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# THE MATHEMATICAL AND ECONOMIC MODELING OF CONTINUOUS ALPHA-AMYLASE PRODUCTION USING THE AIRLIFT FERMENTER

By

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## ABSTRACT

### THE MATHEMATICAL AND ECONOMIC MODELING OF CONTINUOUS ALPHA-AMYLASE PRODUCTION USING THE AIRLIFT FERMENTER

By

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Mathematical and economic modeling were performed on a chemical plant producing alpha-amylase enzyme using an airlift fermenter. Modeling of the airlift fermenter was performed by a BASIC program which used the reactors-in-series flow simulation. The sensitivity of reactor output, conversion, and productivity to various reactor parameters was determined. Economic simulations were run which studied the effect of various reactor parameters and economic factors upon overall economics. Results indicate that a continuous alpha-amylase plant is economically possible with currently available continuous process technology.

This thesis is dedicated to my parents, Norman and Joan Viviano. Without their love and support my dreams would only have been dreams...

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TABLE OF CONTENTS

LIST OF TABLES . . . . . viii

LIST OF FIGURES . . . . . ix

INTRODUCTION . . . . . 1

CHAPTER 1: LITERATURE SURVEY . . . . . 5
1. MICROORGANISM REVIEW . . . . . 5
1.1 *Bacillus stearothermophilus* cell dynamics . . . . . 5
1.2 Recovery of microorganism products . . . . 5
1.3 Thermostable microorganisms and enzymes . . 6
2. SPECIFIC GROWTH MODEL REVIEW . . . . . 6
2.1 The Monod specific growth rate model . . . 6
2.2 Model constants . . . . . 7
3. REACTOR FLOW REGIME REVIEW . . . . . 8
3.1 Models for ideal flow . . . . . 8
3.2 Models for non-ideal flow . . . . . 9
4. OXYGEN INFUSION REVIEW . . . . . 10
4.1 Oxygen as a cell substrate . . . . . 10
4.2 Oxygen transport and $K_l a$ . . . . . 11
5. MATHEMATICAL MODEL REVIEW . . . . . 13
5.1 Substrate, cell and product mass derivations using the Monod model . . . . 13
5.2 Limitations of the Monod model . . . . . 14
5.3 Extensions of the Monod model . . . . . 14
5.4 Other growth models . . . . . 15
6. AIRLIFT AND CSTR REACTOR REVIEW . . . . . 16
6.1 History of airlift fermenters . . . . . 16
6.2 Physical structure of airlift fermenters . 18
6.3 Flow dynamics of CSTR and airlift fermenters . . . . . 18
6.4 Modeling process for airlift fermenters . . 19
7. CONTINUOUS PROCESS REVIEW . . . . . 19
8. MEDIA AND AIR STERILIZATION REVIEW . . . . . 20
8.1 Continuous versus batch sterilization . . . 20
8.2 Continuous sterilization processes . . . . 20
8.3 Air sterilization . . . . . 21
9. SEPARATIONS REVIEW . . . . . 23
9.1 Cell and product separations . . . . . 23
9.2 Ultrafiltration separations . . . . . 23

10. ECONOMIC PROCESS ANALYSIS REVIEW . . . . . 24
10.1 Economic estimations . . . . . 24
10.2 Capital investment estimations . . . . . 24
10.3 Production cost estimations . . . . . 26

CHAPTER II: MODEL DEVELOPMENT SECTION . . . . . 27
1. MATHEMATICAL DERIVATIONS FOR THE AIRLIFT FERMENTER . . . . . 27
1.1 Program variables . . . . . 27
1.2 Cell mass balance . . . . . 27
1.3 Oxygen and carbon mass balance . . . . . 27
1.4 Product mass balance . . . . . 28
1.5 Base case variables . . . . . 29
1.6 Recycle derivations . . . . . 30
2. ECONOMIC MODELS . . . . . 32
2.1 BASIC computer models . . . . . 32
2.2 Supercalc4 computer models . . . . . 36

CHAPTER III: PREVIEW OF MODELING STUDIES . . . . . 42
1. EXPLANATIONS OF MODELING VARIABLES . . . . . 42
1.1 Substrates . . . . . 42
1.2 Yield coefficients and $\mu_{max}$ . . . . . 43
1.3 Monod constants . . . . . 43
1.4 Oxygen mass transfer parameter, $K_La$ . . . . 44
2. COMPUTER MODELING STUDIES PERFORMED . . . . . 44
2.1 Cell parameter comparisons . . . . . 44
2.2 Reactor parameter comparisons . . . . . 44
2.3 Economic parameter comparisons . . . . . 45

CHAPTER IV: RESULTS OF AIRLIFT FERMENTER REACTOR MODELING . . . . . 47
1. CELL PARAMETER STUDIES - YIELD COEFFICIENTS . . . 47
1.1 Yield coefficient - $Y_{x/o}$ . . . . . 47
1.2 Yield coefficient - $Y_{p/s}$ . . . . . 47
2. CELL PARAMETER STUDIES - SUBSTRATES . . . . . 50
2.1 Substrate studies - carbon . . . . . 50
2.2 Substrate studies - oxygen . . . . . 52
3. REACTOR PARAMETER STUDIES - TANKS-IN-SERIES . . . 56
4. REACTOR PARAMETER STUDIES - REACTOR RECYCLE RATIO . . . . . 59
5. REACTOR PARAMETER STUDY - DILUTION RATE . . . . . 61

CHAPTER V: RESULTS OF ECONOMIC PROCESS MODELING . . . . . 63
1. ECONOMIC STUDIES - REACTOR OUTPUT . . . . . 64
1.1 Change in return on investment . . . . . 64
1.2 Change in capital investment . . . . . 66
2. ECONOMIC STUDIES - YEARLY PRODUCTION OUTPUT . . . 68
3. ECONOMIC STUDIES - COST COMPARISONS . . . . . 69
3.1 Equipment cost comparisons . . . . . 69
3.2 Yearly production cost comparisons . . . . 69

CHAPTER VI: DISCUSSION OF RESULTS . . . . . 72
1. PRELIMINARIES . . . . . 72
1.1 Overview of studies performed . . . . . 72
1.2 Relationship between studies . . . . . 72
2. YIELD COEFFICIENT RESULTS . . . . . 73
2.1 Varying $Y_{x/o}$ . . . . . 73
2.2 Varying $Y_{p/s}$ . . . . . 74
3. SUBSTRATE RESULTS . . . . . 74
3.1 Varying the carbon substrate . . . . . 75
3.2 Varying the oxygen substrate . . . . . 76
4. NUMBER OF REACTORS IN SERIES . . . . . 78
5. REACTOR RECYCLE RATIO . . . . . 78
6. REACTOR DILUTION RATE . . . . . 79
7. CONCLUSION OF AIRLIFT FERMENTER DISCUSSION . . . 80
8. INFLUENCE OF REACTOR OUTPUT UPON ECONOMICS . . . 82
9. REACTOR OUTPUT AND RETURN ON INVESTMENT . . . . . 82
10. REACTOR OUTPUT AND CAPITAL INVESTMENT . . . . . 84
11. YEARLY PRODUCTION AND RETURN ON INVESTMENT . . . 85
12. DETERMINING THE LARGEST COSTS . . . . . 86
12.1 Largest capital investments . . . . . 86
12.2 Largest yearly production costs . . . . . 86
13. DISCUSSION SUMMARY . . . . . 87

CONCLUSION . . . . . 89

RECOMMENDATIONS . . . . . 92

APPENDIX A - Derivation of Yield Coefficient, $Y_{p/o}$ . . . . 95

APPENDIX B - Derivation of Oxygen and Carbon Substrate Mass Balances . . . . . 99

APPENDIX C - Price Equations for Capital Investment . . 101

APPENDIX D - Proof of Conversion Fall Off For Low Substrate Concentrations . . . . . 102

APPENDIX E - Base Case Studies . . . . . 103

LIST OF REFERENCES . . . . . 105

## LIST OF TABLES

TABLE 1: Capital Investment vs. Reactor output . . . . . 65

TABLE 2: Capital Investment vs. ROI . . . . . 66

TABLE 3: Capital Investment Factor vs. Capital Inv. . . . 67

TABLE 4: Capital Investment Factor vs. Capital Inv. . . . 67

TABLE 5: Fermentation Costs vs. Yearly MFG. Costs . . . . 70

## LIST OF FIGURES

Figure 1: Principle of An Airlift Reactor . . . . . 17

Figure 2: Alpha-amylase Process Diagram . . . . . 22

Figure 3: Simulation Flow Diagram . . . . . 32

Figure 4: Cell Parameter Comparisons, Yield Coefficient $Y_{x/o}$ . . . . . 48

Figure 5: Cell Parameter Comparisons, Yield Coefficient $Y_{p/s}$ . . . . . 49

Figure 6: Cell Parameter Comparisons, Carbon Substrate Level Studies - Monod Constants: Set 1 . . . . 52

Figure 7: Cell Parameter Comparisons, Carbon Substrate Level Studies - Monod Constants: Set 2 . . . . 52

Figure 8: Cell Parameter Comparisons, $K_la$ Transfer Studies - Monod Constants: Set 1 . . . . . 54

Figure 9: Cell Parameter Comparisons, $K_la$ Transfer Studies - Monod Constants: Set 1 . . . . . 54

Figure 10: Cell Parameter Comparisons, $K_la$ Transfer Studies - Monod Constants: Set 2 . . . . . 55

Figure 11: Reactor Parameter Comparisons, Ideal Tank Series - Monod Constants: Set 1 . . . . 57

Figure 12: Reactor Parameter Comparisons, Ideal Tank Series - Monod Constants: Set 2 . . . . . 58

Figure 13: Reactor Parameter Comparisons, Recycle Ratio - Monod Constants: Set 1 . . . . . 59

Figure 14: Reactor Parameter Comparisons, Recycle Ratio - Monod Constants: Set 2 . . . . . 60

Figure 15: Reactor Parameter Comparisons, Dilution Rate Studies - Monod Constants: Set 1 . . . . 61

Figure 16: Economic Comparison Study, Change in % of Return on Investment . . . . . 64

Figure 17: Economic Comparison Study, Change in Capital Investment . . . . . 66

Figure 18: Economic Comparison Study, Yearly Production Output . . . . . 68

Figure 19: Equipment Comparative Study, % of Direct Costs . . . . . 69

Figure 20: Production Comparative Study, % of Manufacturing Costs . . . . . 70

## INTRODUCTION

Alpha-amylase, an industrially important enzyme used for starch degradation, is produced today in large quantities and for a variety of specialized purposes.[1] Total sales for all enzymes in 1977 was $150 million while the production of alpha-amylase totaled 380 tons per year and accounted for 27% of total enzyme sales in 1982 alone.[1,2]

This enzyme breaks starches into smaller sugar units, called dextrans.[3] Alpha-amylase cleaves the starch molecule randomly at the 1,4 sugar linkages.[3] The enzyme is thermostable, meaning it is chemically stable in temperatures of up to 100°C.[4] It can be stabilized for storage by using $Ca^{2+}$ ions in the enzyme solution.[5] Alpha-amylase solutions are sold in concentrations ranging from 2% to 10% pure. This enzyme is also sold in solid form.[5,6]

The uses of alpha-amylase in industry are quite extensive and varied. The main use of the enzyme is to liquify starch. Alpha-amylase is used primarily in the production of sugars such as glucose, fructose, and maltose from the starch molecule.[3] Alcohol production demands that the starch be saccharified before the addition of malt to the fermentation broth. Paper production uses alpha-amylase

for the sizing of the paper pulp. Textiles need a thermostable enzyme in the high temperature during the desizing process. Sizing and desizing refer to the breaking down of the starch residues between the fibers in both paper and cloth. The grain feed industry uses alpha-amylase to treat barley and other grains. Other uses include the filtering of cane sugar via the breakdown of starches in the liquid and for applications with laundry and dishwater detergents for removal of starch residues.[1,5,7,8,9]

Many different microorganisms produce alpha-amylase; the one studied in this thesis research was *Bacillus stearothermophilus*.[1] Two types of microorganisms are now being used commercially to make this enzyme: fungus and bacteria.[1] The bacterial family of alpha-amylase producing micro-organisms includes *Bacillus subtilus, B. cereus, B. polymyxa, B. stearothermophilus, B. caldolyticus, B. acidocaldarius*, and others.[1]

Fermentations for alpha-amylase production are run either in the batch or fed-batch mode. Batch fermentation is more widely used in industry while continuous fermentations are still in experimental stages.[10] Large stirred tank reactors (CSTR) are used in the production because of simplicity in operation.[10] These reactors can range in size from 1 L bench-top scale up to 120 $m^3$.[11] Presently, no commercial processes use continuous fermentations to produce any enzymes.[12]

The economics of any fermentation process are a function of many variables. A partial list of these variables includes:

1) Batch or continuous fermentations
2) Various cell parameters
3) Various reactor parameters
4) Equipment and manufacturing costs

The production cost of a process is the yearly cost to produce the product. Variable costs are a subset of production costs and are a function of production rate. The cost of raw materials can be as high as 50% of the variable production costs.[10] Utilities such as steam, electricity, process water and cooling water, along with waste treatment account for another large portion of the variable costs.

Cost analysis based upon an engineering and economical analysis of these different factors will allow predictions of the capital and production costs. In order to make an accurate economic model, current science, engineering, and process economics function collectively. Economic feasibility studies are based upon the projected profitability of producing the given product, in this case alpha-amylase from *B. stearothermophilus*. Different methods can be used to calculate the profitability; a direct calculation of Return on Investment - Discounted Cash Flow Rate was used.[15,44] This technique, also known as the "Engineer's method", allows a simple comparison between different case studies and plant configurations.[13]

The objectives of this study were as follows:

1) To study the effects of kinetic and reactor parameters on alpha-amylase production in fermenters. The kinetic parameters included stoichiometric yield coefficients ($Y_{x/o}$ and $Y_{p/s}$), Monod constants ($K_s$ and $K_o$) and substrate concentrations of carbon and oxygen. Reactor parameters studied included the dilution rate, recycle ratio, and extent of backmixing.

2) To ascertain the most cost-intensive aspects of the capital investments and production costs, including studying the effect of previous capital investments. To determine the costliest parts of both the total capital investments and variable production costs.

3) To predict if a proposed continuous process would be profitable.

This study combined computer analysis using two different software packages. Turbo-BASIC™ and Supercalc4™ were used to create a process and economic simulation model of the alpha-amylase project. Programs were written in BASIC that used user defined system variables to calculate the sizes and costs of the equipment needed to produce a given quantity of alpha-amylase per year. The program calculated the process equipment costs using correlations from various sources.[13,14,15,16] This information was then imported into the Supercalc4 spreadsheet which calculated overall capital and production costs along with cash flow tables for a plant lifetime of ten years. Profitability was estimated using Discounted Cash Flow Rate analysis.[17]

# Chapter I

## LITERATURE SURVEY

## 1. MICROORGANISM REVIEW

### 1.1 *Bacillus stearothermophilus* cell dynamics

The *Bacillus stearothermophilus* strain studied produces alpha-amylase extracellularly and only during the growth phase of the cell.[18] Other strains of *B. stearothermophilus* may produce the alpha-amylase enzyme in a combination of phases, such as during both growth and lag phases. This study confines production to only the growth phase. This microorganism is capable of growing in a simple salt solution on one of many different carbon sources.[19] *B. stearothermophilus* has an oxygen uptake of 200 nmol/min/mg of cell at 60°C.[19] The microorganism is also capable of producing products other than alpha-amylase such as superoxide dismutase, rhodanase, tyrosyl-tRNA synthetase, and tryptophanyl-tRNA.[20] These other products are separable from the broth and could be important to the economics of the process although they will not be taken considered here.

### 1.2 Recovery of microorganism products

When enzymes are produced intracellularly, as with *Escherichia coli*, the cell must be lysed to extract the enzyme or product of interest. This extra step can add considerable expense to the separation process because additional equipment is required. Product losses also increase. Up to 90% of product is lost in an intracellular recovery while only 10% is lost in an extracellular recovery.[21]

## 1.3 Thermostable microorganisms and enzymes

Thermostable bacteria are, by nature, typically hearty. *B. stearothermophilus* is able to resist various denaturing agents and is more tolerant to changes in solute concentrations than mesophilic organisms.[22] The Arrhenius' law predicts that higher temperatures will increase the rate of cell reactions. Increases in both enzyme activity and growth of the microorganism have been observed.[22] Thermophilic bacteria are also known to be more physically stable and have a higher oxygen uptake than mesophiles.[22] Most cells cannot grow at thermophilic temperatures; therefore, the chances of microbial contamination are lower. The costs for cooling the sterilized media are also smaller because of the higher reactor temperature.[22] Overall, the fact that *B. stearothermophilus* is thermophilic improves the possibility for continuous alpha-amylase production.

