- TOTAL PRESSURE DROP	3	4	4	4	4	1	PSI

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III.11.4. CHILLED WATER CONSUMPTION

CHILLED WATER FLOWS							
CONVERTED STREAM STERILIZATION	6993	9324	10684	12627	14569	176	LB/HR
UNCONVERTED STREAM STERILIZATION	3862	5149	5900	6973	8046	97	LB/HR
CELL GROWTH FERMENTOR	34543	46068	52792	62397	72002	860	LB/HR
ENZYME PRODUCTION FERMENTOR	1851976	2468642	2828305	3342050	3855736	46836	LB/HR
AIR COOLING HEAT EXCHANGER	9025	12104	13908	16493	19087	214	LB/HR
BIOMASS COOLING HEAT EXCHANGER	7488	9984	11440	13520	15599	189	LB/HR
CHILLED WATER COST	1.35	1.35	1.35	1.35	1.35	1.35	$/1000 GAL
ANNUAL CHILLED WATER COST	2314	3085	3534	4177	4819	58	M$/YR

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II.12. PUMPS

CELL GROWTH FERMENTOR PUMP (J-460)							
TOTAL HEAD	8	8	8	8	8	5	PSIa
TOTAL HEAD	18	19	19	19	19	12	FT
CAPACITY	21	28	32	38	44	1	GPM
INSTALLED COST (93,94)	2	2	2	2	3	2	M$
POWER CONSUMTION	0.38	0.44	0.48	0.53	0.58	0.00	HP

ENZYME PRODUCTION FERMENTOR PUMP (J-457)

TOTAL HEAD	5	5	5	5	5	5	PSIa
TOTAL HEAD	12	12	12	12	12	12	FT
CAPACITY	30	40	45	54	62	1	GPM
INSTALLED COST (93,94)	2	2	2	2	2	2	M$
POWER CONSUMTION	0.28	0.33	0.35	0.39	0.43	0.00	HP
pH AJUSTMENT PUMP (J-330)							
-N1+N2- NaOH 50% FLOW	0.17	0.22	0.25	0.30	0.35	0.00	KG/HR
CAPACITY	0.000	0.001	0.001	0.001	0.001	0.000	GPM
INSTALLED COST (16)	19	22	24	26	28	3	M$
SALTS' ADDITION TO CONV STREAM PUMP (J-350)							
-H1*-	13	17	20	23	27	0	KG/HR
CAPACITY	0	0	0	0	0	0	GPM
INSTALLED COST (16)	227	264	283	309	333	34	M$
SALTS' ADDITION TO UNCONV STREAM PUMP (J-355)							
-H2*-	12	15	18	21	24	0	KG/HR
CAPACITY	0.1	0.1	0.1	0.1	0.1	0.0	GPM
INSTALLED COST (16)	215	250	269	293	316	32	M$
ANTIFOAM AND BASE ADDITION PUMP (J-453)							
CAPACITY	0.29	0.39	0.44	0.52	0.61	0.01	GPM
INSTALLED COST (16)	532	618	663	723	779	78	M$
INOCUMUM FERMENTOR PUMP (J-465)							
TOTAL HEAD	5	6	6	7	7	2	FT
TOTAL HEAD	2	3	3	3	3	1	PSIa
CAPACITY	11	15	17	20	23	0	GPM
INSTALLED COST (93,94)	2	2	2	2	2	2	M$

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III.13. MIXERS AND TANKS

pH AJUSTMENT MIXER (M-340)							
VOLUME	0.00	0.00	0.00	0.00	0.00	0.00	M3
VOLUME	0.02	0.02	0.02	0.03	0.03	0.00	GAL
INSTALLED COST	0.08	0.09	0.10	0.11	0.11	0.01	M$
CONVERTED STREAM SALTS' ADDITION MIXER (M-352)							
VOLUME	0.01	0.01	0.01	0.01	0.01	0.00	M3
VOLUME	1.87	2.49	2.85	3.37	3.89	0.05	GAL
INSTALLED COST	1.01	1.18	1.27	1.40	1.51	0.14	M$
UNCONVERTED STREAM SALTS' ADDITION MIXER (M-356)							
VOLUME	0.01	0.01	0.01	0.01	0.01	0.00	M3
VOLUME	1.69	2.25	2.58	3.04	3.51	0.04	GAL
INSTALLED COST	0.96	1.12	1.21	1.32	1.43	0.13	M$
ANTIFOAM MIXER (M-451)							
VOLUME	0.00	0.00	0.00	0.00	0.01	0.00	M3
VOLUME	0.63	0.85	0.97	1.15	1.32	0.02	GAL
INSTALLED COST	0.6	0.7	0.7	0.8	0.8	0.1	M$
NaOH MIXER (M-452)							
VOLUME	0.29	0.38	0.44	0.52	0.60	0.01	M3
VOLUME	76	101	116	137	159	2	GAL
INSTALLED COST	8	9	10	10	11	1	M$

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III.14. POWER CONSUMTION

TOTAL POWER CONSUMED	362	471	533	619	705	11	HP
ANNUAL POWER COST	81	105	119	138	157	3	M$/YR

IV. SEPARATION

IV.1. DATA

-F2- FLOW OF BROWTH	6759	9012	10326	12203	14081	170	KG/HR
-F2- FLOW OF BROWTH	7	9	10	12	14	0	M3/HR
-X2=CF2- CELL CONC IN F2	25.31	25.31	25.31	25.31	25.31	25.31	G/LIT
-PF2- ENZYME CONCENTRATION IN F2	0.16	0.16	0.16	0.16	0.16	0.16	mMOL/LIT
B-LACTAMASE CONCENTRATION IN F2	4.74	4.74	4.74	4.74	4.74	4.74	G/LIT
MOLECULAR WEIGHTS							
B-LACTAMASE	29000	29000	29000	29000	29000	29000	G/MOL-G
B. PROTEASES (55)	27500	27500	27500	27500	27500	27500	G/MOL-G
CALF CHYMOSIN (56)	40777	40777	40777	40777	40777	40777	G/MOL-G
GLUCOSE ISOMERASE (58)	49740	49740	49740	49740	49740	49740	G/MOL-G
CONCENTRATIONS IN THE BROWTH							
B. PROTEASES	2.25	2.25	2.25	2.25	2.25	2.25	G/LIT
CALF CHYMOSIN	3.33	3.33	3.33	3.33	3.33	3.33	G/LIT
GLUCOSE ISOMERASE	4.07	4.07	4.07	4.07	4.07	4.07	G/LIT
-DF2- DENSITY OF F2	1001	1001	1001	1001	1001	1001	KG/M3
E. COLI DENSITY	1.03	1.03	1.03	1.03	1.03	1.03 *	G/CM3
- - CELL VOLUME FRACTION	0.08	0.08	0.08	0.08	0.08	0.08	%
VISCOSITY IN F2 (21)	1.20	1.20	1.20	1.20	1.20	1.20	Cp

IV.2. STREAMS' DEFINITION AND YIELDS

-CY- CENTRIFUGATION YIELD	0.95	0.95	0.95	0.95	0.95	0.95	UNITS OUT/UNITS IN
-UFY- ULTRAFILTRATION YIELD	0.95	0.95	0.95	0.95	0.95	0.95	UNITS OUT/UNITS IN
-P/CY- PREC/CENTR YIELD	0.90	0.90	0.90	0.90	0.90	0.90	UNITS OUT/UNITS IN
-DY- DIAFILTRATION YIELD	0.95	0.95	0.95	0.95	0.95	0.95	UNITS OUT/UNITS IN
OVERALL YIELD	0.77	0.77	0.77	0.77	0.77	0.77	UNITS OUT/UNITS IN
PROTEASES OVERALL YIELD	0.81	0.81	0.81	0.81	0.81	0.81	UNITS OUT/UNITS IN
STREAMS							
-F4-	6291	8389	9612	11360	13107	158	KG/HR
-F4-	6	8	10	11	13	0	M3/HR
-FM2/F4-	0.05	0.05	0.05	0.05	0.05	0.05 *	-
-FM2-	315	419	481	568	655	8	KG/HR
-FM2-	0.31	0.42	0.48	0.57	0.66	0.01	M3/HR
-FM1-	5977	7969	9131	10792	12452	151	KG/HR
-FM1-	6	8	9	11	12	0	M3/HR
-F4*-	5977	7969	9131	10792	12452	151	KG/HR
-F4*-	6	8	9	11	12	0	M3/HR
-F5-	12592	16789	19238	22736	26234	317	KG/HR
-F5-	11	14	16	19	22	0	M3/HR
-F6-	18569	24759	28369	33527	38685	468	KG/HR
-F6-	17	22	25	30	34	0	M3/HR
-F7/F6-	0.0015	0.0015	0.0015	0.0015	0.0015	0.0015 *	-
-F7-	28	37	43	50	58	1	KG/HR
-F7-	0.02	0.03	0.03	0.04	0.04	0.00	M3/HR
-F8-	18541	24722	28327	33477	38627	467	KG/HR
-F8-	17	22	25	30	34	0	M3/HR
-F9-	12232	16310	18688	22086	25484	308	KG/HR
-F9-	10	14	16	19	22	0.26	M3/HR
-F10-	5949	7932	9089	10741	12394	150	KG/HR
-F10-	5	7	8	9	11	0.13	M3/HR
-F11-	18541	24722	28327	33477	38627	467	KG/HR