The alpha-amylase enzyme is capable of operating at temperatures as high as 100°C which gives several advantages to the engineer.[22] For example, reaction rates are higher, and the risks of contamination are reduced.

# 2. SPECIFIC GROWTH MODEL REVIEW

## 2.1 The Monod specific growth rate model

A model has been created to simulate the growth of the cell. Empirical studies provided by Monod [23] allow the prediction of cell growth rate to be predicted based upon known system variables:

$$\mu = \mu_{max} \cdot (S/K_s+S)$$

where $\mu_{max}$ is the maximum specific growth rate of the cell under unlimited carbon substrate conditions ($S>>K_s$), and $\mu$ is the actual specific growth rate measured. The variable S is the substrate concentration, and $K_s$, the Monod constant, is the substrate concentration at which the growth rate is at one-half of the maximum.[23]

## 2.2 Model constants

In the assumed model, concentration of product is directly related to the concentration of cells in solution.[23] The sizes of the process equipment components were based upon the production rate of the reactor, which itself was based on growth rate of the cells. The Monod model uses various kinetic parameters to determine growth rate.

Estimates of the kinetic parameters used in the Monod equation were found in literature. Some constants were specific for *B. stearothermophilus*; others were taken from other thermophilic bacteria. The following data were used to model the cellular growth and production:

1) $\mu_{max} = 2.1\ hr^{-1}$
2) $K_o = 0.000114$ (g/L)
3) $K_s = 0.0025$ (g/L)
4) $Y_{x/s} = 0.33$
5) $Y_{x/o} = 1.36$
6) $Y_{p/o} = 1.22_{calculated}$

Kuhn et. al.[24] reported a $\mu_{max}$ value of 2.1 $hr^{-1}$ for *B. caldotenax*, a similar thermophilic organism. Glassner et. al.[6] reported a $\mu_{max}$ calculated value of 2.18 $hr^{-1}$ for *B. stearothermophilus*.[6] The yield coefficient $Y_{x/s}$ is the ratio of grams of cells made to

the grams of carbon substrate used. The yield coefficient $Y_{x/o}$ is the ratio of grams of cells made to the grams of oxygen used. The yield coefficient $Y_{p/o}$ is the ratio of grams of product made to grams of oxygen used. The value of the yield coefficient $Y_{x/s}$ is 0.33 and was acquired from Coultate et. al.[19] for *B. stearothermophilus*. $Y_{x/o}$ was not found for *B. stearothermophilus*; therefore a value for *B. caldotenax* from Kuhn et. al.[24] was used. A calculation based upon the oxygen uptake of *B. stearothermophilus* yielded a $Y_{x/o}$ value of 1.24. Both values were well within error tolerances of each other. The yield ratio $Y_{p/o}$ was calculated from an electron balance based upon the other two yield coefficients.(See Appendix A.) The Monod constants were from bacteria roughly the same size as *B. stearothermophilus*, which was the separating factor in the reference.[25]

## 3. REACTOR FLOW REGIME REVIEW

### 3.1 Models for ideal flow

The ability to merge an accurate growth model with that of a reliable reactor fluid dynamics model allows the prediction of product concentration. Within a biological reactor, many different types of flow regimes exist simultaneously.[26] Several mixing models exist to predict liquid residence times. Two mixing models, the Continuous Stirred Tank Reactor (CSTR) and the Plug Flow Reactor (PFR), describe the extremes of complete backmixing and no backmixing, respectively.

The ideal mixing models involve the concept of backmixing within the reactor. The ideal CSTR reactor model assumes that the concentration of the effluent is the same as that within the tank itself.[27] Therefore, this assumption also presumes that all concentrations throughout the reactor are the same. The Plug Flow Reactor (PFR) assumes no backmixing within the reactor.[28,29] Concentration gradients exist throughout the length of the reactor.[28] Since these gradients are infinitely small, differential material balances must be used. An equation to describe the PFR reactor follows:

$$\frac{V}{F_{Ao}} = \int \frac{dX_A}{-r_A}$$

where V is the volume of the reactor, $F_{Ao}$ is the initial flow of reactant A in mol/time, $X_A$ is the conversion of reactant A, and $-r_A$ is the volumetric reaction rate of reactant A.[30]

## 3.2 Models for non-ideal flow

To model a non-ideal reactor, the amount of reactor deviation from ideality must be first quantified. Salt tracer studies have been used for this purpose.[31,32] This type of study may also be done with other types of tracers such as radioactive isotopes, or heated fluid elements.[32]

Two models are commonly used to describe non-ideal mixing, the stirred tanks-in-series model and the dispersed plug flow (dispersion) model. The dispersion model predicts that axial mixing occurs in addition to convective flow through the reactor.[33,34] The effective dispersion coefficient ($D_z$) is used to describe the relative degree of

axial mixing.[33] The larger this parameter, the farther from idea PFR reactor flow, and the greater the backmixing. $D_z$ is a function of the flow properties of the system. The steady state dispersion model can be written:[33]

$$u \cdot (dC/dZ) = D_z \cdot (d/dZ(dC/dZ)) + r$$

where u is the axial velocity of the fluid in the reactor, dC/dZ is the substrate concentration gradient, and r is volumetric reaction rate.[33]

The stirred tanks-in-series model assumes that non-ideal flow can be approximated by flow through several stirred tank reactors connected in series. Each of these reactors is assumed to be of equal volume and perfectly mixed.[35] The greater the mixing, the fewer the tanks that are required for an accurate model.[36,37] An infinite number of tanks-in-series would give a liquid residence time distribution identical to that of a PFR. However, since real reactor sizes are not differential, a finite measurable amount of backmixing occurs across the reactor.[27,36] Tracer inputs, as discussed above, can be used to determine the number of tanks needed. In general, the tanks-in-series model is superior to the dispersion model when the degree of mixing is relatively large and was therefore used in the simulations.[36]

## 4. OXYGEN INFUSION REVIEW

### 4.1 Oxygen as a cell substrate

All chemical reactions use substrate(s). Biological reactions are no different and, in fact complexity is

magnified because of the inherent sophistication of the cell metabolism. Many different compounds are needed to make the cell grow and produce product.[23,36,39] In an aerobic cell, the two main rate-limiting substrates are the carbon and oxygen sources. Carbon sources include many different forms of sugars ranging from pure glucose to relatively impure molasses.[40] Oxygen is needed by *B. stearothermophilus* as a terminal electron acceptor. It is typically introduced in gaseous form and dissolved into the liquid phase.

4.2 Oxygen transport and Ka

For oxygen to reach the cell, it is introduced in gaseous form and diluted into the liquid phase. Because of low solubility in the aqueous phase, the oxygen gas/liquid mass transfer rate is the limiting factor for growth in most aerobic fermentations.[27,41,42] Different physical resistances are encountered during oxygen transfer from the bubble to the cell:[43]

1) Diffusion from bulk gas to gas-liquid interface
2) Movement through the gas-liquid interface
3) Diffusion of the gas through the unmixed liquid boundary layer
4) Transport of the oxygen through the bulk liquid to a boundary layer surrounding the cell
5) Transport through the second cell boundary layer to the cell surface
6) Diffusion into the cellular floc or individual cells

7) Transport to the intracellular reaction site

The relative resistance of each of the above factors is different.[43] In general, the rate-limiting oxygen transport step lies in the flux of the dissolved oxygen through the liquid boundary layer of the bubble.[43,44] To transport oxygen across the boundary layer, a driving force or concentration gradient must exist. A low driving force is caused by the low oxygen solubility in the aqueous phase.[45] The rate of oxygen transport across the boundary layer is given by the equation below,

$$\text{Oxygen flux} = N_{O2} \text{ in units of } \underline{\text{mol } O_2/(cm^2 \cdot hr)}$$

$$= K_l \cdot (C_l^* - C_l)$$

where $O_2$ is the molar amount of oxygen transported across the boundary layer. $K_l$ is the mass transfer coefficient in cm/hr that relates flux to concentration differentials. The values $C_l^*$ and $C_l$ represent oxygen concentration in the boundary layer and bulk phases, respectively. The overall oxygen transport rate ($Q_{O2}$) between the gas and the liquid can be expressed in terms of an overall coefficient $K_la$.

$$\text{Volumetric Oxygen Uptake Rate} = (\text{flux}) \cdot (\text{area}) / (\text{VOLUME}_{\text{Reactor}})$$

$$= K_la \cdot (C_l^* - C_l)$$

The constant a is the interfacial surface area of the bubble divided by the volume of the bubble.

# 5. MATHEMATICAL MODEL REVIEW

## 5.1 Substrate, cell, and product mass derivations using the Monod model

Mass balance equations are used to predict the fermenter performance.[23] There are three types of mass balances used: substrate (oxygen and carbon) balances, cell balances, and product formation balances.[23]

The steady state mass balance on the substrate (carbon) in a CSTR is shown below:[23]

$$D\cdot(S_F-S)-(Y_{x/s})^{-1}\cdot\mu\cdot X = 0$$

or

$$D\cdot(S_F-S)-(Y_{x/s})^{-1}\cdot(\mu_{max}*S)\cdot X/(K_s+S) = 0$$

where D is the dilution rate, or inverse of the mean residence time, of the reactor. $S_F$ is the substrate feed concentration; S is the bulk substrate concentration. X is the concentration of the cells.

The mass balance for the cells is shown below:[23]

$$(\mu-D)\cdot X+D\cdot X_F = 0$$

For sterile feed ($X_F$=0) the following equation results:

$$X = Y_{x/s}\cdot(S_F-(D\cdot K_s/(\mu_{max}-D)))$$

where $X_F$ is the concentration of cells in the feed stream.

The mass balance for the product is shown below:[23]

$$D\cdot(P_F-P)+Y_{p/x}\cdot\mu\cdot X = 0$$

where $P_F$ is the product feed into the reactor; P is bulk product concentration; and $Y_{p/x}$ is the yield ratio of product per cell.

## 5.2 Limitations of the Monod model

The mass balance equations are derived by using the Monod model. However, the Monod equation has limitations.[12,46] At either very low or very high system dilution rates, the model breaks down for some microorganisms, because the cell is not under standard reactor growth conditions. At high dilution rates (i.e. low liquid residence times) the carbon sources may be incompletely metabolized.[12,46] Also, the substrate concentration is high and may not be rate limiting. High dilution rates, or low residence times, allow little time for adequate mixing which would in turn cause the concentration of the substrate to vary throughout the reactor. At low dilution rates, or high residence times, maintenance metabolism of the cell must be taken into account within the growth equation. During extended periods of time, the cell must maintain itself and resources are diverted away from growth and product formation. Growth of the cell is no longer just a function of the substrate input.[12] In the case of *B. stearothermophilus*, production occurs only during the growth phase; therefore the maintenance factor does not need to be taken into account.[18]

## 5.3 Extensions of the Monod model

Since oxygen concentration may be a major rate limiting factor, its effect on the kinetics must also be modeled. Oxygen can be treated as a substrate by using the double Monod kinetic expression:[47]

$$\mu = \mu_{max} \cdot (S_1/(S_1+K_{s1})) \cdot (S_2/(S_2+K_{s2}))$$

where the variables $S_2$ and $K_{s2}$ are the substrate concentration and Monod constant for the second substrate, oxygen in this case.

## 5.4 Other growth models

There are other models, such as the Dynamic Lag Response, which compensate for the inadequacies of the Monod model. The Monod model fails during unsteady state fermentations in which conditions change rapidly.[48] Since the actual Monod equation has no time dependencies, any perturbations of the substrate concentrate are assumed to affect the growth rate of the cell instantaneously.[48,49] Since cellular metabolism does not respond instantaneously, models incorporating time lags have been developed.[49,79] However, a steady-state continuous fermentation is assumed in the present study. Time dependent kinetics are not needed.

Other time dependent growth kinetic models are available. Tessier, Moser, and Contois have proposed kinetic growth models.[80] The Monod equation will suffice for these studies, because only moderate dilution rate values will be used in the mathematical model.[80] In addition, detailed kinetic data are not available to warrant the use of more elaborate models.

# 6. AIRLIFT AND CSTR REACTOR REVIEW

## 6.1 History of airlift fermenters

The bioreactor model developed in this study is capable of describing both airlift as well as stirred tank fermenters. The airlift fermenter, shown in Figure 1, has been used widely in the fermentation industry. This type of fermenter was first patented by Lefrancois in 1955 and is currently used for brewing and waste treatment.[82] ICI, a British chemical company, uses a tubular loop reactor 100 m tall while two German companies have constructed two 30 m airlift fermenters for aerobic sewage treatment. In Japan, a company is using a 1000 L airlift fermenter, and Gulf Research and Development has experimented with a 50,000 L airlift fermenter.[81,82]

**SCHEMATIC OF AN AIRLIFT REACTOR**

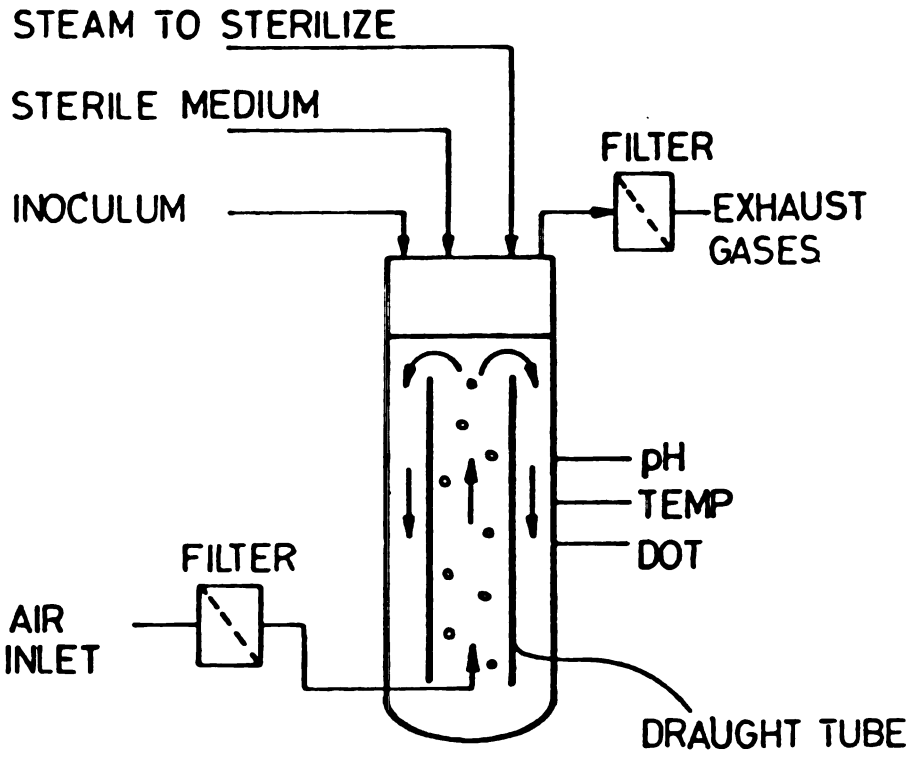


**Figure 1**[42]

## 6.2 Physical structure of airlift fermenters

The airlift fermenter, Figure 1, has three regions within the reactor: the head region where two phases, gas and liquid, coexist, the inner tube or draft region, and the annular region, which lies outside the draft region. Air is sparged by a compressor into the bottom of the draft region causing the density of the liquid at this point to drop below that of the annular region. Lower density causes the fluid in the draft region to rise while drawing in the fluid from the annular region. Gas separates from the liquid in the head region and the liquid flows down the annular region to complete the cycle. The cycle can be reversed by sparging air into the annular region. Other designs include a split cylinder airlift fermenter and an external loop airlift fermenter.[51]

## 6.3 Flow dynamics of the CSTR and airlift fermenters

The stirred tank fermenter (chemostat) is simple, relatively easy to model, and used widely throughout the chemical industry.[10] There are different reactor requirements in the biochemical industry than that of the chemical industry. The reaction occurs in an aqueous medium; oxygen must often be transported through this medium; enzymes and some microbial cells are shear sensitive and therefore hydrodynamic shear can cause enzyme denaturation or cell destruction.[10,52] The chemostat agitator produces high shear rates and thus is not well suited for some fermentations.[53,54] The airlift fermenter uses less

energy to mix the contents while causing less agitation and shear stress to the cell.[10] Agitation and kinetic energy are provided by sparging air into the system. The chemostat maximizes volumetric productivity (mass of product per unit volume of reactor and time), while the airlift fermenter maximizes specific productivity (mass of product per unit substrate input, time, and power input). The latter will have lower production costs.[55]

### 6.4 Modeling process for airlift fermenters

Liquid mixing in airlift fermenters lies between the extremes of perfect mixing and plug flow. Various mathematical models have been used to simulate the airlift fermenter. It has been proposed to model both the annular and draft regions as a PFR.[56] Others propose that it be modeled as a series of CSTRs.[51] The number of CSTRs chosen depends on the degree of axial mixing in the reactor.[36,57] This number must be determined experimentally.