-F11-	17	22	25	30	34	0	M3/HR
CONCENTRATIONS							
-EF2-	4.74	4.74	4.74	4.74	4.74	4.74	G B-LACT/LIT
-EF4-	4.44	4.44	4.44	4.44	4.44	4.44	G B-LACT/LIT
-EFM1-	4.44	4.44	4.44	4.44	4.44	4.44	G B-LACT/LIT
-EF4*-	4.44	4.44	4.44	4.44	4.44	4.44	G B-LACT/LIT
-EF6-	1.61	1.61	1.61	1.61	1.61	1.61	G B-LACT/LIT
-EF7-	1115	1115	1115	1115	1115	1115	G B-LACT/LIT
-EF7-	86	86	86	86	86	86	%

IV.3. CENTRIFUGATION

-PCTF3- % CELL IN F3 (48)	14	14	14	14	14	14 *	%
-DF3- DENSITY OF F3	1004	1004	1004	1004	1004	1004	KG/M3
-X3- CELL CONC IN F3	141	141	141	141	141	141	G/LIT
-F3- FLOW OUT OF THE FIRST CENTR	1.22	1.62	1.86	2.20	2.53	0.03	M3/HR
-F3- FLOW OUT OF THE FIRST CENTR	1221	1628	1865	2204	2543	31	KG/HR
-Vc- CELL VOLUME FLOW IN F2	0.55	0.74	0.85	1.00	1.15	0.01	M3/HR
-Vb- CELL FREE BROWTH FLOW IN F2	6.20	8.27	9.47	11.19	12.92	0.16	M3/HR
- - CELL VOLUME FRACTION IN F3	0.45	0.45	0.45	0.45	0.45	0.45	-
-F3b- CELL FREE BROWTH FLOW IN F3	0.66	0.88	1.01	1.20	1.38	0.02	M3/HR
-x- RAT CF BROWTH TO CELL VOL IN F3	1.20	1.20	1.20	1.20	1.20	1.20	-
-CY- CENTRIFUGATION YIELD	0.95	0.95	0.95	0.95	0.95	0.95	-
-W- WATER FLOW RATE	0.75	1.00	1.15	1.36	1.57	0.02	M3/HR
-W- WATER FLOW RATE	754	1005	1151	1361	1570	19	KG/HR
-PF4- ENZYME CONC IN F4	4.44	4.44	4.44	4.44	4.44	4.44	G/LIT
-PCTC- % CELLS IN C (48)	14.00	14.00	14.00	14.00	14.00	14.00 *	%
-DC- DENSITY OF C	1004	1004	1004	1004	1004	1004	KG/M3
-Xc- CELL CONC IN C	141	141	141	141	141	141	G/LIT
-F3'- FLOW TO SECOND CENTRIFUGE	1974	2633	3017	3565	4114	50	KG/HR
-PCTF3'- % CELL IN F3'	8.66	8.66	8.66	8.66	8.66	8.66	%
-DF3'- DENSITY OF F3'	1003	1003	1003	1003	1003	1003	KG/M3
-XF3'- CELL CONC IN F3'	87	87	87	87	87	87	G/LIT
-F3'- FLOW TO SECOND CENTRIFUGE	1.97	2.63	3.01	3.56	4.10	0.05	M3/HR
-C-	1.22	1.62	1.86	2.20	2.53	0.03	M3/HR
-C-	1221	1628	1865	2204	2543	31	KG/HR
-F4-	6291	8389	9612	11360	13107	158	KG/HR
CELL MASS WASTED	171	228	261	309	356	4	KG DRY CELLS/HR
PROCESS WATER COST (74)	0.02	0.02	0.02	0.02	0.02	0.02	$/1000 GAL
ANNUAL WATER COST	0.03	0.04	0.05	0.05	0.06	0.00	M$/YR
FIRST CENTR COST (FF-502) (13)	245	275	290	310	329	56	M$
SECOND CENTR COST (FF-504) (13)	150	168	177	190	201	34	M$

IV.4. CELL KILLING

CELL MASS WASTED	171	228	261	309	356	4	KG/HR
-C+FM2- DISCARTED STREAM	1535	2047	2346	2772	3199	39	KG/HR
% CELL IN DISCARTED STRM	11	11	11	11	11	11	%
pH IN C	7.2	7.2	7.2	7.2	7.2	7.2 *	-
pH TO KILL THE CELLS	3	3	3	3	3	3 *	-
HCL 20 Be REQUIRED	4	6	6	8	9	0	KG/DAY
NaOH 76% REQUIRED	1.94	2.59	2.96	3.50	4.04	0.05	KG/DAY
HCL 20 Be ANNUAL COST	0.09	0.12	0.14	0.17	0.20	0.00	M$/YR
NaOH 76% ANNUAL COST	0.02	0.02	0.03	0.03	0.04	0.00	M$/YR

IV.5. MICROFILTRATION

-F4-	6291	8389	9612	11360	13107	158	KG/HR
MICROFILTR (P-506) INST COST (38)	206	251	275	307	338	17	M$
MICROFILTR (P-506) OPER COST (38)	0.53	0.62	0.67	0.74	0.80	0.07	M$/YR

IV.6. ISOELECTRIC pH AJUSTMENT

PROTEASES ISOELECTRIC POINT	9.4	9.4	9.4	9.4	9.4	9.4 *	-
pH IN FM1	7.2	7.2	7.2	7.2	7.2	7.2	-
-N3- NaOH 0.5% REQUIRED	0.076	0.101	0.116	0.137	0.158	0.002	KG/DAY
NaOH 0.5% ANNUAL COST	0.00	0.00	0.00	0.00	0.00	0.00	M$/YR
-F4*- (FM1+N3) STRM TO PREC REACTOR	5977	7969	9131	10792	12452	151	KG/HR
DIAMETER OF PIPE FOR F4*	1.9	2.1	2.3	2.4	2.6	0.4	IN
MIXER (M-512) INSTALLED COST (13)	2	3	3	3	3	1	M$

IV.7. PRECIPITATION/CENTRIFUGATION

-GO- CAMP NUMBER (50)	100000	100000	100000	100000	100000	100000 *	-
-G- SHEAR RATE (49)	20	20	20	20	20	20 *	1/S
-O- RESIDENCE TIME	1.4	1.4	1.4	1.4	1.4	1.4	HR
-F4*- STREAM TO PREC REACTOR	5977	7969	9131	10792	12452	151	KG/HR
-Na2SO4F5- Na2SO4 CONC IN F5 (50)	32	32	32	32	32	32 *	%
-Na2SO4F6- Na2SO4 CONC IN F6 (50)	21.7	21.7	21.7	21.7	21.7	21.7 *	%
-F5- FLOW OF SATURATED SOLUTION	12592	16789	19238	22736	26234	317	KG/HR
-F6- FLOW OUT OF THE PREC REACTOR	18569	24759	28369	33527	38685	468	KG/HR
PREC REACTOR VOLUME (R-530)	24	32	37	43	50	1	M3
VISCOSITY IN PREC REACTOR	1.1	1.1	1.1	1.1	1.1	1.1 *	CP
POWER CONVERSION EFFICIENCY	0.92	0.92	0.92	0.92	0.92	0.92 *	%
PREC REACTOR POWER CONSUMPTION	2	2	2	3	3	0	HP
POWER PER UNIT VOLUME	0.3	0.3	0.3	0.3	0.3	0.3	HP/1000 GAL
PREC REACTOR (R-530) INST COST ( )	149	162	169	178	186	49	M$
CENTRIFUGE (FF-535) INST COST (13)	350	393	415	444	470	80	M$

IV.8. SODIUM SULFATE SUPPLY AND RECUPERATION

-F5- FLOW OF SATURATED SOLUTION	12592	16789	19238	22736	26234	317	KG/HR
-F6- FLOW OUT OF THE PREC REACTOR	18569	24759	28369	33527	38685	468	KG/HR
-F7- FLOW OF ENZYME SOLUTION	28	37	43	50	58	1	KG/HR
-F8- FLOW OF NaSO4 SOLUTION	18541	24722	28327	33477	38627	467	KG/HR
-F11-	18541	24722	28327	33477	38627	467	KG/HR
-Na2SO4F5-	32.0	32.0	32.0	32.0	32.0	32.0	%
-Na2SO4F6-	21.7	21.7	21.7	21.7	21.7	21.7	%
-Na2SO4F8-	21.7	21.7	21.7	21.7	21.7	21.7	%
-Na2SO4F9-	30.0	30.0	30.0	30.0	30.0	30.0 *	%
-Na2SO4F10-	30.0	30.0	30.0	30.0	30.0	30.0	%
-Na2SO4F11-	21.7	21.7	21.7	21.7	21.7	21.7	%

Na2SO4 RECIRCULATION LOOP CONVERGENCE

-F9-	12232	16310	18688	22086	25484	308	KG/HR
-F10-	5949	7932	9089	10741	12394	150	KG/HR
-V-	1791	2388	2736	3233	3731	45	KG/HR
-S-	360	480	550	650	750	9	KG/HR
-F10-	5949	7932	9089	10741	12394	150	KG/HR
-F9-	12232	16310	18688	22086	25484	308	KG/HR
-F10/F11- PERCENTAGE OF PURGE	32	32	32	32	32	32	%

EVAPORATOR (FE-550)

-F10- PURGE STREAM	5949	7932	9089	10741	12394	150	KG/HR
-F9- RECYCLED STREAM	12232	16310	18688	22086	25484	308	KG/HR
-V- EVAPORATED WATER	1791	2388	2736	3233	3731	45	KG/HR
-Q9- HEAT GIVEN BY F9	1	1	1	2	2	0	MILLIONS BTU/HR
-TF11-	38	38	38	38	38	38	C
-U- HEAT TRANSFER COEFF (13)	104	104	104	104	104	104 *	BTU/(HR*F*FT2)
-Qev- HEAT SUPPLIED BY THE EVAP	5	7	8	9	11	0	MILLIONS BTU/HR

-A- HEAT TRANSFER AREA	215	287	329	388	448	5	FT2
INSTALLED COST	11469	14028	15430	17344	19172	872	M$
EVAP STEAM (150 PSIg) CONSUMPTION	2789	3718	4261	5035	5810	70	KG/HR
EVAP ANNUAL STEAM (150 PSIg) COST	156	209	239	282	326	4	M$/YR
SPECIFIC POWER CONSUMPTION	0.12	0.12	0.12	0.12	0.12	0.12 *	KW/M2
POWER CONSUMTION	3	4	5	6	7	0	HP
-W- WATER TO CONDENSER	45843	61125	70039	82773	95507	1154	KG/HR
COOLING WATER (80 F) COST (10)	0.03	0.03	0.03	0.03	0.03	0.03	$/1000 GAL
ANNUAL COOLING WATER (80 F) COST	3	4	4	5	6	0	M$/YR
AIR DISCARTED BY THE EJECTOR	0	1	1	1	1	0	KG AIR/HR
-S- STEAM CONS BY THE EJECTOR	5	6	7	8	9	0	KG/HR
STEAM (150 PSIg) COST	3.4	3.4	3.4	3.4	3.4	3.4	KG/HR
EJECT ANNUAL STEAM COST	0.3	0.3	0.4	0.5	0.5	0.0	$/1000 LB

PREHEATING HEAT EXCHANGER (E-545)

DUTY	1	1	1	2	2	0	MILLIONS BTU/HR
-TF11- TEMP OF F11	38	38	38	38	38	38	C
Tln	24	24	24	24	24	24	F
OVERALL HEAT TRANSFER COEF (10)	50	50	50	50	50	50	BTU/(HR*F*FT2)
AREA	708	944	1082	1279	1476	18	FT2
INSTALLED COST	89	108	118	131	144	8	M$

FINAL COOLING HEAT EXCHANGER (E-525)

DUTY	0.4	0.6	0.6	0.8	0.9	0.0	MILLIOS BTU/HR
OVERALL HEAT TRANSFER COEF (10)	50	50	50	50	50	50 *	BTU/(HR*FT2*F)
Tln	22	22	22	22	22	22	F
AREA	380	506	580	686	791	10	FT2
INSTALLED COST (10)	60	72	79	88	96	5	M$
CHILLED WATER CONSUMPTION	7024	9366	10731	12682	14634	177	KG/HR
ANNUAL CHILLED WATER COST	19	25	29	34	39	0	M$/YR

SUPPLY OF SATURATED SOLUTION OF Na2SO4

-F5- FLOW OF SATURATED SOLUTION	12592	16789	19238	22736	26234	317	KG/HR
-F9- RECYCLED STREAM	12232	16310	18688	22086	25484	308	KG/HR
-Na2SO4F5-	32	32	32	32	32	32	%
-Na2SO4F9-	30	30	30	30	30	30	%
-S- Na2SO4 ADDITION	360	480	550	650	750	9	KG/HR
Na2SO4 ANNUAL COST	7	9	10	12	14	0	M$/YR

SEWAGE

Na2SO4 DISPOSAL COST (74)	3	3	3	3	3	3	$/1000 GAL
-F10- Na2SO4 (30%) TO SEWAGE	5949	7932	9089	10741	12394	150	KG/HR
Na2SO4 ANNUAL DISPOSAL COST	30	40	46	54	63	1	M$/YR
DISCARTED STREAM	1535	2047	2346	2772	3199	39	KG/HR
% CELLS IN DISCARTED STREAM	11	11	11	11	11	11	%
CELL STRM DISP COST (74)	5	5	5	5	5	5	$/1000 GAL
ANNUAL CELL STRM DISP COST	15	20	23	27	32	0	M$/YR

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IV.9. DIAFILTRATION AND STABILIZATION

-F7-	28	37	43	50	58	1	KG/HR
DIAFITRAFILTR (P-542) INST COST (38)	43	49	52	56	60	8	M$
DIAFILTRAFILT (P-542) OPER COST (38)	0.07	0.08	0.09	0.10	0.11	0.01	M$/YR
SODIUM BENZOATE CONCENTRATION	1	1	1	1	1	1 *	%
SODIUM BENZOATE CONSUMPTION	0.28	0.37	0.43	0.50	0.58	0.01	KG/HR
SODIUM BENZOATE ANNUAL COST	4	5	6	7	9	0	M$/YR

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IV.10. PUMPS

CELL RECOVERY PUMP (J-505)

CENTRIFUGE PRESSURE DROP	5	5	5	5	5	5 *	PSI
TOTAL HEAD	5	5	5	5	5	5	PSI
TOTAL HEAD	12	12	12	12	12	12	FT
CAPACITY	9	12	13	16	18	0	GPM
INSTALLED COST (93,94)	2	2	2	2	2	2	M$/YR
POWER CONSUMPTION	0	0	0	0	0	0	HP
pH AJUSTMENT PUMP (J-510)							
MOTIONLESS MIXER PRESS DROP (13)	9	9	9	9	9	9 *	PSI
TOTAL HEAD	9	9	9	9	9	9	PSI
TOTAL HEAD	21	21	21	21	21	21	FT
CAPACITY	26	35	40	48	55	1	GPM
INSTALLED COST (93,94)	2	2	3	3	3	2	M$/YR
POWER CONSUMPTION	0	1	1	1	1	0	HP
PRECIPITATION REACTOR PUMP (J-532)							
TOTAL HEAD	5	5	5	5	5	5	PSI
TOTAL HEAD	12	12	12	12	12	12	FT
CAPACITY	73	97	111	131	152	2	GPM
INSTALLED COST (93,94)	3	3	3	3	3	2	M$/YR
POWER CONSUMPTION	0	1	1	1	1	0	HP
Na2SO4 SUPPLY PUMP (J-526)							
TOTAL HEAD	5	5	5	5	5	5	PSI
TOTAL HEAD	12	12	12	12	12	12	FT
CAPACITY	47	63	72	85	98	1	GPM
INSTALLED COST (93,94)	2	3	3	3	3	2	M$/YR
POWER CONSUMPTION	0	0	0	1	1	0	HP
Na2SO4 RECYCLE PUMP (J-540)							
TOTAL HEAD (64)	7	7	7	7	7	7	PSI
TOTAL HEAD	16	16	16	16	16	16	FT
CAPACITY	73	97	111	131	151	2	GPM
INSTALLED COST (93,94)	3	3	3	3	3	2	M$/YR
POWER CONSUMPTION	1	1	1	1	1	0	HP
EVAPORATOR PUMP (J-552)							
TOTAL HEAD (64)	14	14	14	14	14	14	PSI
TOTAL HEAD	32	32	32	32	32	32	FT
CAPACITY	46	61	70	83	95	1	GPM
INSTALLED COST (93,94)	3	3	3	3	3	2	M$/YR
POWER CONSUMPTION	1	1	1	1	2	0	HP
CELL STREAM PUMP (J-528)							
HEAD	5	5	5	5	5	5	PSI
HEAD	12	12	12	12	12	12	FT
CAPACITY	93	124	143	168	194	2	GPM
INSTALLED COST (93,94)	3	3	3	3	3	2	M$
pH AJUSTMENT PUMP (J-522)							
CAPACITY	0.00001	0.00002	0.00002	0.00003	0.00003	0.00000	GPM
INSTALLED COST	3	4	4	4	4	0	M$
FINAL PRODUCT PUMP (J-546)							
CAPACITY	1	1	1	1	1	0	GPM
HEAD	15	15	15	15	15	15	FT
HEAD	7	7	7	7	7	7	PSI
INSTALLED COST (93,94)	2	2	2	2	2	2	M$