## 7. CONTINUOUS PROCESS REVIEW

A chemical production process can be run either in a continuous or batch mode. Batch mode is more commonly used for industrial fermentation processes for the following reasons:[10]

1) Most laboratory experiments today are only capable of running continuous fermentations of up to 200 hours; to be economical, fermentations must be kept running for at least 1000 hours.

2) Maintaining sterility is difficult over long periods of time because of the enormous quantities of media introduced into the continuous system.

3) The substrates used in batch sterilization must be maintained at a constant concentration. This is a problem in industrial size continuous fermentations.

4) Genetically produced strains may be mutated into cells which can out compete their forebearers.

However there are many advantages to running a process in continuous mode:[10,58]

1) Lower cost for the continuous sterilization of the media.[58]

2) Constant output of product and no downtime needed for the 'turn around' of a batch reactor system.[58]

3) Maintaining steady state.[59]

## 8. MEDIA AND AIR STERILIZATION REVIEW

### 8.1 Continuous versus batch sterilization

Media sterilization is necessary to prevent contamination by unwanted microorganisms. Batch sterilization is expensive, takes a long time (up to 3-4 hours), and can alter the nutrient composition.[59] Continuous sterilization allows higher temperatures to be attained thus decreasing sterilization times. Continuous sterilization is less costly, safer for the nutrients, and requires less plant space.[59]

### 8.2 Continuous sterilization processes

Different types of continuous sterilizations are possible. One method uses direct steam input into the media. This method is highly efficient and lowers capital investment; a flash tank is the only major piece of equipment required.[60] However this method causes foaming in

certain mixtures.[60] One of the more efficient recycling systems involves two heat exchangers, a pre-heater and a main heater (see Figure 2). The pre-heater uses the output of hot media from the main heater to pre-heat the cooler incoming liquid. The warmed media from the pre-heater is then sent to the main heater where it is further heated to 121°C using steam. Again the output from the main heater is sent to the pre-heater to conserve energy.[60] Up to 90% of the heat can be recovered with this recycle system. In addition, no impurities are added to the media through the steam. One of the disadvantages is the need for a pipe to hold the media for the predetermined sterilization time. Also, under some circumstances, the proteins and starches may coagulate and cause fouling problems in the heat exchangers.

There are other, less conventional methods for the sterilization of the media such as radiation and chemical sterilization. Radiation is dangerous to human life and is not used widely by industry.[60] Chemical sterilization methods include formaldehyde, hydrogen peroxide, and ethylene oxide.[60] Another method uses filtration sterilization to remove microorganisms from the media. Both depth and screen filters are used for this type of sterilization.[60,61]

## 8.3 Air sterilization

Air fed to the reactor must also be sterilized.[62] The quantity of air being sparged into any aerobic fermentation

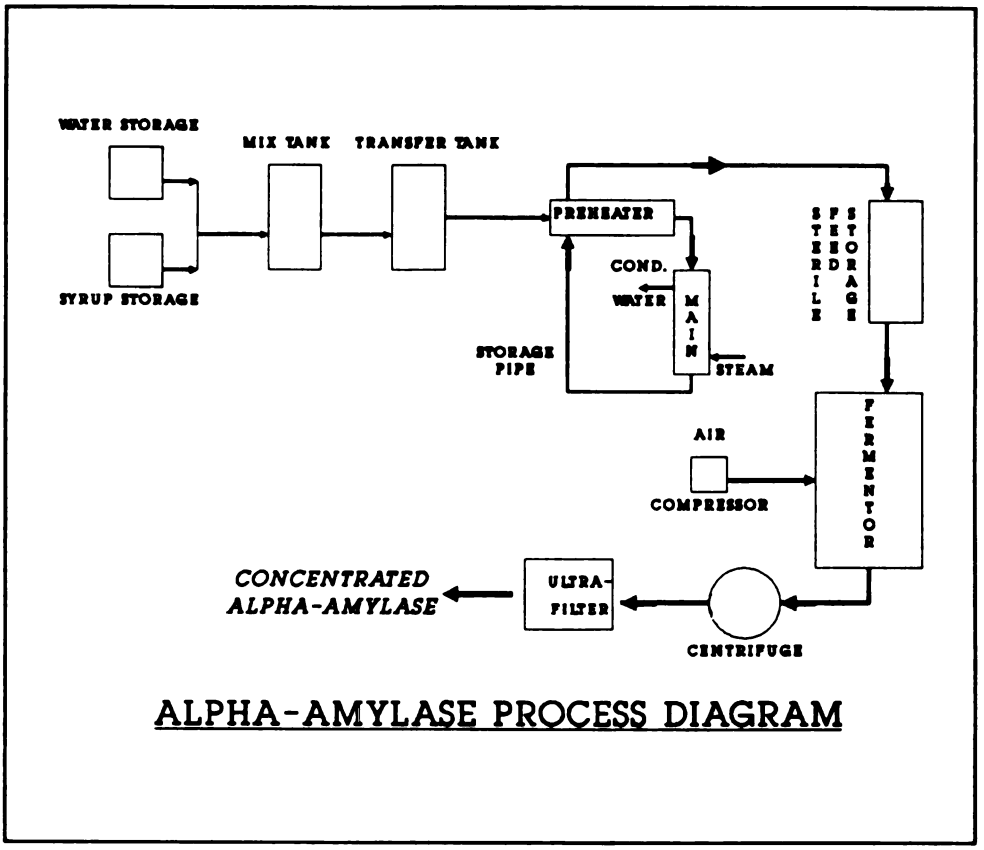


**Figure 2**

is usually very large, sometimes up to 600 $m^3$/min in 10,000 L reactors. Normal sparging usually ranges from 0.5 to 1 volume of air per volume of reactor per minute (VVM). The contaminants in air sometimes reach up to 2000 microorganisms/$m^3$. [59] Previous methods of air sterilization include electrically heated elements and glass wool fibers. Due to the high cost of electricity and clogging of the glass wool fibers, both methods have been replaced.[59,62] Newer methods use steam sterilizable glass fiber filters that do not shrink or solidify.[59] The air is pumped into the reactor by air compressors of various types.

## 9. SEPARATIONS REVIEW

### 9.1 Cell and product separations

Alpha-amylase is sold in a 2% to 10% pure solution.[63] The following separation and purification steps are typical of most processes:

1) Cells and other solids are removed from the broth.

2) The cell free broth is concentrated to the desired concentration.

To remove the cells, micro-filtration or centrifugation is used.[64] When large volumes of solutions are processed, the accepted procedure is centrifugation.[20] Flocculation of the cells, although taking longer in time, could replace the centrifugation step with possible enhancements to the economics.

### 9.2 Ultrafiltration separations

Concentrating the enzyme solution leaving the reactor reduces packaging and shipping costs.[65] Increased recovery will, however, increase the expense and processing time. Ultra-filtration is the recovery method of choice, since it can concentrate a solution 100 to 1000 times. Ultra-filtration techniques are used because of the low cost and ease of sterilization.[66,67] In spite of deactivation of enzymes due to shear stress, it is one of the more popular methods of biological downstream product concentration. Both hollow fiber and flat plate ultrafilters are commonly used for recovery and concentration. Hollow fiber systems are able to filter up to 500 $m^3$/hr but are prone to protein

fouling at low enzyme concentrations.[66,67] Flat bed ultrafiltration systems have less tendency to bind the proteins but are able to handle only up to 50 $m^3$/hr of solution.[66,67]

## 10. ECONOMIC PROCESS ANALYSIS REVIEW

### 10.1 Economic estimations

Economic analysis can be used to estimate the profitability of a chemical plant.[68,69,70,71] There are different gauges of profitability for plant economics.[72] The "Engineer's Method" uses the future worth of cash (the worth of present cash in future terms) to determine the return on investment. Appropriately called the "Return on Investment -- Discounted Cash Flow" method, this procedure calculates the amount of interest or profit that would be made on a given capital investment.[13] Other methods are specific to individual corporations.[13,14] The discounted cash flow method uses a cash balance to determine the cash flux into and out of a project. Cash flow projects the amount of capital a process will generate or lose. The cash flow is calculated by using the capital investment, production costs, and depreciation. It is a function of the interest rate assumed by the company.[13]

### 10.2 Capital investment estimations

The sum of the future worth during the lifetime of the plant is equal to the initial capital investment. For the company to make the chosen percentage profit (return on investment) the above future worth calculation must be

valid. Capital investment refers to the initial equipment and set up costs during construction.[73] The capital investment is a one time cost and is recovered through the use of depreciation and salvage value.[74,75]

The capital investment required for a biochemical process is usually large when compared to standard chemical plants. Higher quality of construction materials are needed for cell growth and maintenance which causes this cost increase.[14] For instance, 316 stainless steel is used in fermenters and piping systems instead of normal carbon steel or iron; use of stainless steel causes higher construction costs.

The equipment sizes may be estimated based upon the desired yearly mass product output. Since the price correlations for the equipment are not current, Marshall and Swift Indices are used to adjust for inflationary changes.[76] For prices of accessory equipment such as piping, process control, electrical equipment, building and construction, Lang approximations are used.[77] Direct costs are the sum of the equipment costs and the costs of putting the equipment on-line (i.e. Lang factors). Capital costs that are not directly related to the construction of the plant are called indirect costs. Indirect costs are not related to the size of the plant or the equipment within. The sum of the direct costs and indirect costs is the total capital investment of the project.[74,75]

## 10.3 Production cost estimations

Yearly production costs are also calculated in the cash balance. These costs are incurred during actual production of the plant. They are broken down into two different forms: variable and fixed costs. Variable costs are a function of the amount of product produced. The variable costs include media for the biochemical process, utilities, maintenance, labor, or costs are not existent during plant shutdown. Fixed costs, such as rent of the building, local taxes, and plant overhead are calculated by well known approximations from various sources.[13,74,75,78] These costs are not variable and must be paid even during plant shutdown.

Depreciation is lost investment due to equipment usage. This cost is spread over a predetermined period of time. There are many different methods to calculate the depreciation. The simplest is the straight line method which divides the total cost of the equipment depreciable (Total purchase cost - salvage value) by the lifetime of the equipment to give the annual depreciation cost.

# CHAPTER II

## MODELING SECTION

## 1. MATHEMATICAL DERIVATIONS FOR THE AIRLIFT FERMENTER

### 1.1 Program variables

In this study, mathematical models were developed to predict airlift fermenter performance and alpha-amylase process economics.[83] The airlift fermenter model takes into account differences in $K_La$ for the annular and draft regions, differences in size between the annular and draft regions, the recycle ratio (flow in annular region/flow into reactor) and the number of CSTRs in the series.

### 1.2 Cell mass balance

The model uses the tanks-in-series theory presented in section 1.3.1. For each tank, four different mass balances were solved: carbon substrate, oxygen, cells, and product. The steady-state cell mass balance is shown below:

$$X=(S_f-S)\cdot Y_{x/s}+X_f$$

where $Y_{x/s}$ is the yield coefficient for gram of cell produced per gram of substrate used.

### 1.3 Oxygen and carbon mass balance

For each of the tanks, both the concentration for oxygen and carbon substrates were calculated simultaneously by using an iterative fixed point method. The oxygen mass balance (derived in Appendix B) is shown below.

$$O = (-K2+((K2^2-(4\cdot K1\cdot K3))^{.5})) / (2\cdot K1)$$

where K1, K2, and K3 are lumped parameters defined below.

$$K1= (1+K_La/D)$$

$$K2 = (K_o - O_f + (Y_{o/x} \cdot X \cdot \mu_{max}/D) \cdot (S/S(S+K_s)) + K_l a/D \cdot (K_o - O_s))$$

$$K3 = -(O_f \cdot K_o + K_l a/D \cdot (O_s \cdot K_o)).$$

O is the oxygen concentration within the reactor in g/L; $O_f$ is the oxygen concentration flowing into the reactor in g/L and $O_s$ is the saturated oxygen concentration at the given temperature.

The following is the carbon substrate balance used in the model (derived in Appendix B):

$$S = (K4/2) + ((K4^2 + 4 \cdot (S_f \cdot K_s))^{.5})/2.$$

where,

$$K4 = S_f - K_s - (\mu_{max} \cdot X)/((Y_{x/s} \cdot D) \cdot (O/(K_o + O))$$

It is necessary to solve the carbon and oxygen mass balances simultaneously because of their interdependence. The equations are similar except that the oxygen balance uses the $K_l a$ transport factor. This factor takes into account the oxygen transfer across the gas-liquid interface. Without the addition of oxygen gas the reaction would stop soon after the initial dissolved oxygen was depleted. Modeling studies indicated that oxygen may be the rate limiting factor for reaction.

## 1.4 Product mass balance

The amount of product made in each tank was calculated using the Monod equation along with a yield coefficient, $Y_{p/x}$. Below is the product mass balance equation:[23]

$$P = P_f + (Y_{p/x}/D) \cdot \mu_{max} \cdot (S/K_s + S) \cdot (O/K_o + O) \cdot X$$

where P is the product concentration inside the reactor; $P_f$ is the product feed concentration entering the reactor; and

other variables are the same as those defined above. Using this mass balance, the output from the ($N^{th}$) reactor in the draft region was used as the product feed of the (N+1) reactor.

## 1.5 Base case variables

The following parameters were used as a base case for the modeling:

Substrate Concentrations
- Carbon(S) = 10.0 g/L (1%)
- Oxygen(O) = $5.0 \times 10^{-3}$ g/L

Reactor Configurations
- Ratio of Draft vol/Overall vol = 0.66
- Recycle Ratio(R) = 0.5
- Dilution Rate = 0.4 $hr^{-1}$
- Number of Tanks-in-series = 10

Cell Constants
- $Y_{x/s}$ = 0.33 g/g
- $Y_{p/o}$ = 1.22 g/g
- $Y_{x/o}$ = 1.36 g/g
- $\mu_{max}$ = 2.1 $hr^{-1}$
- $K_s$ = 0.0025 g/L
- $K_o$ = 0.000114 g/L
- $K_la_{draft}$ = 200 $hr^{-1}$
- $K_la_{annular}$ = 0.0 $hr^{-1}$

$K_la_{draft}$ and $K_la_{annular}$ refer to the gas mass transfer constant in the draft and annular regions, respectively. Most gas exits the reactor at the head region; $K_la$ is therefore assumed zero in the annular region. The recycle ratio is the amount of fluid recycled through the annular region divided by the amount of fluid entering the reactor. These constants provided a base case scenario for comparison with various permutations of the system parameters. The conversion of carbon in the system provides a measure of how completely the reaction consumed the substrate.

## 1.6 Recycle Derivations

Recycle of the various substrates and products through the annular region of the reactor required specialized equations. The recycle (R) was defined as the amount of liquid flowing through the annular region divided by the flow into the reactor. Below are the various equations used to model the inlet concentrations to the draft region. They are simply adaptations of simple mass balances:

Substrate:

$$S_R = (1 - X) \cdot S_f$$

$$S_{draft} = (S_f + R \cdot S_R)$$

Where $S_R$ is the concentration of substrate exiting the annular region; $S_{draft}$ is the substrate concentration entering the draft region.

Oxygen:

$$O_R = 0.0 \text{ g/L}$$

$$O_{draft} = (O_f + R \cdot O_R)/(1+R)$$

where the oxygen concentration, $O_R$, exiting the annular region is zero; $O_{draft}$ is the oxygen concentration entering the draft region.

Cell:

$$X_R = (Y_{x/s} \cdot (S_{draft} - S_R)) + X_f$$

$$X_f = (R \cdot X_R)/(1+R)$$

where $X_R$ is the concentration of cells leaving the annular region. The solution is iterated within the model.

Product:

$$P_R = (Y_{p/s} \cdot (S_{draft} - S_R)) + P_f$$

$$P_f = (R \cdot P_R)/(1+R)$$

where $P_R$ is product concentration exiting the reactor. The solution is iterated within the model.

Since the dilution rate of each of the tanks changes according to whether in is in the draft or annular region, the model must take this into account.

The actual dilution rate, $D_{actual}$, per tank is shown below:

$$D_{actual} = D_{overall} \cdot \text{number of tanks-in-series.}$$

where $D_{overall}$ is the inputted dilution rate of the system.

If the tank is in the draft region:

$$D_{actual\ draft} = D_{actual} \cdot (1 + R)$$

If the tank is in the annular region:

$$D_{actual\ annular} = D_{actual} \cdot R$$

where $D_{actual\ draft}$ and $D_{actual\ annular}$ are the dilution rates used in the calculations in the draft and annular regions, respectively.

# 2. ECONOMIC MODELS

## 2.1 BASIC computer models

Nine computer programs, shown schematically in Figure 3, were written to simulate industrial alpha-amylase production and estimate the process economics. The simulation programs are described below.

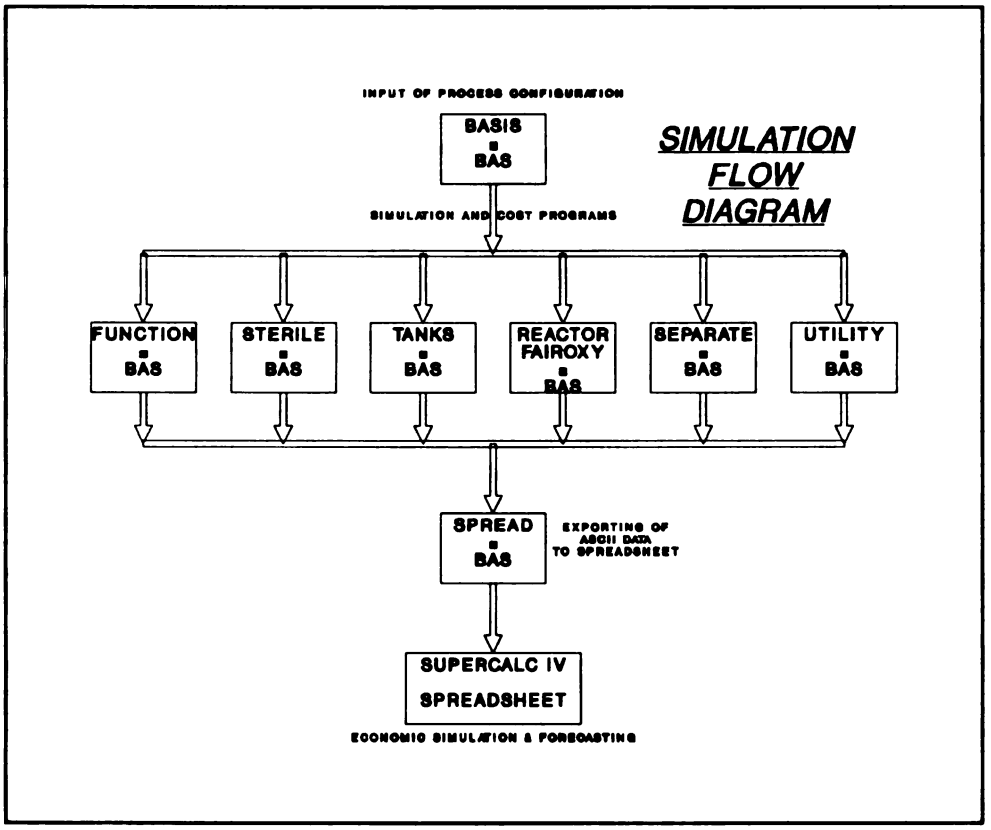


**Figure 3**

BASIS.BAS controls the programs in this package. This module allows the user to model either an airlift fermenter or CSTR fermenter. The user inputs the following data to cost and size the equipment:

a) Current Marshall & Swift indices

b) Yearly amount of alpha-amylase to be produced

c) Stream Factor for system

d) % of reactor occupied by foam

e) % concentration of carbon substrate in feed stream.

FUNCTION.BAS contains the cost functions for the various pieces of equipment. These costs are linearized on graphs (Appendix C). The following is a list of the equipment used in the various sections of system.

STERILIZATION SECTION

* Heat exchangers, pre-heater, and main heater are used to heat the media.

* Storage pipe is used to hold the media at an elevated temperature until sterilization is complete.

* Hangers are used to hold the storage pipe.

* Tanks are used for storage of pre-sterilized and sterilized media. Also, an agitator is needed to mix the fluid.

REACTOR SECTION

* Fermenters are the main part of this section. Two forms of fermenters can be purchased: on-site and off-site constructed. On-site constructed fermenters are more expensive than off-site constructed. The cut-off point is 50,000 L above which the reactor must be constructed off-site. Both CSTRs and airlift fermenters are assumed to have the same price.

* Air compressors are used to deliver pressurized air into the reactor.

SEPARATION SECTION

* Centrifugation removes the cells from the broth. A continuous flow disk centrifuge will be used in this step.

* Ultrafiltration is used to concentrate the cell-free enzyme solution. The user inputs the required flux across the membrane. The size of the membrane is then calculated based upon the flux.

STERILE.BAS calculates the sizes of the heat exchangers and the storage pipe used to sterilize the media. Below is the information provided by the user. Recommended values are offered in parentheses.

a) Temperature from transfer tank (60°F)

b) Sterilization Temperature (250°F)

c) Temperature input to reactor (170°F)

d) Overall Heat Transfer Coefficients:

$U_{o\ preheater}$=200 Btu/hr-F-Ft$^2$

(liquid to liquid transfer)

$U_{o\ mainheater}$=100 Btu/hr-F-Ft$^2$

(liquid to condensing gas transfer)

e) Steam pressure and temperature are supplied by the user.

Heat recycle was used to save on heating costs. The inlet from the transfer tank (unsterilized media) is put through a pre-heater which itself is heated by effluent from the main heater at 250°F. The purpose of the pre-heater is two-fold:

a) To preheat the cold media so the amount of steam needed in the main heater is conserved.

b) To eliminate the need for a media cooler thereby reducing the capital investment and process cooling water costs.

The main heater uses steam to heat the media to the sterilization temperature. The effluent from the main heater is sent through a pipe of sufficient length to allow a residence time of 20 min.

TANKS.BAS calculates the costs and sizes of the various tanks, mixers, and pumps. The user inputs the average residence time for liquid in each tank and agitator power consumption in HP/1000 Gal. The user must give the pressure differential across each pump to calculate pump sizes. The storage and pumping equipment for which costs and sizes are calculated include:

Storage:
- Mix tank
- Transfer tank
- Sterile feed tank
- Agitators for each tank

Pumping: Six main pumps are assumed to be used for this process.
- Pre-mix tank
- Pre-transfer tank
- Pre-sterilization
- Pre-reactor(s)
- Pre-separation
- In-line separation

REACTOR.BAS calculates the sizes and costs of the reactor, air compressor, and agitator (if a CSTR was used). The reactor size is based upon data used in BASIS.BAS, including production of alpha-amylase each year.

The air compressor size is based upon the volume of air per volume of reactor per minute (VVM) factor. Using this

value and the calculated size of the reactor, the amount of air provided by the air compressor is calculated in the following equation:

$$\text{Compressor output } (ft^3/min) = \text{Reactor Volume } (ft^3) \cdot VVM.$$

Agitators are used to keep the contents of the reactor well mixed but are not needed in an airlift fermenter where sparging replaces mechanical agitation. The size of the agitator is based upon the desired HP/1000 gal.

SEPARATE.BAS sizes and costs the following separation equipment: the disk centrifuge, the ultrafiltration membrane, and the ultrafiltration equipment.

PRINTER.BAS prints out the sizes and the costs for all equipment.

SPREAD.BAS creates an ASCII file containing all the cost and size data. This file is imported into the SuperCalc4 spreadsheet and then used to calculate the total capital investment, production costs, and cash flow table.

FAIROXY.BAS is the airlift fermenter simulation program. It was used only if the user decided to simulate an airlift fermenter.

## 2.2 Supercalc4 economic models

The balance of the process simulation is performed using the SuperCalc4 spreadsheet. The spreadsheet calculates total capital investment (TCI), yearly production costs, and the cash flow tables.

The direct costs table includes the following: the equipment used in the process as well as the size, quantity, unit cost, total cost, and the percentage of the cost of each piece of equipment to the total cost. The direct costs table has five parts and includes the following alpha-amylase process equipment:

1) Pre-fermentation:
   - Syrup storage tank
   - Syrup mix tank
   - Syrup transfer tank
   - Syrup pre-heater
   - Syrup main heater
   - Sterile feed storage tank (& agitators for above)
   - Hot storage pipe

2) Fermentation:
   - Air compressor
   - CSTR (or airlift) fermenter

3) Recovery:
   - Centrifuge
   - Ultra-filtration equipment

4) Ancillary
   - Pumps and smaller equipment

5) Lang factors are used to account for further process costs. Factors are multiplied by 50% of total capital costs from above.[74]

| | |
|---|---|
| Equipment Installation: | 0.49 |
| Instrumentation & Cont: | 0.18 |
| Piping: | 0.66 |
| Electrical: | 0.11 |
| Buildings: | 0.28 |
| Service Facilities: | 0.70 |
| Land: | 0.06 |
| Total: | 2.48 |

Indirect costs take into account actual building costs which include:[74]

Engineering and Construction:
- 10% of direct costs

Contingency:
5% of total capital investment

Construction:
10% of direct costs

Working capital is included in the total capital investment. The working capital includes a 30 day inventory of final product and production chemicals needed to make the product. Additional chemicals needed for production will account for 20% of the working capital.

The production cost table summarizes the yearly cost for the production of alpha-amylase. The yearly production cost is the difference between gross and net profit. The production cost table is composed of variable and fixed costs which are costs that are and are not dependent upon production costs, respectively.

The variable costs for the process are as follows:

a) Materials & Supplies:[7,18,19,84,47,85,86]

Raw Materials:

| | |
|---|---|
| Carbon Source (Starch) | (10 g/L) |
| Nitrogen Source (Corn Steep Liq.) | (4 g/L) |
| Peptone | (4 g/L) |
| $MgSO_4 \cdot H_2O$ | (0.5 g/L) |
| KCl | (1 g/L) |
| $(NH_4)_2 \cdot H \cdot PO_4$ | (4 g/L) |
| Anti-Foam | (1 g/L) |

(Costs are based upon current supply)

Separation: Size of membrane is based upon flux provided by user.
Ultra-filtration Membranes

b) Utilities:

Electrical:
Sum of electrical use by all equipment multiplied by two.

Process Water:
Amount is based upon total flow through the system. Cost is variable depending on current Marshall and Swift index.

Steam:
Amount of steam for the main heater is calculated by the media flow rate and the required temperature rise.

c) Labor:
Operating labor: 10% of total yearly production costs
Quality control: 20% of operating labor costs
Supervisor Labor: 20% of operating labor costs

d) Miscellaneous:
Various costs incurred during production.
Maintenance Materials: 2.5% of fixed capital investment
Operating Materials: 10% of operating labor costs
Maintenance & Repairs: 10% of operating labor costs
Laboratory Expenses: 10% of operating labor

Raw material amounts are based upon a variety of sources. The fixed costs, those costs which do not vary with a change in production, are calculated as follows:

| | |
|---|---|
| Local Taxes: | 1% of fixed capital investment |
| Insurance: | 0.4% of fixed capital investment |
| Plant Overhead: | 50% of operating labor costs plus supplies and maintenance |
| Rent: | Assumed to be 0 |

Depreciation, in this case, is calculated by the total capital investment spread evenly over the first five years of operation. This cost is taken as a production expense for these first five years. Other methods are available but for simplicity linear depreciation was chosen.

General expenses are incurred during normal operation. These include administrative costs, distribution and marketing, and research and development. These three categories are assumed to be 2% of the yearly manufacturing costs.

The cash flow table represents the flow of monies into and out of the corporate structure. The profitability of a

plant can be determined from the cash flow table and present value. The profitability will depend on the Return on Investment desired by the company. The optimal Return on Investment will be that value in which the sum of the future values of cash flow will be equal to the present expenditure of the capital investment. Below is a list of definitions that are used in the experimental cash flow table.

Capital Outlays:

This is the initial capital investment of the plant which is taken into account in the cash flow table two years before the start of production.

Revenue:

Gross revenue is calculated by multiplying the amount of alpha-amylase produced by the current price. The price of alpha-amylase used for the simulation is $55/kg. This prices assumes a 7.5% pure solution of alpha-amylase.

Start-Up Costs:

This was assumed to be zero.

Manufacturing Costs:

This is also known as total yearly production costs. These costs will change after the fifth year when depreciation is removed from the yearly costs.

Operating Expenses:

This factor is the sum of:

Manufacturing costs, excluding depreciation, and general expenses.

Total Expenses:

This factor is the sum of the operating expenses and the working capital and the depreciation.

Income Tax:

This tax is given by the formula:

(Gross Revenue-Total Costs)·Tax%

Net Profit:

This is calculated by:

Gross Revenue minus the Total Expenses minus the Income Tax.

Yearly Cash Flow:

This factor is the sum of:

Net Profit and Depreciation

Total Cash Flow:

This factor is the sum of:

Previous Yearly Total Cash Flow and Present Yearly Cash Flow

Present Worth:

This is calculated by using the future worth equation:

$$\text{Present Value} = \text{Future Value} \cdot (1 + i)^n$$

# CHAPTER III

## PREVIEW OF MODELING STUDIES

### 1. EXPLANATIONS OF MODELING VARIABLES

The results section is divided into two parts. The first contains the basis for the mathematical simulations. The second presents the data gathered through the simulations.

Future research will update the constants previously defined. Some constants are specifically for *B. stearothermophilus* while others are an estimate from other bacteria. Although some numbers are estimated, they will serve to demonstrate the important economic trends. As more accurate data become available, the model may be updated.

#### 1.1 Substrates

The two substrate sources for the cell are starch and oxygen. The carbon substrate is provided through the induction of a glucose or starch-rich syrup such as molasses, corn syrup, or a defined media. Oxygen is provided by sparging air through a frit, bubble plate, or sparger.[87] The oxygen transfer is limited by the combination of surface area of the bubble and the flux across the bubble interface. $K_La$ represents the mass transfer constant. For the simulations, the carbon substrate concentration was chosen to be 10 g/L, an average concentration found in most literature references. The oxygen concentration in the liquid feed was chosen to be $5.00 \times 10^{-3}$ g/L. This was somewhat below that of the maximum soluble oxygen

concentration ($O_s$) of fluid at 60°C calculated to be $5.85 \times 10^{-3}$ g/L.

1.2 Yield coefficients and $\mu_{max}$

The Monod growth model uses certain cell specific constants. In order to accurately simulate the airlift fermenter, the Monod growth model was used to determine cell productivity. For *B. stearothermophilus*, the average $\mu_{max}$ was found to be 2.1 $hr^{-1}$. Yield coefficients of the cell determine the relative mass of one substrate used to another substrate, $Y_{s/o}$, the relative mass of cells produced to substrate used, $Y_{x/o}$ or $Y_{x/s}$, and the relative mass of product made per mass of substrate used, $Y_{p/o}$ or $Y_{p/s}$. Cellular output is either in cell or product mass. Maintenance metabolism is not taken into account in this Monod model. For $Y_{x/s}$, a value of .33 was found in literature. For $Y_{x/o}$, 1.36 was also found in the literature. A ratio relating the mass of product made to the mass of substrate used was calculated from an electron balance based upon the previous two yield coefficients. The value calculated from the electron balance for $Y_{p/o}$ is 1.22 (See Appendix A).