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IV.11. MIXERS AND TANKS

CENTRIFUGATION MIXER (M-503)							
VOLUME	0.11	0.14	0.17	0.20	0.23	0.00	M3
INSTALLED COST (47,16,54)	30	32	34	35	37	10	M$
PRECIPITATION SURGE TANK (TT-508)							
VOLUME	4	5	6	7	8	0	M3
INSTALLED COST (47,16,54)	120	146	161	180	198	10	M$
CELL KILLING SURGE TANK (TT-514)							
VOLUME	7	9	11	13	15	0	M3
INSTALLED COST (47,16,54)	190	231	253	283	312	16	M$
CELL KILLING MIXER (M-516)							
VOLUME	7	9	11	13	15	0	M3
INSTALLED COST (47,16,54)	52	63	69	77	85	4	M$
EVAPORATOR SURGE TANK (TT-538)							
VOLUME	9	12	14	16	19	0	M3
INSTALLED COST (47,16,54)	225	273	300	336	370	18	M$
STANDARIZATION/STABILIZATION MIXER (M-544)							
VOLUME	0.05	0.06	0.07	0.09	0.10	0.00	M3
INSTALLED COST (28)	3	3	4	4	4	0	M$
FINAL PRODUCT STORAGE TANKS (TT-548 ^ TT-549)							
VOLUME (ONE TANK)	4	5	5	6	7	0	M3
INSTALLED COSTS (16)	81	93	99	107	113	5	M$
pH AJUSTMENT TANK (TT-520)							
VOLUME	3.63E-06	4.84E-06	5.54E-06	6.55E-06	7.56E-06	9.14E-08	M3
INSTALLED COST (47, 16, 54)	10	12	13	15	17	1	M$
Na2SO4 SUPPLY MIXER (M-524)							
VOLUME	6	8	9	11	12	0	M3
INSTALLED COST (47,16,54)	171	202	219	241	262	20	M$

IV.12. TOTAL POWER CONSUMPTION

POWER CONSUMED	8	10	11	13	15	0	HP
ANNUAL COST	2	2	3	3	3	0	M$/YR

V. COST SUMMARY

V.1. LACTOSE HYDROLYSIS

MAJOR EQUIPMENTS							
I-120+RESIN	184	220	239	263	286	49	M$
I-130+RESIN	222	253	268	288	311	53	M$
R-170	29	35	38	42	46	3	M$
E-160	70	79	84	90	96	15	M$
P-195	454	516	548	591	630	88	M$
TOTAL	960	1102	1176	1274	1369	208	M$
PUMPS							
J-110	3	3	3	3	3	2	M$
J-141	145	168	181	197	212	21	M$
J-150	2	2	2	2	2	2	M$
J-180	2	2	2	2	2	2	M$
J-192	2	2	2	2	2	2	M$
J-193	2	2	2	2	2	2	M$

TOTAL	157	181	193	210	225	34	M$
MIXERS AND TANKS							
M-142	5	6	6	7	7	1	M$
M-140	27	31	34	37	40	4	M$
M-121	197	231	248	272	294	27	M$
M-190	59	69	74	81	87	8	M$
TT-100	48	54	57	61	64	4	M$
TT-171	72	78	81	84	87	10	M$
TT-172	72	78	81	84	87	10	M$
TOTAL	479	546	580	625	665	63	M$
RAW MATERIALS AND SUPPLIES							
HCL 20 Be	30	40	46	54	62	1	M$/YR
NaOH 76%	126	168	192	227	262	3	M$/YR
IMMOBILIZED LACTASE	311	415	475	562	648	8	M$/YR
ACETIC ACID	0	0	0	0	0	0	M$/YR
UF MEMBRANES	260	300	321	349	375	41	M$/YR
TOTAL	726	922	1034	1192	1348	53	M$/YR
UTILITIES							
PROCESS WATER (DEIONIZED)	0	0	0	0	0	0	M$/YR
COOLING WATER (80 F)	2	2	2	3	3	0	M$/YR
ELECTRICITY	14	19	21	25	29	0	M$/YR
TOTAL	16	21	24	28	32	0	M$/YR
DISPOSAL							
BRAKISH WATER	18	24	27	32	37	0	M$/YR
******TOTAL INSTALLED COST	1597	1830	1950	2109	2259	305	M$
******TOTAL OPERATING COST	760	967	1085	1252	1417	54	M$/YR

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V.2. FERMENTATION

MAJOR EQUIPMENTS							
E-311	43	48	51	55	58	9	M$
E-312	73	82	87	93	99	16	M$
E-313	189	214	226	243	258	40	M$
E-321	63	71	75	81	86	13	M$
E-322	63	71	75	81	86	13	M$
E-323	64	72	77	82	87	14	M$
E-440	243	299	331	374	415	18	M$
E-470	54	61	65	69	74	12	M$
R-450	94	102	106	113	119	73	M$
R-455	335	423	475	548	621	79	M$
R-460	2	2	3	3	4	0	M$
J-410	188	239	268	308	347	9	M$
F-447	3	4	4	5	6	0	M$
F-457	2	3	3	4	4	0	M$
F-450	3	4	4	5	6	0	M$
F-458	2	3	3	4	4	0	M$
TOTAL	1422	1699	1853	2066	2273	295	M$
PUMPS							
J-330	19	22	24	26	28	3	M$
J-350	227	264	283	309	333	34	M$
J-355	215	250	269	293	316	32	M$
J-453	532	618	663	723	779	78	M$
J-465	2	2	2	2	2	2	M$
J-460	2	2	2	2	3	2	M$
J-457	2	2	2	2	2	2	M$
TOTAL	1001	1161	1246	1359	1463	154	M$
MIXERS AND TANKS							
M-340	0	0	0	0	0	0	M$
M-352	1	1	1	1	2	0	M$

M-380 + M-385	4	5	5	5	6	2	M$
M-390 + M-395	3	3	4	4	4	2	M$
M-356	1	1	1	1	1	0	M$
M-451	1	1	1	1	1	0	M$
M-452	8	9	10	10	11	1	M$
TOTAL	18	20	21	23	25	5	M$
RAW MATERIALS AND SUPPLIES							
NaOH 76%	36	48	55	65	75	1	M$/YR
SALTS	553	737	844	998	1151	14	M$/YR
ANTIFOAM	13	17	20	23	27	0	M$/YR
AIR FILTRATION CARTRIDGES	155	207	237	280	323	4	M$/YR
TOTAL	757	1009	1156	1366	1577	19	M$/YR
UTILITIES							
CHILLED WATER	2314	3085	3534	4177	4819	58	M$/YR
STEAM	24	33	37	44	51	1	M$/YR
ELECTRIC ENERGY	81	105	119	138	157	3	M$/YR
TOTAL	2420	3223	3691	4359	5027	62	M$/YR
******TOTAL INSTALLED COST	2440	2880	3121	3448	3761	454	M$
******TOTAL OPERATING COST	3176	4232	4847	5725	6604	81	M$/YR

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V.3. SEPARATION

MAJOR EQUIPMENTS							
FF-502	245	275	290	310	329	56	M$
FF-504	150	168	177	190	201	34	M$
P-506	206	251	275	307	338	17	M$
R-530	149	162	169	178	186	49	M$
FF-535	350	393	415	444	470	80	M$
P-542	43	49	52	56	60	8	M$
FE-550	11469	14028	15430	17344	19172	872	M$
E-545	89	108	118	131	144	8	M$
E-525	60	72	79	88	96	5	M$
TOTAL	12762	15506	17005	19049	20996	1131	M$
PUMPS							
J-505	2	2	2	2	2	2	M$
J-510	2	2	3	3	3	2	M$
J-528	3	3	3	3	3	2	M$
J-540	3	3	3	3	3	2	M$
J-546	2	2	2	2	2	2	M$
J-522	3	4	4	4	4	0	M$
J-526	2	3	3	3	3	2	M$
J-552	1	1	1	1	2	0	M$
TOTAL	19	20	20	21	21	15	M$
MIXERS AND TANKS							
M-503	30	32	34	35	37	10	M$
M-516	52	63	69	77	85	4	M$
M-544	3	3	4	4	4	0	M$
M-524	171	202	219	241	262	20	M$
M-512	2	3	3	3	3	1	M$
TT-508	120	146	161	180	198	10	M$
TT-514	190	231	253	283	312	16	M$
TT-538	225	273	300	336	370	18	M$
TT-548 + TT-549	81	93	99	107	113	5	M$
TT-520	10	12	13	15	17	1	M$
TOTAL	883	1059	1154	1282	1402	86	M$
RAW MATERIALS AND SUPPLIES							
PROCESS WATER (DEIONIZED)	0	0	0	0	0	0	M$/YR
SODIUM BENZOATE	4	5	6	7	9	0	M$/YR
AMONIUM SULFATE	7	9	10	12	14	0	M$/YR

HCL 20 Be	0	0	0	0	0	0	M$/YR
NaOH	0	0	0	0	0	0	M$/YR
UF MEMBRANES	1	1	1	1	1	0	M$/YR
TOTAL	12	15	18	21	24	0	M$/YR
UTILITIES							
STEAM	157	209	239	283	326	4	M$/YR
ELECTRICAL ENERGY	2	2	3	3	3	0	M$/YR
CHILLED WATER	19	25	29	34	39	0	M$/YR
COOLING WATER	3	4	4	5	6	0	M$/YR
TOTAL	180	240	275	325	374	5	M$/YR
DISPOSAL							
BIOMASS STREAM (~12% CELLS)	15	20	23	27	32	0	M$/YR
Na2SO4	30	40	46	54	63	1	M$/YR
TOTAL	45	60	69	82	94	1	M$/YR
******TOTAL INSTALLED COST	13664	16584	18179	20351	22419	1232	M$/YR
******TOTAL OPERATING COST	237	315	361	427	493	6	M$/YR