1.3 Monod constants

Since a double substrate Monod model is assumed, there are two different Monod constants, one for oxygen and one for carbon. The Monod constant for the carbon substrate, $K_s$, is 0.0025 g/L for other bacteria.[25] The Monod constant for the oxygen substrate, $K_o$, was found to be 0.000114 g/L.

### 1.4 Oxygen transfer parameter, $K_l a$

The oxygen mass transfer parameter $K_l a$ determines the amount of oxygen in solution. Values of 100 $hr^{-1}$ to 300 $hr^{-1}$ were found in literature for various reactors. An average value of the 200 $hr^{-1}$ was chosen for the draft region in the model. When the gas in the draft region of the airlift fermenter reaches the top of the reactor, it is discharged. Therefore, the amount of air reaching the annular region is negligible. $K_l a$ is assumed to be 0.0 $hr^{-1}$ in the annular region.

## 2. COMPUTER MODELING STUDIES PERFORMED

The mathematical modeling results are broken into three sections:

### 2.1 Cell Parameter Comparisons

These comparisons study the effect of changing various cell constants. These include the yield coefficients ($Y_{x/o}$, $Y_{p/s}$) and the two substrate levels, carbon and oxygen. Changing these cell parameters illustrates the relative sensitivity of the reactor output and the conversion of carbon substrate.

1) A study of $Y_{x/o}$
2) A study of $Y_{p/s}$
3) Carbon substrate level studies
4) $K_l a$ (oxygen transfer) studies

### 2.2 Reactor Parameter Comparisons

Various computer studies were performed that show the effect of changing reactor parameters on both reactor

output and productivity or conversion. The following parameters were studied:

1) Number of reactors in series studies
2) Recycle ratio of reactor studies
3) Dilution rate of reactor study.

## 2.3 Economic Parameter Comparisons

The success or failure of an industrial process depends on economic factors of the plant. The final part of the computer simulations incorporates the previous cell and reactor studies to create an economic picture of the production of alpha-amylase by *B. stearothermophilus*. Two economic studies plot Return on Investment and capital investment versus reactor output. The model allows the process to incorporate previously used and installed equipment. Each study has four different categories which account for the attainment of previously purchased and installed process equipment. The 0% category represents the corporation having all equipment already owned and previously installed; the fixed capital investment is $0.00. In the 25% category, the corporation purchases and installs only 25% of the necessary process equipment. At 100% the corporation must purchase and install all of the equipment and, therefore, is the least profitable scenario.

The following economic studies were performed on the alpha-amylase production process:

1) Return on Investment vs. Reactor Output

2) Capital Investment vs. Reactor Output

3) Return on Investment vs. Yearly Production (MM Kg)

4) A percentage cost comparison of process equipment

5) A percentage cost comparison of manufacturing costs.

Each of these studies was completed using the SuperCalc4 spreadsheet.

# CHAPTER IV

## AIRLIFT FERMENTER REACTOR MODELING

### 1. CELL PARAMETER STUDIES - YIELD COEFFICIENTS

The cell parameter comparisons, Figures 4 and 5, show the various trends that occur if two yield coefficients are changed. The airlift reactor model using the tanks in series method was used.

#### 1.1 Yield coefficient - $Y_{x/o}$

In Figure 4, the yield coefficient $Y_{x/o}$ was varied from 0.05 to 2.0. The trends show a distinct and parallel correlation between the reactor output and the conversion of the carbon substrate. This was expected since reactor output and conversion increase as the cell production efficiency increases. The reactor output reaches an effective maximum at a yield coefficient of 1.65. At this point conversion of the carbon substrate was at 1.0 (or 100%), and reactor output also was at the maximum. For $Y_{x/o}$ values less than 1.65, the model predicts that oxygen transport is rate limiting. Above 1.65, the carbon substrate availability is rate limiting. Since $Y_{x/o}$ has a direct effect upon the reactor output, it thus needs to be optimized in industrial production.

#### 1.2 Yield Coefficient - $Y_{p/o}$

Another yield coefficient studied was $Y_{p/o}$, which relates the amount of product made to the change in this yield coefficient. Shown in Figure 5, reactor output increases with an increase in this yield coefficient. The

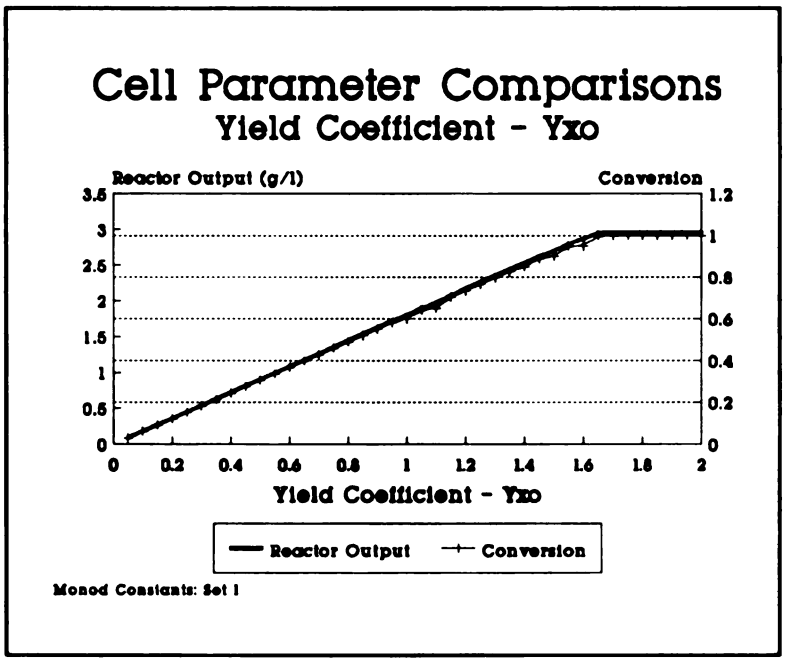


**Figure 4**

airlift fermenter simulation model uses. $Y_{p/x}$ to calculate the amount of alpha-amylase made in each CSTR. This coefficient was calculated directly from $Y_{p/s}$ and therefore results in a linear graph. Since product inhibition was not considered in the model, conversion of the substrate was not affected by the amount of product in the broth. The conversion line has a zero slope and illustrates this concept. Average carbon substrate conversion of a biochemical reaction is 50%; however, the recycle of the airlift reactor will cause the conversion to increase.[10]

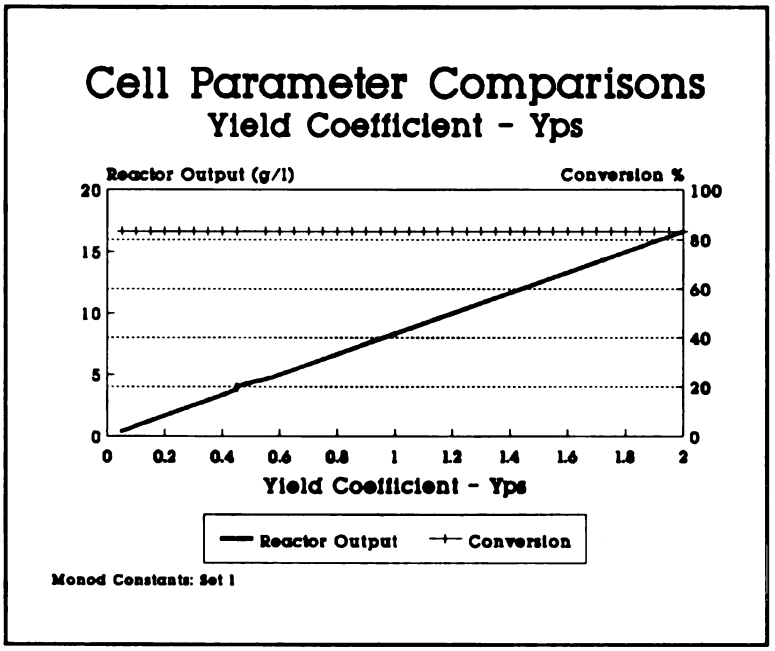
Cell Parameter Comparisons
Yield Coefficient - Yps
Reactor Output (g/l)
Conversion %
20
15
10
5
0
100
80
60
40
20
0
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
2
Yield Coefficient - Yps
Reactor Output
Conversion
Monod Constants: Set 1

**Figure 5**

## 2. CELL PARAMETER STUDIES - SUBSTRATES

Two forms of substrate are needed in the biomolecular process: carbon and oxygen. Figures 6 - 10 show the effects of changing substrate amounts in the airlift fermenter on reactor product output and conversion. The amount of carbon substrate entering the reactor was varied from 0.1 to 100 g/L. The solubility of oxygen was near the maximum at the entrance of the reactor ($[O_i]$= 5.00 x$10^{-3}$ g/L while $[O_e]$=5.85 x$10^{-3}$ g/L). $K_La$ was changed from 0.0 $hr^{-1}$ to 1000 $hr^{-1}$ to vary the oxygen substrate availability.

The model uses additional approximations for $K_s$ and $K_o$ that were not specific to *B. stearothermophilus*. These were listed previously in section 2.1.5. Therefore, along with the above factors for the Monod constants, some simulations were also run with the Set 2 Monod factors.

$$K_{s\,Set\,2} = 0.2 \text{ g/L}$$

$$K_{o\,Set\,2} = 0.002 \text{ g/L}$$

Since the Set 1 bacterial Monod factors were small when compared with actual substrate concentrations, this would cause $\mu$ to be approximately $\mu_{max}$ during the simulations:[3]

$$\mu_{max} = \mu/(S/(K_s+S))$$

$$\text{If } K_s << S \text{ then } \mu_{max} \approx \mu$$

Using these higher values of the Monod factors, the effects of substrate depletion on reactor performance can be seen.

### 2.1 Substrate studies - carbon

In Figures 6 and 7 the carbon substrate concentration was varied from 0.1 to 100 g/L. Figure 6 uses the Set 1

Monod constants for bacteria. The maximum reactor output and the beginning of the decline of conversion begin at the same point in Figure 6. This was expected since the system is saturated with the carbon substrate at about 9 g/L and can no longer produce the product quickly enough to use the extra carbon substrate. Conversion declines to about 10% for a feed concentration of 100 g/L.

In Figure 7, where the Monod constants are much higher, a maximum was shown for conversion near 3 g/L. This maximum was hidden in Figure 6. As the amount of substrate goes to zero, the conversion goes to zero as driving force for the reaction is reduced:

$$-r_s = \mu_{max}(S/(S+K_s)) \cdot X \cdot Y_{s/x}$$

$$\text{At } S = 0, \; -r_s \approx 0.$$

When S goes to the limit of zero, the reaction and conversion go to zero also (See Appendix D). Simulations such as those shown if Figures 6 and 7 ensure that the substrate is utilized to maximum conversion.

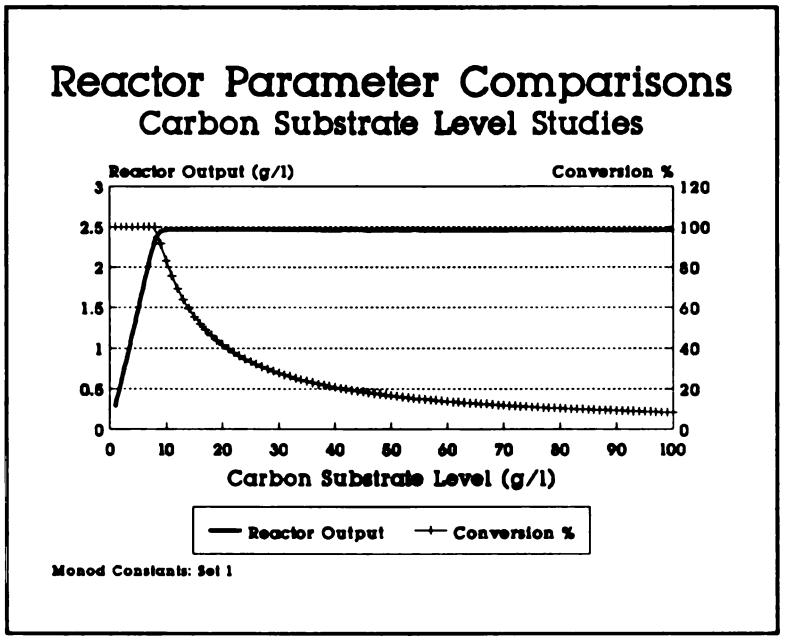


**Figure 6**

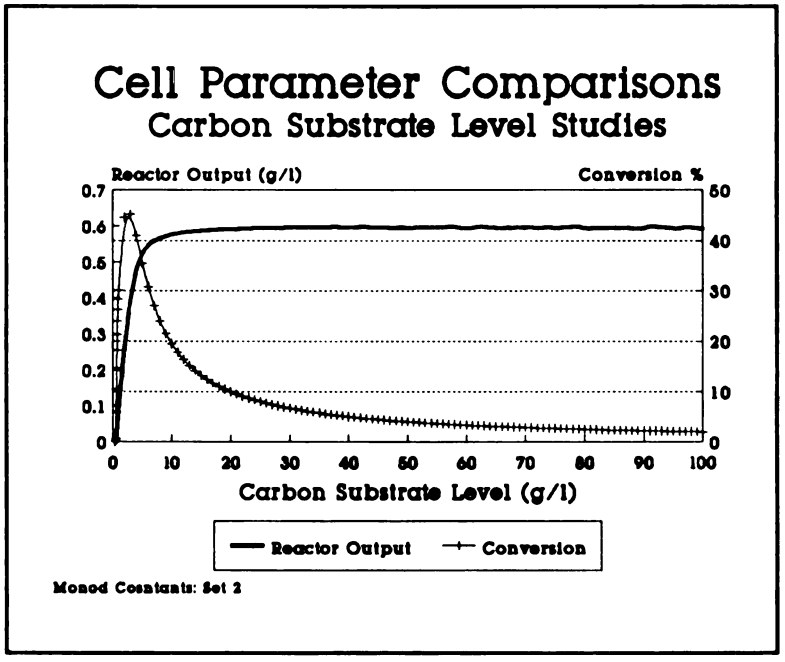


**Figure 7**

## 2.2 Substrate studies - oxygen

The second substrate of a biological process, oxygen, was almost completely introduced by the flux across the

bubble interface. A small amount was introduced initially through the inlet liquid. However, due to the recycle effect, which dilutes the inlet, and the demand of the cells, this small concentration was quickly depleted. Figures 8 - 10 show a zero or near zero reactor outlet concentration for a $K_l a$ of 0.0 $hr^{-1}$. Each figure depicts the reactor output and carbon substrate conversion over a $K_l a$ range from 0.0 $hr^{-1}$ to 1000 $hr^{-1}$.

Figure 8 uses the Set 1 Monod values for bacteria at a normal model dilution rate of 0.4 $hr^{-1}$. Product concentration was directly proportional to $K_l a$ up to a value of 240.0 $hr^{-1}$. At this point the system was saturated with oxygen, the carbon substrate conversion was at 100%, and the reaction operated at the maximum production level. At this low dilution rate, the oxygen was adequate and is no longer the rate-limiting step.

Figure 9 shows the effect of $K_l a$ on rector performance when the dilution rate was increased to 1.2 $hr^{-1}$. At this dilution rate the $K_l a$ transfer can no longer provide the cell with the oxygen saturated broth. Asymptotic conversion and reactor output value were attained. A linear region exists below the $K_l a$ values of 300 $hr^{-1}$ which indicates that the cell was using most of the $O_2$ transferred. Above this factor, further increasing $K_l a$ would result in a lower return on the aeration power investment.

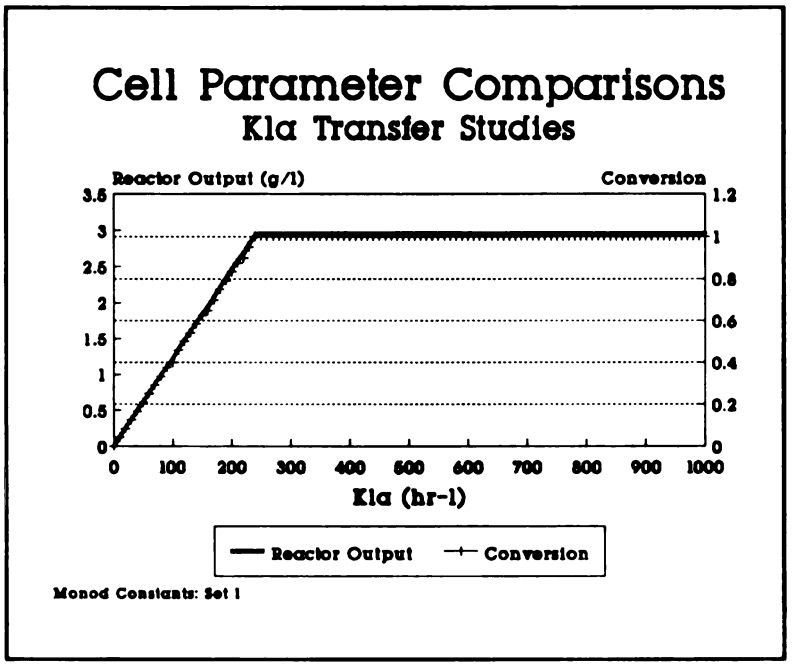


**Figure 8**

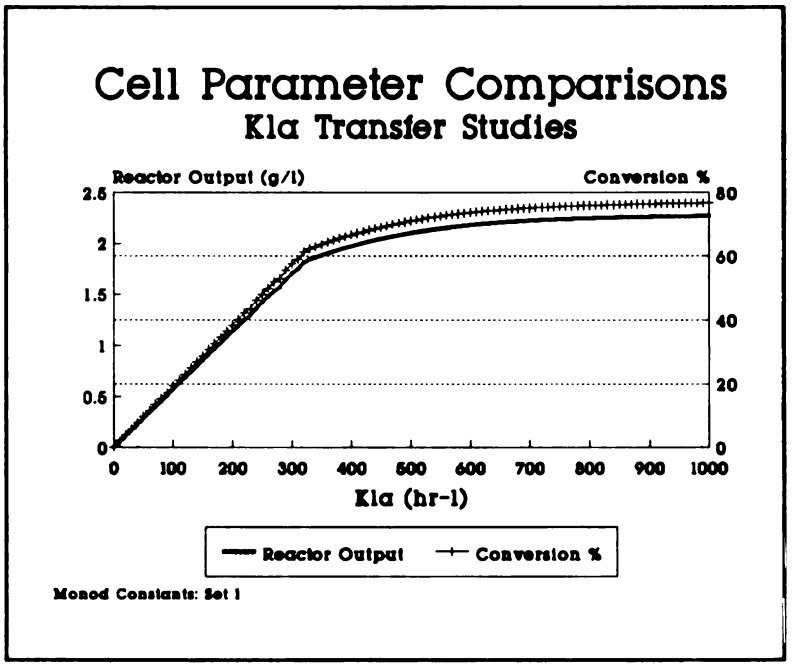


**Figure 9**

Figure 10 illustrates the reactor performance when the higher Set 2 Monod constants are used. The graphs illustrate the reactor output is sensitive to $K_l a$ at low

values where oxygen is rate-limiting, but insensitive at high $K_La$ values where oxygen is in ample supply. At high $K_La$ values further increases yield little benefit to the system and may drive the power and equipment costs to an unprofitable level.

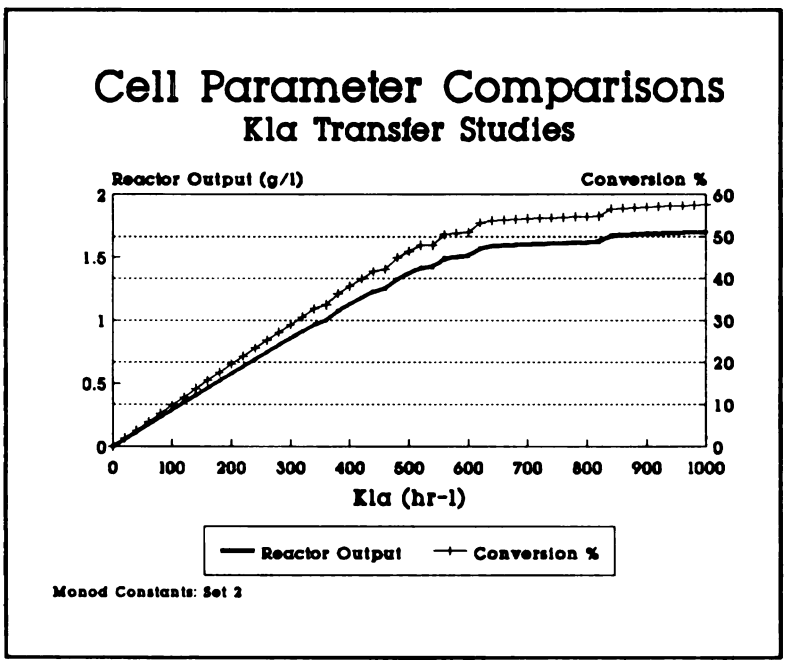


**Figure 10**

## 3. REACTOR PARAMETER STUDIES - TANKS-IN-SERIES

The tanks-in-series model was used to describe the internal mixing of the airlift fermenter. As discussed by Levenspiel[20], for positive order kinetics, the greater the number of tanks-in-series (i.e. the closer to plug flow), the greater the conversion and productivity. The airlift fermenter simulation package was modified to take a zero recycle (R = 0.0) into account, which implies that the reactor has no return flow down the annular region. This simulation confirmed the previously mentioned references with respect to the substrate conversion and alpha-amylase production based upon the number of tanks used in the model. However, when normal recycle was added into the airlift fermenter model, opposite results occurred.

Figures 11 and 12 show the effect of an increased number of tanks-in-series on fermenter performance for a recycle rate (R) of 0.5. The conversion and product concentration drop as the number of CSTR reactors are increased. The trend is due to the recycle of the substrate back into the draft region of the reactor. Schugerl confirms this interesting phenomenon for airlift fermenters and explains it as a result of the substrate concentrations in the recycle.[86] Figure 11 depicts the model using the Set 1 Monod factors for bacteria. The differences in reactor output and conversion are small, insignificant in Figure 11, but apparent for the changes in the number of CSTR tanks-in-series.

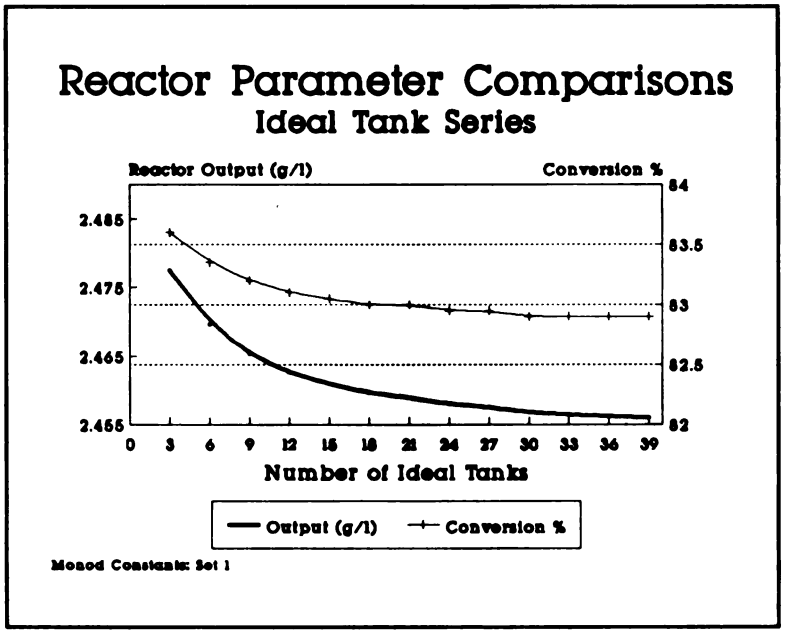


**Figure 11**

However, these conditions do not illustrate this strange phenomenon as well as Figure 12 which uses the higher Set 2 Monod constants. (Note the large difference between the Y axis spans for each figure.) Figure 12 shows distinctly that as the number of CSTR tanks-in-series was increased production and conversion drop. Backmixing of the reactor fluid should no longer be avoided when using this type of reactor but encouraged through the use of baffles and other physical modifications.

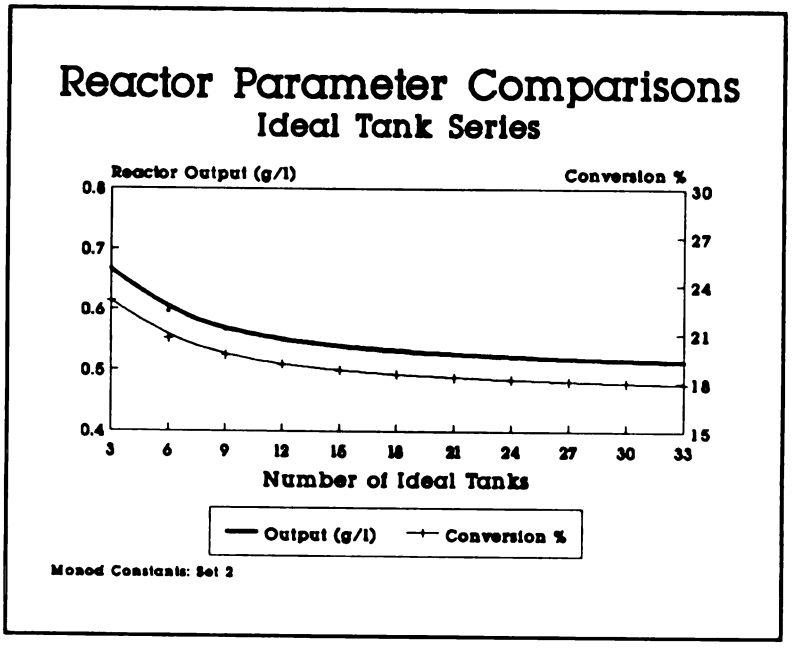
Reactor Parameter Comparisons
Ideal Tank Series
Reactor Output (g/l)
Conversion %
0.8
0.7
0.6
0.5
0.4
30
27
24
21
18
15
3
6
9
12
15
18
21
24
27
30
33
Number of Ideal Tanks
Output (g/l)
Conversion %
Monod Constants: Set 2

**Figure 12**

## 4. REACTOR PARAMETER STUDIES - REACTOR RECYCLE RATIO

The recycle ratio of the reactor was defined as the amount of the flow rate into the annular region divided by the amount of fresh feed flowing into the reactor. In Figure 13, the recycle ratio was varied from 0.1 to 1.0. Both output and conversion remain constant within this region. Schugerl predicts that at lower recycle rates a PFR is modeled and higher conversion and product levels are expected. At higher recycle rates a CSTR is modeled and the opposite is true.[88] The slight variations between recycle ratios 0.1 and 0.2 are caused by computer iteration error. They do not reflect actual airlift fermenter reactions.

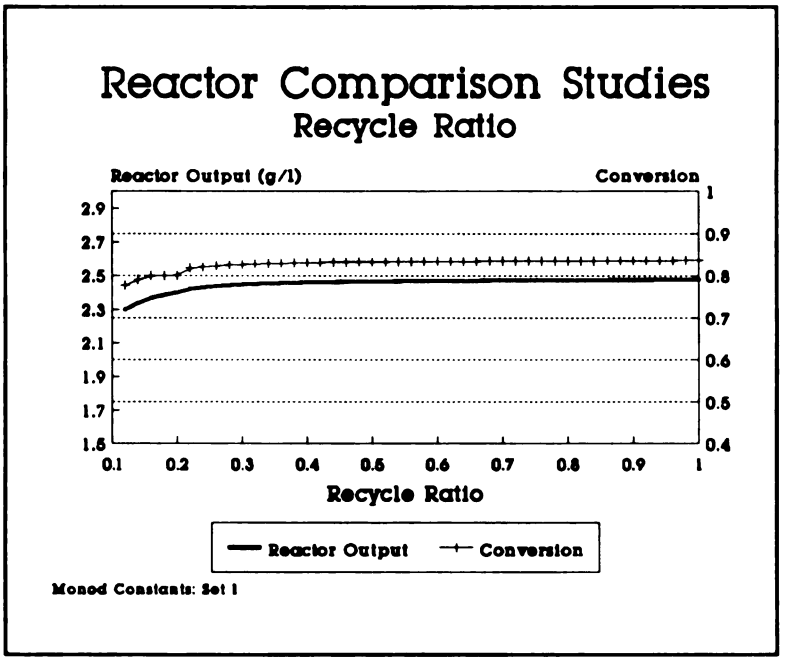


**Figure 13**

In Figure 14 the Monod constants were increased to higher Set 2 levels. This causes the effect that Schugerl describes and shows that at lower recycle rates conversion

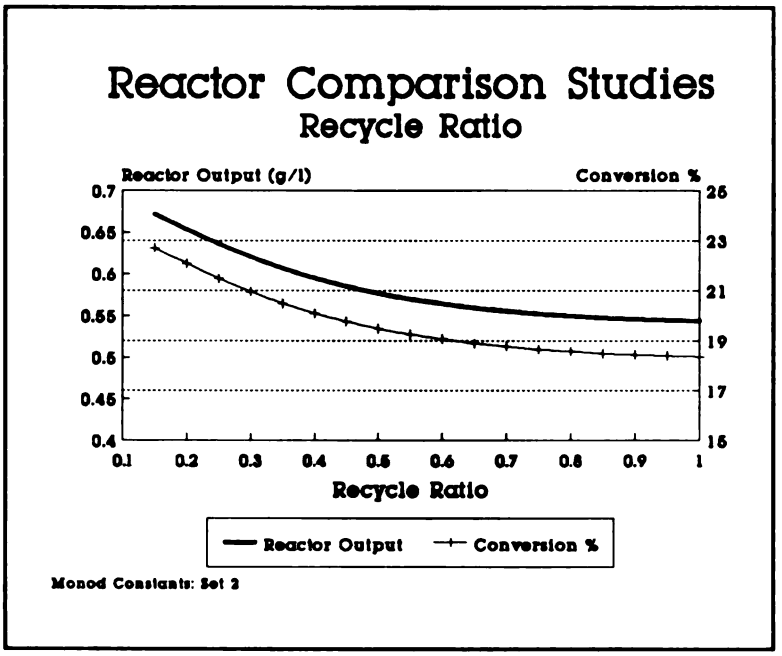


**Figure 14**

and production increase due to PFR flow.[86] Figure 13 uses the lower Monod constants which causes the trend to be hidden. This is not to be confused with Figures 11 and 12 which show a specific physical characteristic of using the airlift fermenter is not a true effect of an ideal PFR or a CSTR reactor.

## 5. REACTOR PARAMETER STUDY - DILUTION RATE

The overall dilution rate of the reactor was changed from 0.1 $hr^{-1}$ to 1.5 $hr^{-1}$ in Figure 15.

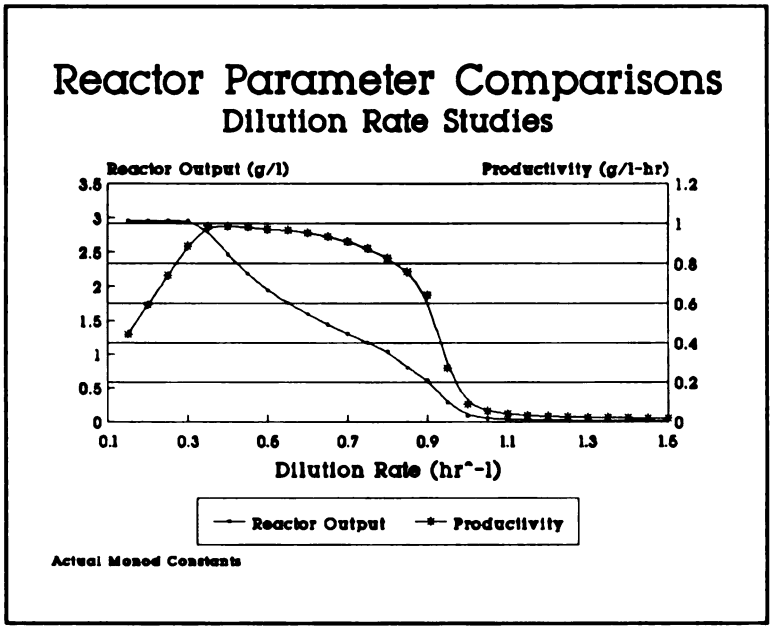


**Figure 15**

The productivity is given by the product of the product concentration and the overall dilution rate. The concentration of the product drops steadily as the dilution rate was increased. However, the more important parameter was the productivity of the reactor. At a dilution rate of 0.4 $hr^{-1}$, the productivity reaches a maximum of approximately 1 g/L-hr. In this case increasing the Monod constants have little effect in changing the figure trends. Doing such a study before actual construction of a plant enables designers to accurately determine the size of the reactor. Each of the previous parametric studies were run at the

dilution rate of 0.4 $hr^{-1}$ in order to increase the productivity.