V.4. TOTALS

MAJOR EQUIPMENTS	15143	18307	20035	22389	24637	1634	M$
PUMPS	1177	1362	1460	1589	1710	202	M$
MIXERS AND TANKS	1380	1625	1756	1930	2093	154	M$
TOTAL EQUIPMENTS' INSTALLED COST	17701	21294	23250	25908	28439	1990	M$
INSTRUMENTATION AND CONTROLS (28)	2160	2598	2837	3161	3470	243	M$
PIPING (28)	7948	9561	10439	11633	12769	894	M$
ELECTRICAL (28)	1328	1597	1744	1943	2133	149	M$
BUILDINGS (28)	2160	2598	2837	3161	3470	243	M$
TOTAL DIRECT PLANT COST (28)	31296	37648	41106	45806	50280	3519	M$
ENGINEERING AND SUPERVISION (28)	3965	4770	5208	5803	6370	446	M$
CONSTRUCTION EXPENSES (28)	4939	5941	6487	7228	7934	555	M$
TOTAL DIR AND INDIRECT PLANT COSTS	40200	48360	52801	58838	64585	4520	M$
CONTRACTOR'S FEE (28)	2010	2418	2640	2942	3229	226	M$
CONTINGENCY (28)	4020	4836	5280	5884	6459	452	M$
FIXED CAPITAL INVESTMENT (28)	46230	55613	60721	67664	74273	5198	M$
WORKING CAPITAL (28)	10355	12457	13601	15156	16637	1164	M$
TOTAL CAPITAL INVESTMENT (28)	56585	68071	74323	82820	90910	6363	M$
START-UP COST (28)	3698	4449	4858	5413	5942	416	M$

V.5. TOTAL PRODUCTION COSTS

RAW MATERIALS AND SUPPLIES	1495	1947	2208	2579	2948	72	M$/YR
UTILITIES	2615	3483	3989	4712	5434	67	M$/YR
DISPOSAL	63	84	96	114	131	2	M$/YR
TOTAL PRODUCT COST (28,10)	22468	28088	31253	35666	39976	1821	M$/YR

VI. REVENUES

WORLD MARKET (75,76,77)							
B. PROTEASE	2	2	2	2	2	2 *	MILLIONS KG/YR
GLUCOSE ISOMERASE	0.152	0.152	0.152	0.152	0.152	0.152 *	MILLIONS KG/YR
CALF RENNET	0.0138	0.0138	0.0138	0.0138	0.0138	0.0138 *	MILLIONS KG/YR

SELLING PRICE (77)

B. PROTEASE	100	100	100	100	100	100 *	$/KG PURE ENZYME
GLUCOSE ISOMERASE	250	250	250	250	250	250 *	$/KG PURE ENZYME
CALF RENNET	5000	5000	5000	5000	5000	5000 *	$/KG PURE ENZYME
PRODUCTION TIME							
B. PROTEASE	65	65	65	65	65	65 *	%
GLUCOSE ISOMERASE	30	30	30	30	30	30 *	%
CALF RENNET	5	5	5	5	5	5 *	%
CONTAMINATION LOSS	2	2	2	2	2	2 *	%
BROWTH CONCENTRATIONS							
B-LACTAMASE	4.74	4.74	4.74	4.74	4.74	4.74	G/LIT
B. PROTEASE	2.25	2.25	2.25	2.25	2.25	2.25	G/LIT
GLUCOSE ISOMERASE	4.07	4.07	4.07	4.07	4.07	4.07	G/LIT
CALF RENNET	3.33	3.33	3.33	3.33	3.33	3.33	G/LIT
-EF7-	1115	1115	1115	1115	1115	1115	G/LIT
F7	0.021	0.029	0.033	0.039	0.045	0.001	M3/HR
TOTAL ENZYMES' PRODUCED							
B. PROTEASE	0.0540	0.0720	0.0825	0.0975	0.1126	0.0014	MILLIONS KG/YR
GLUCOSE ISOMERASE	0.0451	0.0601	0.0689	0.0814	0.0940	0.0011	MILLIONS KG/YR
CALF RENNET	0.0062	0.0082	0.0094	0.0111	0.0128	0.0002	MILLIONS KG/YR
WORLD MARKET SHARE							
B. PROTEASE	3	4	4	5	6	0	%
GLUCOSE ISOMERASE	30	40	45	54	62	1	%
CALF RENNET	45	60	68	81	93	1	%
REVENUES							
B. PROTEASE	5403	7204	8254	9755	11256	136	M$/YR
GLUCOSE ISOMERASE	11275	15034	17226	20358	23490	284	M$/YR
CALF RENNET	30812	41082	47073	55632	64191	776	M$/YR
ENZYMES REVENUES	47490	63319	72554	85745	98937	1196	M$/YR
DISPOSAL REVENUES	56	74	85	101	116	1	M$/YR
TOTAL REVENUES	47545	63394	72639	85846	99053	1197	M$/YR

===

VII. PROFITABILITY ANALYSIS

ANNUAL PROFITS	25078	35306	41385	50179	59076	-624	M$/YR

YEAR				CASH FLOWS			
-3	-15410	-18538	-20240	-22555	-24758	-1733	M$/YR
-2	-15410	-18538	-20240	-22555	-24758	-1733	M$/YR
-1	-15410	-18538	-20240	-22555	-24758	-1733	M$/YR
0	-14054	-16906	-18459	-20570	-22579	-1580	M$/YR
1	25078	35306	41385	50179	59076	-624	M$/YR
2	25078	35306	41385	50179	59076	-624	M$/YR
3	25078	35306	41385	50179	59076	-624	M$/YR
4	25078	35306	41385	50179	59076	-624	M$/YR
5	25078	35306	41385	50179	59076	-624	M$/YR
6	25078	35306	41385	50179	59076	-624	M$/YR
7	25078	35306	41385	50179	59076	-624	M$/YR
8	25078	35306	41385	50179	59076	-624	M$/YR
9	25078	35306	41385	50179	59076	-624	M$/YR
10	35433	47763	54987	65336	75713	540	M$/YR

RATE OF RETURN	26	29	31	33	35	-54	%
PAYOUT TIME	1.99	1.70	1.58	1.46	1.36	-9.00	YR
RETURN ON INVESTMENT	44	52	56	61	65	-10	%
TOTAL CAPITAL INVESTMENT	56585	68071	74323	82820	90910	6363	M$

=== PRECAUTION: LOTUS GETS THE RATE OF RETURN BY TRIAL AND ERROR CALCULATIONS.
THE SPREADSHEET HAS AN INITIAL GUESS THAT LOTUS USES AS FIRST APPROXIMATION.
IF THE TRUE VALUE OF THE RATE OF RETURN IF TOO FAR FROM THE INITIAL GUESS,
LOTUS WILL PRINT AN ERROR. IF THAT HAPPENS SIMPLY CHANGE THE INITIAL
GUESS AND RUN THE CALCULATIONS AGAIN. THIS ONLY HAPPENS WHEN THE INITIAL
GUESS IS MORE THAN 35% OFF FROM THE TRUE VALUE.

Appendix 2

Experimental

The Cornell Excretion System (CES) has only been tested with isopropyl ß-D-thiogalactoside (IPTG) as inducer. Batch fermentations were performed to find out how it responds to lactose as inducer. The strains employed were:

> RB791(pKN). The lac i (repressor) gene is not in the pKN plasmid. So, the cells are strongly induced and die relatively fast.
>
> W3110(pKNI). The presence of the lac i gene in the plasmid allows the formation of lac i molecules when the cells are induced. This results in lower expression levels, and higher growth rates.
>
> RB791(Host). It has similar growth rates as W3110(Host).

The medium consisted of several concentrations of glucose (Sigma; G-8270), galactose (Sigma; G-0750), and lactose (Mallinckrodt; 5652), in Tanaka salts at 7.2 pH. Selective pressure was applied by the inclusion of 250 µg/mL of neomycin (Sigma; N-1876). The pKN and pKNI plasmids had a neomycin resistance gene.

Cell density was determined by measuring the optical density at 600 nm in a spectrophotometer (Bausch & Lomb, spectronic 20). Extracellular ß-lactamase was determined by measuring the rate of absorbance decrease of penicillin G (Sigma; PEN-K) at 240 nm. Penicillin was dissolved in 50 mM phosphate buffer, pH 7.0. One mmol of penicillin G cleaved per minute at 25 °C, was defined as one unit of ß-lactamase. The extinction coefficient was 0.57 A_{240} units of ß-lactamase/mmol of penicillin G[1]. A Beckman Acta MV2 spectrophotometer was employed in these determinations.

Figures A2.1 trough A2.3 confirm that lactose was metabolized as a nutrient. Figure A2.4 shows that there is a direct relationship between the lactose concentration and the amount of ß-lactamase excreted. Maximum enzyme excretion and lower growth rates were obtained with RB791(pKN) (figure A2.5). Opposite trends were observed in W3110(pKNI). This is easily explained by the location of the lac i gene in both strains. Figure A2.6 compares the response of the CES when IPTG and lactose are employed as inducers.

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1. Togna, A. (1991). Population Dynamics of E. coli Overproducing a Plasmid Encoded Protein in Batch and Continuous Culture. Ph.D. Thesis. Cornell University. Ithaca, NY.