Running the reactor at lower dilution rates would increase conversion. Experiments would have to be performed to determine whether economic advantages would be found at higher conversion or higher productivity.

# CHAPTER V

## ECONOMIC PROCESS MODELING

The economic comparison studies show the effect of various economic and industrial process factors upon the profitability of the process. These factors include Return on Investment and capital investment based on reactor output and yearly production of alpha-amylase. The various equipment and production costs were also compared with each other to demonstrate relative costs. This method allows the highest costs to be identified.

## 1. ECONOMIC STUDIES - REACTOR OUTPUT

Figures 16 and 17 depict the effect of reactor output concentration in g/L upon return on investment and capital investments. These figures include the capital investment factors that allow all, some, or none of the fixed capital investment to be neglected. The output from the reactor determines the sizes of the equipment, the amount of production materials, and the yearly production costs. It is the one variable of the system that must be fully optimized.

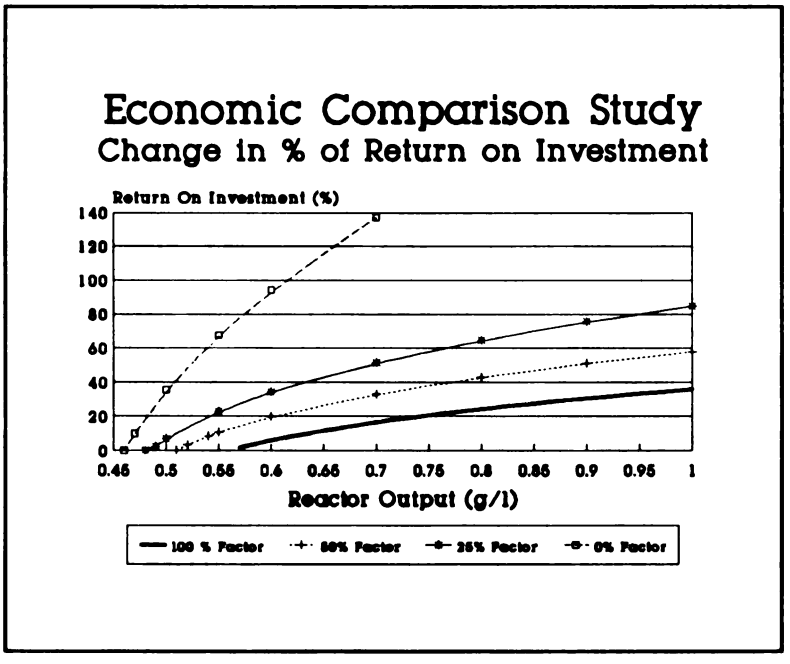


**Figure 16**

### 1.1 Change in return on investment

In Figure 16, return on investment (ROI) is plotted against reactor output concentration. Reactor output values ranged from 0.45 to 1.0 g/L. At the 100% factor, 100% of the equipment must be bought; at the 0% factor, no equipment

must be bought. Four lines were included that represent the different amounts of fixed capital investment already available for use. Depending on which factor was chosen, the minimum reactor outputs varied at a return on investment of 0%. At a return on investment of 0%, the following reactor outputs have been calculated.

Table 1: Capital Investment Factor vs. Reactor Output

| CAPITAL INVESTMENT FACTOR | MINIMUM REACTOR OUTPUT G/L |
|---|---|
| 0 % | .46 |
| 25 % | .48 |
| 50 % | .51 |
| 100 % | .57 |

The table above demonstrates how the reactor output concentration at the break-even point rises depending on the amount of equipment required for the process. At the 100% capital investment factor, where all equipment must be purchased, the process must produce 0.57 g/L to break even. If the capital investment factor lowers to the 0% level, where all equipment is owned and currently installed, then the process need only produce a concentration of 0.46 g/L. The difference of 0.11 g/L between the two studies was significant when compared to the differential increases of product output from enhancements due to microbiological and product recovery research. The importance of attaining used equipment can be easily justified.

At 1.0 g/L output from the reactor, the Return on Investment varies with the capital investment factor. Thus,

by having equipment that is already paid for and installed, profit margins will be substantially higher.

Table 2: Capital Investment Factor vs. ROI

| CAPITAL INVESTMENT FACTOR | RETURN ON INVESTMENT |
|---|---|
| 100 % | 36 % |
| 50 % | 59 % |
| 25 % | 84 % |
| 0 % | >200 % |

For each of the fixed capital investment factors, in Figure 11, a leveling off was apparent near the 1.0 g/L reactor output.

1.2 Change in capital investment

Figure 17 demonstrates that the concentration of the product greatly influences the capital investment needed for the plant.[80] The reactor output in this study spans 0.1 to 1.0 g/L.

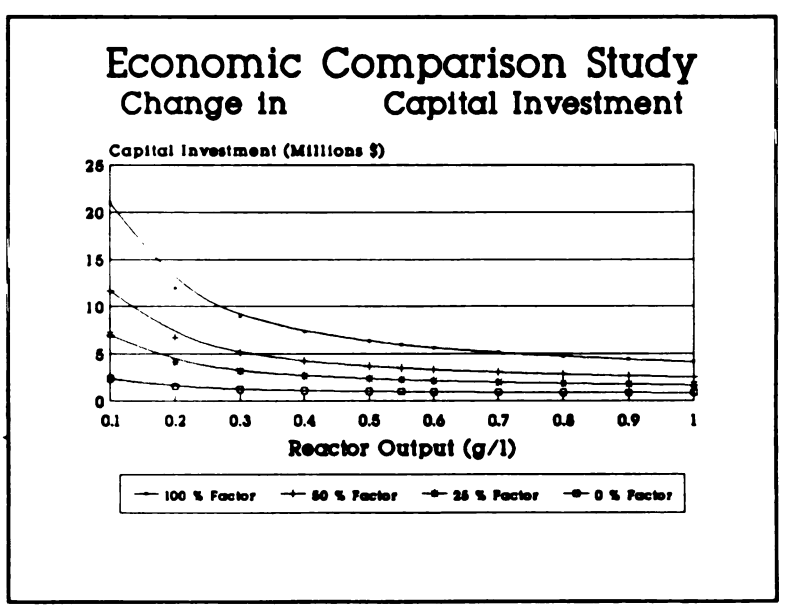


Figure 17

At lower concentrations the size of the equipment must be very large. As the amount of product concentration was increased, the capital investment dropped. For these studies a yearly production of 100,000 kg was assumed. This value is 33% of the total yearly production of alpha-amylase. The difference between the capital investments for 0.1 g/L and 1.0 g/L was enormous. At 0.1 g/L reactor output, the following capital investments have been calculated:

Table 3: Capital Invesmtent Factor vs. Capital Inv.

| CAPITAL INVESTMENT FACTOR | CAPITAL INVESTMENT ($ X 1E6) |
|---|---|
| 100 % | 21.0 |
| 50 % | 11.5 |
| 25 % | 7.3 |
| 0 % | 2.5 |

While the variation was large between the different factors for the capital investments and the need for previously used equipment is apparent, at 1.0 g/L this variation diminishes. The following table is similar to the above except that the reactor output is 1.0 g/L.

Table 4: Capital Investment Factor vs. Capital Inv.

| CAPITAL INVESTMENT FACTOR | CAPITAL INVESTMENT ($ X 1E6) |
|---|---|
| 100 % | 4.1 |
| 50 % | 2.5 |
| 25 % | 1.7 |
| 0 % | 0.9 |

## 2. ECONOMIC STUDIES - YEARLY PRODUCTION OUTPUT

Figure 18 demonstrates the Return on Investment as a function of yearly production of alpha-amylase. The product concentration was assumed to be 1.0 g/L. The return on investment showed growth as the yearly production was increased. The line rises sharply at low production rates and levels off. Average ROI lies between 5% to 25%. At the 1,000,000 kg/yr point, the ROI was an unrealistic 54%.

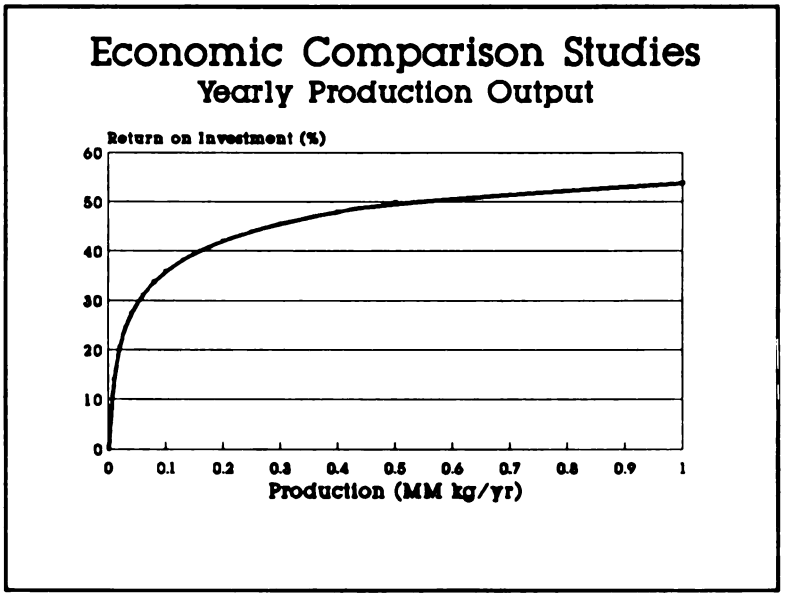


**Figure 18**

# 3. ECONOMIC STUDIES - COST COMPARISONS

## 3.1 Equipment cost comparisons

Figure 19 illustrates the relative costs of the various pieces of equipment and indicates the outstanding and overriding capital costs of a process. This study showed which parts of the process need to be modified to reduce capital expenditures. The single most costly piece of equipment is the ultra-filtration apparatus. The use of flocculation combined with a spray dryer may reduce this cost.

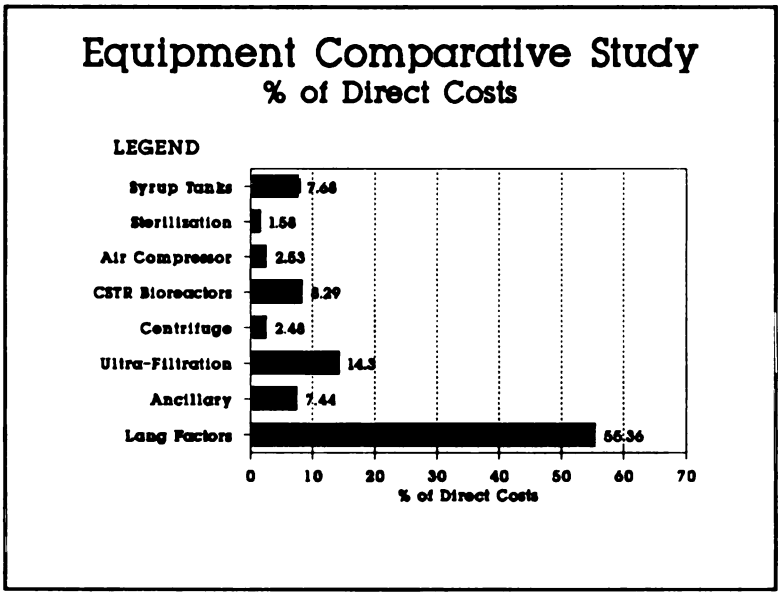


**Figure 19**

The highest costs in the process capital investment are the installation and preparation costs taken into account in the Lang factors.

## 3.2 Yearly production cost comparisons

In Figure 20, the yearly manufacturing costs are shown. The production costs of a process are the most influential

costs of any system.[14] The ability of a process to financially succeed depends on reducing the variable costs. The fermentation costs are the largest component of the production costs.[10,40] The following is a breakdown of the fermentation costs.

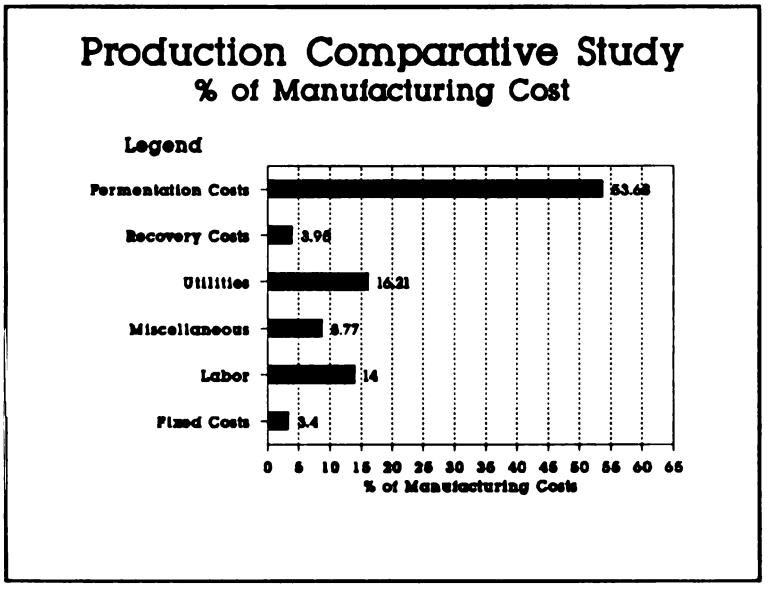


**Figure 20**

Table 5: Fermentation Costs vs. Yearly MFG. Costs

| FERMENTATION COSTS | % OF YEARLY MFG. COSTS |
|---|---|
| Fixed Costs | 3.4 |
| Recovery Costs | 3.95 |
| Miscellaneous | 8.77 |
| Labor | 14.0 |
| Utilities | 16.21 |
| Fermentation Costs | 53.68 |
| | 100% |

Modeling the return on investment versus a cell or reactor parameter is possible. The slopes of the reactor output concentration versus the reactor parameter can be

multiplied by the slope of the ROI versus the reactor output curve. The sensitivity of the ROI to one of the reactor or cell parameters can be readily determined from the results of this type of study. For example, ROI can be plotted against the mass transfer coefficient $K_La$, to find the change of ROI from a change in $K_La$.

$$d(ROI)/d(K_La) = (d(Output)/d(K_La)) \cdot (d(ROI)/d(Output))$$

# CHAPTER VI

# DISCUSSION OF RESULTS

## 1. PRELIMINARIES

"Fermentation *per se* is an expensive process. In fact, it is this expense that has so far prevented the spread of biotechnology very far beyond the pharmaceutical field, into commodity and specialty chemicals."[10]

### 1.1 Overview of studies performed

The success of a chemical plant is based upon its ability to produce a reasonable profit. The investment in a plant must yield a ROI obtainable in the stock market or in any competitive bank. Process and economic models were developed to evaluate the possibility of using an airlift fermenter in a continuous process to produce the alpha-amylase enzyme from *B. stearothermophilus*. The process and economic studies were run independently. The airlift fermenter studies explored the effect of changing various cell, substrate, and reactor parameters on reactor output, conversion, and cell productivity. The economic studies looked at the effect of capital investment factors and annual production on the return on investment. The effect of product concentration on capital investments and yearly production costs were also studied.

### 1.2 Relationship between studies

The studies involving the airlift fermenter performance and the economics of the continuous process would seem to be

unrelated. Studies of yield coefficients appear to have little effect upon, say, the total capital investment of a continuous alpha-amylase plant. Yet each of the previous reactor studies have an intimate and direct effect upon the economics. While total capital investment is not directly plotted against the yield coefficients, it was shown in Figures 16 and 17 that product concentration is the major factor in both capital investment and profits (return on investment). Although not all of the unit operations for a continuous plant were as closely studied as the airlift fermenter, the others are also critically important. The airlift fermenter was studied in greater depth because of its unique design and the new advantages it offers over other bioreactor configurations. The process studies of the airlift fermenter are as important to the economics as any of the economic variables. While this study may seem to gather a large amount of unrelated data together, it actually shows the connection between a variable specific to the cell, a yield coefficient, to that of the overall process economics.