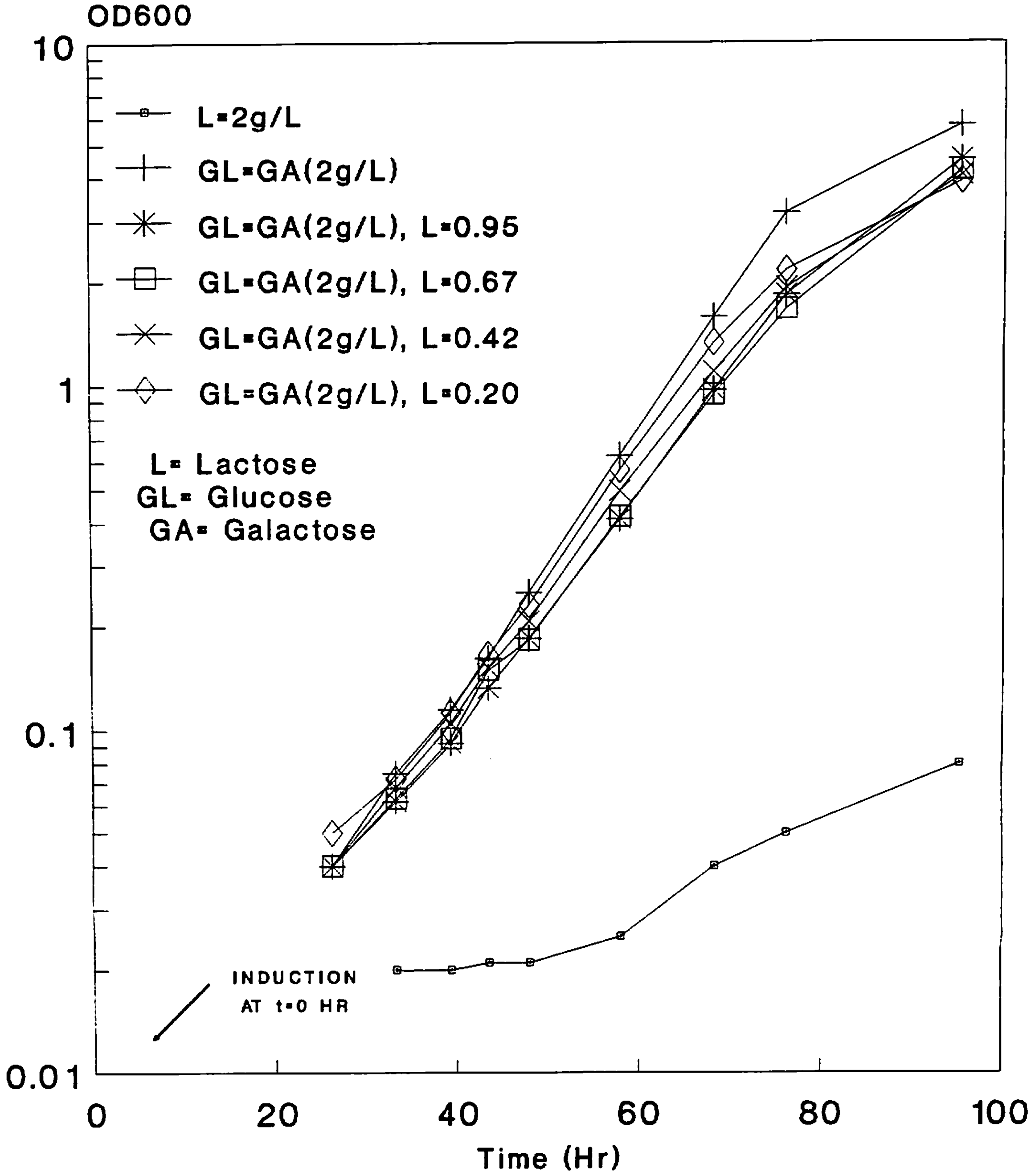

Figure A2.1 Enzymes' Production
Cell Growth Experiments (Batch, Initial Induction)
T: 20 °C, Strain: RB791(pKN), Tanaka Medium
Lactose Induction

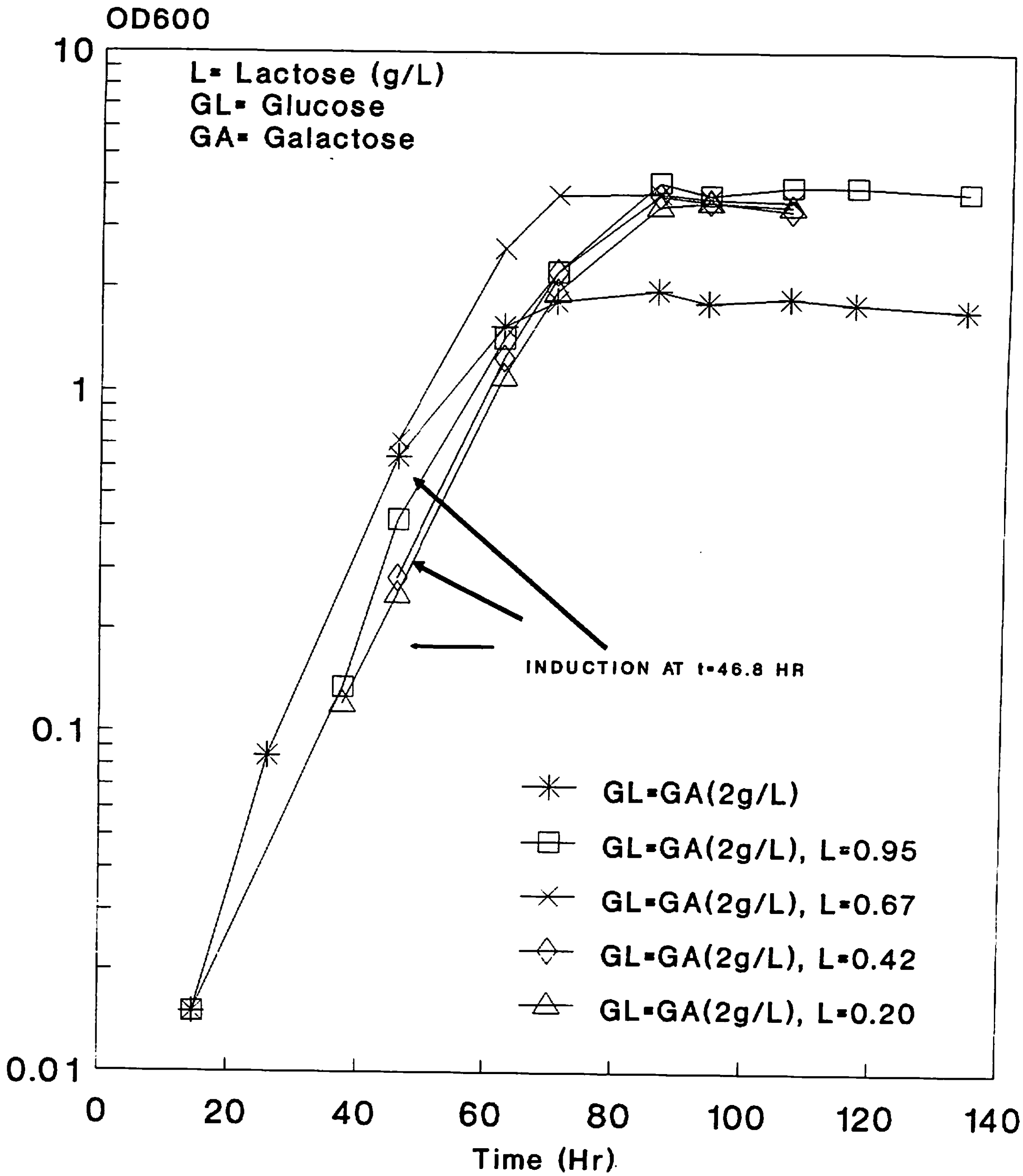

Figure A2.2a Enzymes' Production
Cell Growth Experiments (Batch, Intermediate Induction)
T:20 °C, Strain: W3110(pKNI), Tanaka Medium
Lactose Induction

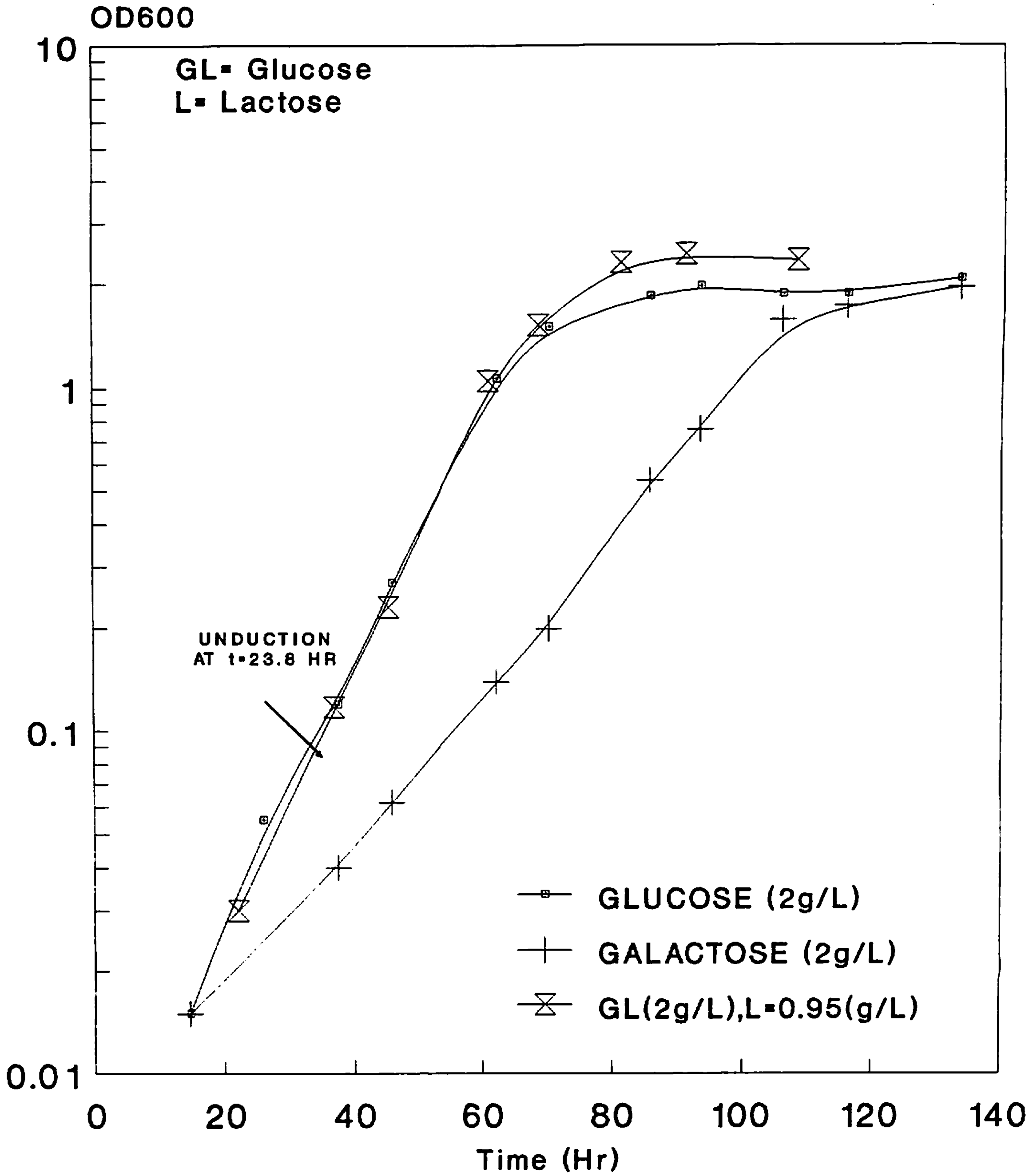

Figure A2.2b Enzymes' Production
Cell Growth Experiments (Batch, Intermediate Induction)
T: 20 °C, Strain: W3110(pKNI), Tanaka Medium
Lactose Induction

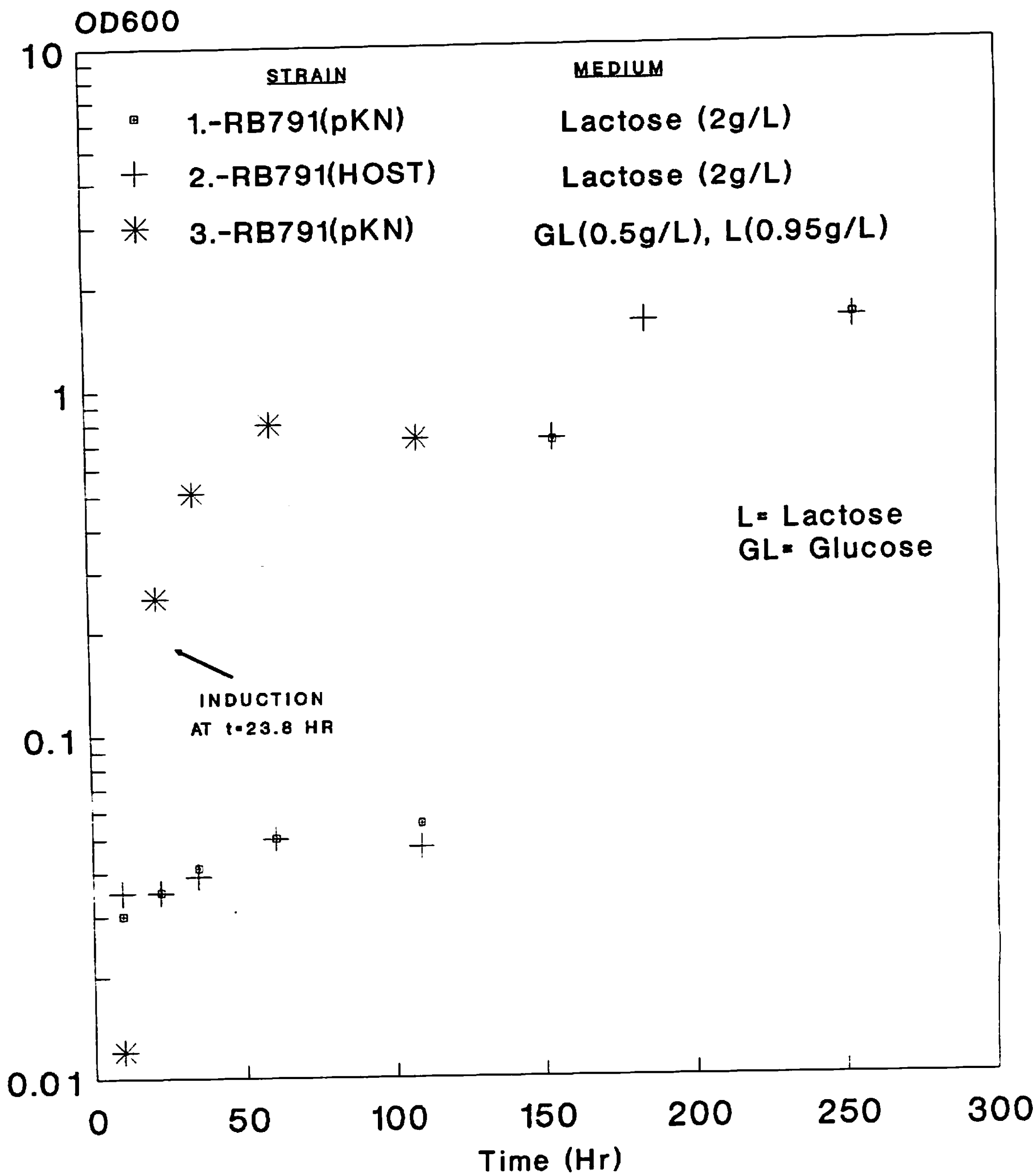

Figure A2.3 Enzymes' Production
Cell Growth Experiments (Batch, Initial (t=0 Hr) and Intermediate Induction)
T: 20 °C, Tanaka Medium, Lactose Induction

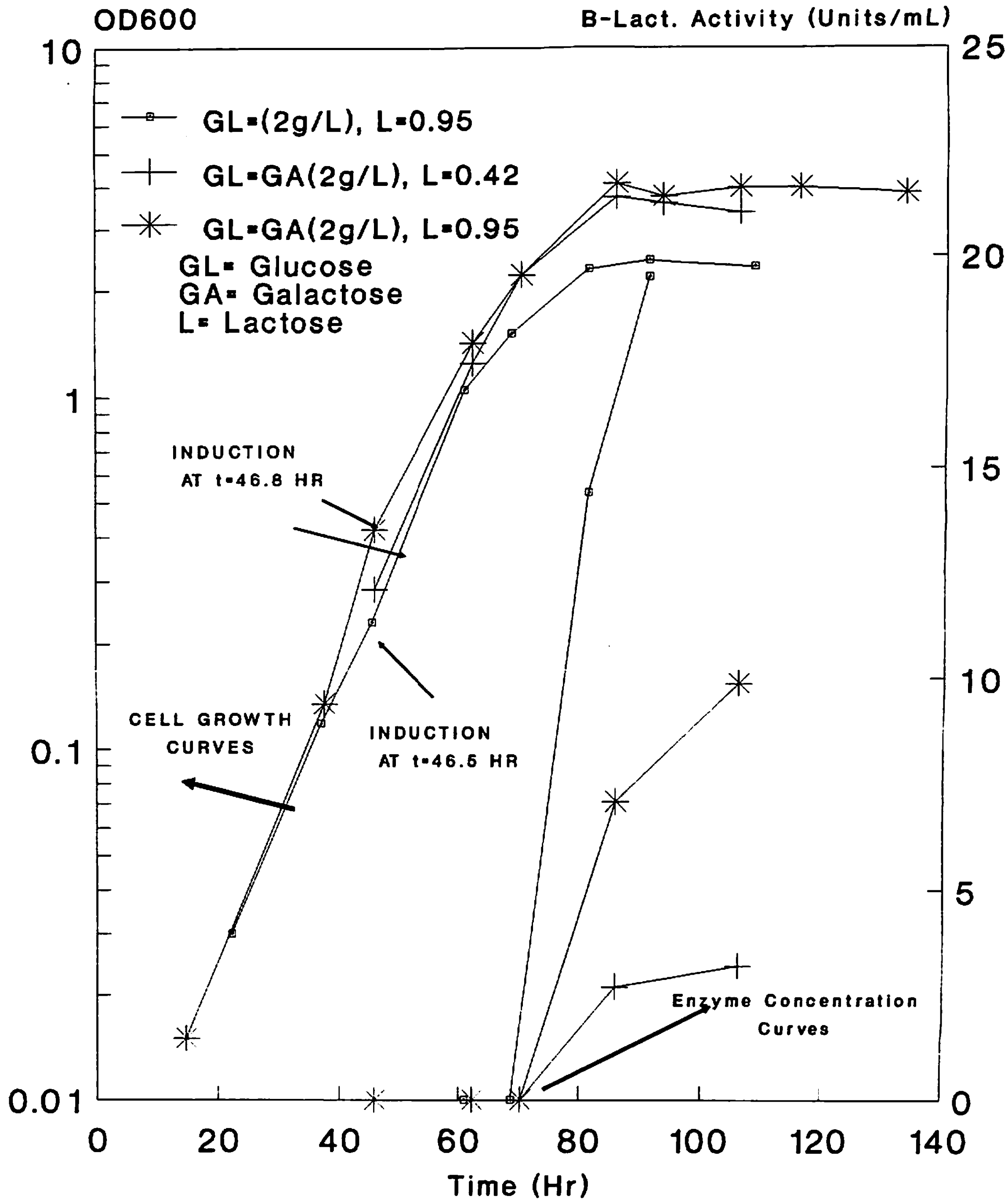

Figure A2.4 Enzymes' Production
Enzyme Excretion (Batch)
T:20 °C, Strain: W3110(pKNI), Tanaka Medium
Lactose Induction

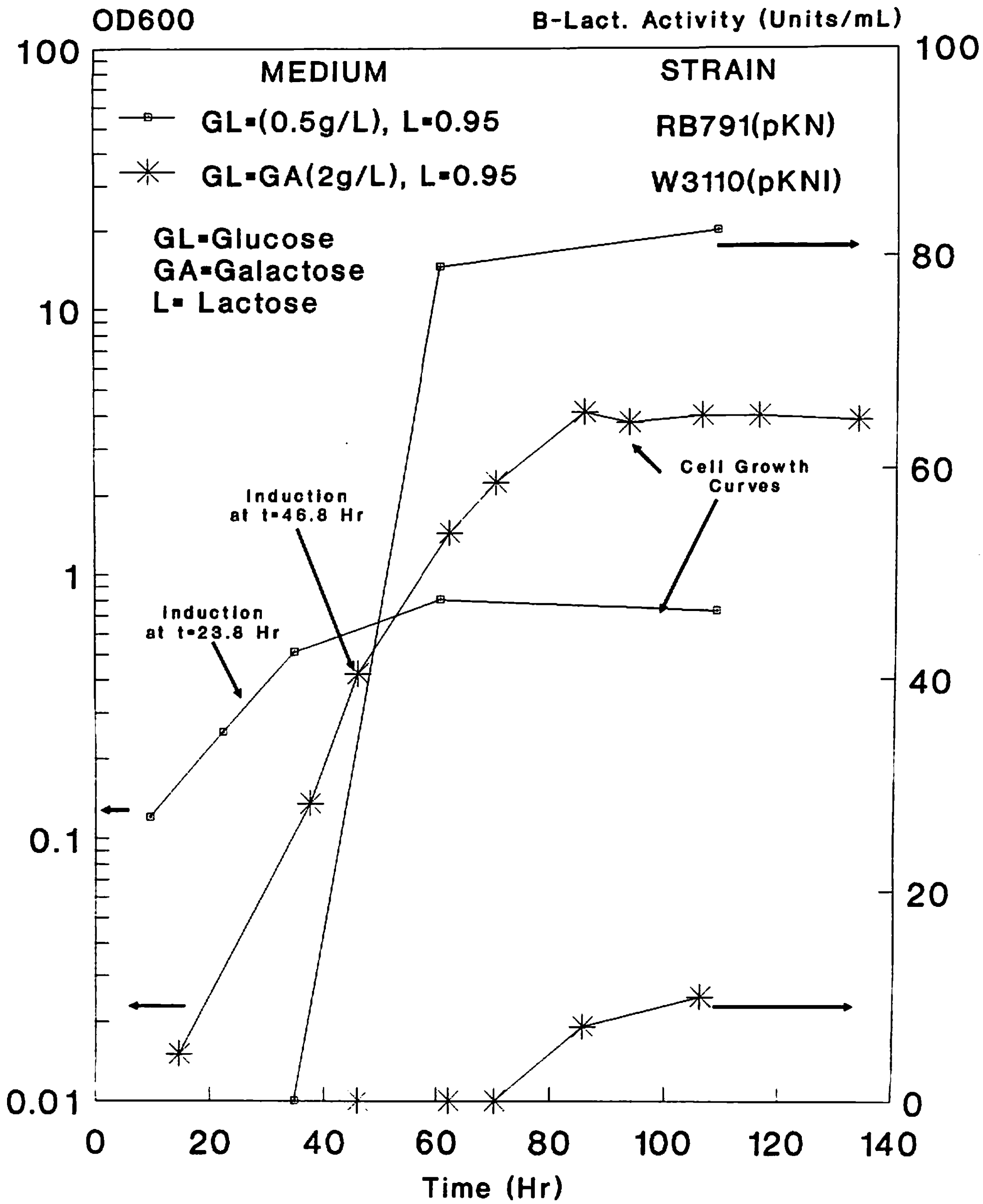

Figure A2.5 Enzymes' Production
Maximum Enzyme Excretion
T:20 °C, Tanaka Medium, Lactose Induction

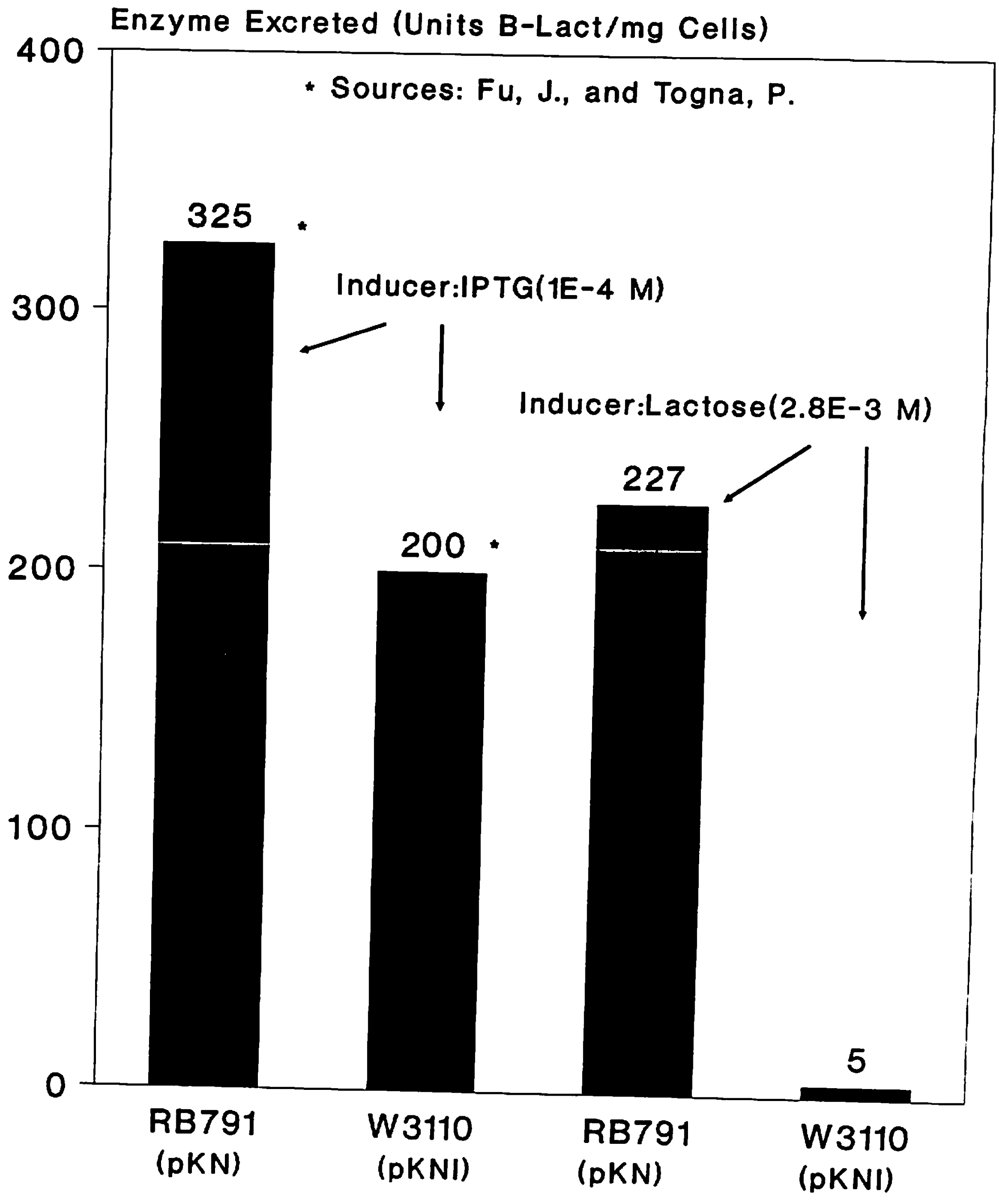

Figure A2.6 Enzymes' Production
Enzyme Excretion Comparison
T: 20 C, Tanaka Medium