## 2. YIELD COEFFICIENT RESULTS

### 2.1 Varying $Y_{x/o}$

Figure 4 illustrates the effects of changing the yield coefficient $Y_{x/o}$ on bioreactor output and the importance of high efficiency of cellular production. As the yield coefficient $Y_{x/o}$ is increased both conversion and reactor output increase linearly. Thus, increases in the yield

coefficient would directly influence the reactor output. Through genetic engineering, it may be possible to make the production of the cells more efficient, i.e., less substrate used per given amount of cells produced. However, there is a point beyond which increasing the $Y_{x/o}$ has no further effect ($Y_{x/o}$ = 1.65). At this point research efforts should be directed towards improving other areas.

2.2 Varying $Y_{p/s}$

The next intrinsic cell constant studied was the yield coefficient $Y_{p/s}$. In Figure 5, $Y_{p/s}$ is plotted against both reactor output and conversion. A linear relationship exists between reactor output and $Y_{p/s}$. Unlike for $Y_{x/o}$, this trend does not reach a plateau but increases indefinitely as $Y_{p/s}$ is increased. The assumed kinetic model does not take into account product inhibition and therefore does not truly illustrate a real life system. Since the system is assumed to not be product limited, this is to be expected. However, the model does serve to illustrate the strong effect that this yield coefficient has upon overall reactor output. Research directed into this area would be advantageous, although an asymptotic limit would certainly be approached at large values for $Y_{p/s}$. The conversion of the carbon substrate was held constant in Figure 5.

## 3. SUBSTRATE RESULTS

Changing the amount of substrate in a substrate-limited system greatly effects the product output and economics of the reactor. One objective of this study is to determine

substrate concentrations that will minimize the overall cost of producing the enzyme. The ideal situation would be to have infinite amounts of all substrates present in solution. No limiting conditions would exist and growth of cell would approach $\mu_{max}$. However, this scenario along with being physically impossible would be incredibly expensive. This biological reaction uses two different substrates, carbon and oxygen. Either substrate may be rate-limiting, depending on the operating conditions. However, both substrates must be taken into account in the growth equation. Figures 6 and 7 illustrate how reactor output and conversion are functions of carbon substrate concentration. Figures 8, 9, and 10 depict the effect of changing $K_la$ on reactor output or conversion.

3.1 Varying the carbon substrate

Each of the substrates, carbon and oxygen, is expensive to administer to the growing cells. The carbon substrate itself is generally expensive, and raw materials can cost as high as 60% of the yearly production costs.[10] However, sufficient quantities must be used that will facilitate cell growth. A concentration at which the carbon substrate optimizes the economics must be found. This is illustrated in Figures 6 and 7. In Figure 6, conversion is at 100% until 10 g/L where the carbon substrate is wasted. Not coincidentally, this is the same point at which the reactor output reaches its maximum value. The cells can utilize the carbon substrate up to a certain concentration,

at which the carbon is no longer growth limiting; the oxygen then becomes the limiting reactant. If the amount of carbon substrate is reduced too low, conversion becomes zero as does reactor output as shown in Figure 7 (Appendix B). A conversion maximum is shown in this figure near 3 g/L. The plant may run at this carbon substrate conversion maximum. To gain conversion while sacrificing productivity, it may run at a lower dilution rate. Although the reactor output is not at the maximum in Figure 7, the expensive substrate is not wasted.

Figure 6 shows that maximum reactor output occurs at the same concentration as decline of conversion. Therefore the inlet carbon substrate should have a concentration corresponding to the conversion maximum. This type of study illustrates the importance of reactor modeling upon the overall economics of a process. Without a model of the substrate utilization, the correct input to the reactor would not be known. If the substrate input was too high, the glucose is wasted and expenses rise. If the substrate inlet is too low then the reactor will not operate at the fullest potential.

### 3.2 Varying the oxygen substrate

The other substrate, oxygen, has been shown to be rate-limiting under most conditions. The oxygen concentration in the liquid feed stream is irrelevant to reactor performance. This small amount of oxygen is depleted quickly when mixed

with the oxygen deficient recycle stream and during the first or second theoretical reactors-in-series.

The parameter $K_l a$ determines the rate of oxygen transferred to the liquid from the bubble. It strongly affects the oxygen concentration in the reactor broth. Figures 8, 9, and 10 depict the change in reactor output and conversion versus $K_l a$. Like the concentration of the carbon substrate, $K_l a$ has an influence upon reactor productivity. At large $K_l a$, the reactor output and carbon substrate conversion reach asymptotic values. Unlike the carbon substrate, however, the oxygen substrate (air) is inexpensive. The expense (capital investment) involves methods of getting this substrate into the liquid. This can be done by increasing the size and speed of an impeller in a CSTR reactor. In an airlift fermenter the quantity of air is increased through the sparger. No matter which reactor is chosen, increasing the $K_l a$ factor increases both capital and production costs of the system. Therefore, like the carbon substrate, choosing a $K_l a$ value that is large enough for the cell growth, yet small enough to keep the process profitable is important. Increasing the $K_l a$ beyond 240 $hr^{-1}$ for Figure 8, which models D = 0.4 $hr^{-1}$ and uses actual Monod constants, would yield no advantage in either reactor output or conversion. Figure 9 which uses a D = 1.4 $hr^{-1}$ shows the same effect at a $K_l a$ of 340 $hr^{-1}$. Figure 10 uses Set 2 Monod constants and has the same trend at 800 $hr^{-1}$. The study of $K_l a$ is of economic necessity. Air compressors are

expensive. Designing a compressor that is too large would waste capital initially and large amounts of production costs over a period of time. Designing a compressor that is too small would mean another would have to be purchased.

## 4. NUMBER OF REACTORS-IN-SERIES

Internal mixing within a reactor determines the actual conversion of the substrates. A PFR reactor is known to be the most efficient for positive order kinetics. In the airlift fermenter this is not the case. For a constant recycle ratio, the more the reactor resembles a backmixed tank, the better the conversion and reactor output. This type of study may not influence the economics of the system directly, but as shown in Figure 12, the reactor design has a great impact on the actual product output. It is assumed that PFR flow, or a large number of CSTR reactors-in-series, will benefit productivity. In an airlift fermenter, changing the amount of CSTR reactors-in-series effects various recycle parameters which cause this inverse trend to appear. Without this type of study the reactor would be designed to maximize the plug flow regime and would optimize the incorrect type of fluid flow.

## 5. REACTOR RECYCLE RATIO

Figures 13 and 14 depict the change in recycle ratio within the airlift fermenter. The recycle ratio is defined as the overall amount of fluid entering the reactor divided by the amount flowing down the annular region. At a recycle

rate of 0.0 the reactor would emulate a PFR reactor; at a recycle rate of 1.0 the reactor would emulate a fully backmixed CSTR. Figure 14 shows the effect of changing the recycle rate upon both conversion and reactor output. At lower recycle rates where the reactor behaves as a PFR both conversion and reactor output are higher. At a recycle rate of 1.0, an ideal CSTR, the reactor output and conversion are at the minimum point. Like the above study of ideal tanks-in-series, this study does have a direct effect upon the economics of the alpha-amylase plant. By designing the reactor in such a way as to maximize the product output and substrate utilization, this continuous process will have a better chance of becoming an economic reality. This type of behavior seems to be opposite of that in the previous study. PFR flow was not the best type of fluid flow to use for maximum conversion and production. In this case, increasing the recycle ratio of the reactor causes the fluid dynamics to move toward a CSTR reactor. Conversion for all reactors using these type of kinetics is smaller in the CSTR region.

### 6. REACTOR DILUTION RATE

The dilution rate of the reactor has a strong influence upon the economics of the plant. At higher dilution rates a greater amount of the carbon substrate is wasted. Substrate recycle may help alleviate the problem, but still a greater amount of substrate is wasted at higher dilution rates. Carbon substrate is very costly, so the dilution rate of the reactor must be optimized. Figure 15 shows reactor output

and productivity. While product output drops as the dilution rate increases, the productivity of the reactor reaches a maximum near 0.4 $hr^{-1}$. This is the dilution rate at which all computer studies were run. The importance of such a study on both reactor and plant design along with the overall economics is enormous. Designing a reactor at a dilution rate far from the optimal value will not only reduce the maximum possible productivity of the system but will also cause an increase in both capital investments and production costs. At dilution rates below 0.4 $hr^{-1}$ the reactor would be oversized (although the reactor product concentration would be higher); dilution rates above that of 0.4 $hr^{-1}$ wastes the carbon substrate. This study influences the sizing of all the equipment along with the prediction of the yearly production costs for the system.

The reactor could be run at a lower dilution rate in order to maximize conversion of the carbon substrate. Doing so would lower product output concentration. However, the carbon substrate would not be wasted through the outlet stream. Studies in this area may yield interesting results.

## 7. CONCLUSION OF AIRLIFT FERMENTER DISCUSSION

The mathematical study of the airlift fermenter involves no economics yet has a direct influence on economic planning. Each of the studies illustrates how mathematical modeling can be used to better understand the effects different parameters have upon reactor output and conversion. These studies are by no means the end, but are

the start of understanding how a fermenter reacts under varying conditions. With these types of studies, an initial size can be found for the reactor and confirmed through lab and pilot plant scale-up.

The production of alpha-amylase is assumed to occur during the growth phase of *B. stearothermophilus*. In other alpha-amylase producing bacteria, the product is made during the substrate-poor stationary phase. For stationary-phase production, a tank may be added between the reactor and the recovery section to induce the stationary phase and enhance enzyme yields.

The main reason these studies were done was to analyze how each of the cell and reactor parameters affected output and conversion. Some of the parameters changed the output dramatically (e.g., the yield coefficients), while others had little or no affect (e.g., the recycle rate). From these parametric studies, areas of most importance can now be singled out for research while the others can be safely neglected.

There are two base cases studied; one uses the actual Monod constants (Set 1) while the other uses the higher constants (Set 2). In Appendix C, the two base cases are presented. The first base case, which uses the actual Monod constants, gives a reactor output of 2.47 g/L. This factor is far and above that which is needed for the plant to be considered profitable. The actual return on investment for this process is over 50%.

Base case 2 uses the higher Monod constants and the results are interesting. The reactor output is 1.13 g/L which is 50% less than base case 1. This case is still profitable with a return on investment of nearly 40% yet this demonstrates the fluctuation that occurs when cell constants are changed.

## 8. INFLUENCE OF REACTOR OUTPUT UPON ECONOMICS

The economic studies in Chapter V yield a great deal of necessary information. The studies examine how different process parameters affect the economics. Reactor output has the greatest influence upon overall economics. From the reactor output, the sizes of all the equipment are calculated. If the output from the reactor is very small, the process equipment capital investment is very large and vice versa. Figures 16 and 17 illustrate how both capital investment and production costs are influenced by the reactor output. The amount of product produced per year also has an effect upon the amount of profit, return on investment, that a plant can attain, as illustrated in Figure 18. Figures 19 and 20 show the highest costs for both equipment and yearly production costs. Both are necessary to reduce the costs that cause the greatest loss of profits, total production costs.

## 9. REACTOR OUTPUT AND RETURN ON INVESTMENT

The return on investment is the most important variable to economically optimize in an industrial system. It is

important to estimate this return, often done by using economic simulations. The most important process factor is the actual output concentration from the reactor. In Figure 16 the return on investment is plotted against reactor output. The figure includes the different capital investment factors that take into account the various amounts of process equipment that have been previously bought and installed. The 100% factor study in Figure 16 shows that to attain a reasonable level of return on investment, say 25%, a reactor output of 0.75 g/L is needed. As the amount of pre-purchased and installed equipment is increasingly available to the company, the need for reactor output drops. At the 0.0% factor, where all equipment is assumed owned and installed, the 25% return on investment mark occurs at 0.48 g/L.

This simulation reveals two interesting conclusions. First, Figure 16 shows the sensitivity of return on investment on reactor output. A point is reached at which continued research and genetic development will not outweigh the cost investment in such research. Initially, increasing the reactor output will significantly change the return on investment. However, the change becomes less dramatic as reactor output increases. The point of decreasing return on investment could be calculated from this type of study.

Second, many chemical companies are known to reuse existing equipment.[10] Figures 16 and 17 show the dramatic effect upon the economics in using existing equipment.

Corporations that must purchase all equipment have a much higher bottom level of reactor output than do those which reuse older equipment. The slope of the 100% capital investment factor is also much lower than that of the 0% factor. The advantages of using old equipment, even though the production results might not be as good, are apparent. For example, a new company must produce 0.75 g/L to attain the a 20% ROI while another established company, owning 75% of the needed equipment, need only product 0.54 g/L to reach the same profit level. Even if the old reactor were not as efficient or could not produce as high a product concentration, a 0.21 g/L difference is quite large.

If current technology of *B. stearothermophilus* and alpha-amylase production could produce up to 0.50 g/L enzyme in production stream, a new plant could not produce a 25% return on investment. However, a reconstructed plant using existing equipment might be able to produce the necessary profit.

## 10. REACTOR OUTPUT AND CAPITAL INVESTMENT

Figure 17 is shows the capital investment necessary at different reactor outputs. A subset of the return on investment, the capital investment shows some interesting trends as the reactor output is changed. As in Figure 16, four different cases are shown that represent the amount of previously used equipment. If all of the equipment is owned and has been previously installed, (i.e. the 0.0% factor

used in the graphs), capital investment is almost independent of reactor output (accounted for only in the change of stocked chemicals). However, as more process equipment must be purchased, the capital investment becomes more sensitive to output. The ability of a plant to attain used equipment is more vital when the output concentration is low as shown by Figure 17. The change between the 0% factor and the 100% factor is much greater at 0.1 g/L than at 1.0 g/L which illustrates the importance of attaining the old equipment when reactor output is lower.

The costliest piece of equipment was the ultra-filtration apparatus. This may be replaced with the newer technique of recovery using ionization gels[93] or through the use of cell flocculation.

## 11. YEARLY PRODUCTION AND RETURN ON INVESTMENT

The return on investment for a chemical company producing alpha-amylase versus the yearly output is shown in Figure 18. The annual production of alpha-amylase is approximately 300,000 kilograms per year. Depending on the market share that a company produces (for these studies it was assumed to be 100,000 kg per year) the return on investment will vary. It is unreasonable to presume that any one company will have a monopoly on a commodity chemicals; however, amounts of up to 1,000,000 kg per year were studied. The more chemical produced, the less expensive the product is per unit, up to a point. This trend is shown in Figure 18 where the return on investment

or profit margin increases as the amount of production increases.

## 12. DETERMINING THE LARGEST COSTS

Determining the largest costs of both capital investment and production indicates where efforts should be made to trim costs. For example, there is no use in optimizing a $1000.00 piece of equipment when a reactor may cost $500,000.00 or more. Determining the "priority" for reducing certain costs will save both time and expense. Figures 19 and 20 show the capital and production costs, respectively.

### 12.1 Largest capital investments

Figure 19 shows overriding capital costs of a process are the bioreactors and the ultra-filtration equipment. The bioreactors are taken to be normal CSTR reactors. While airlift fermenter prices were not available, they are less complicated and easier to manufacture. Because of these characteristics, the capital cost would most likely be lower. The ultra-filtration equipment could be replaced with more current technology. Another possibility is to use flocculation to remove the cells from solution. The broth could then be concentrated through spray drying.

### 12.2 Largest yearly production costs

Figure 20 illustrates the various relative amounts of the different yearly production costs. Fermentation costs account for 54% of the yearly cost. The carbon substrate is the most expensive part of the yearly variable fermentation

costs. Finding a more inexpensive substitute for the starch or glucose, such as molasses, would greatly reduce the cost of running the alpha-amylase plant.

## 13. DISCUSSION SUMMARY

The two cost analyses illustrated above are not an "end all" to the economic optimization that must be completed. Each of the costs, both capital and production, must be reduced. The study gives a detailed indication of which costs influence the profit.

Finally, the economics of a plant are based upon many different factors which range from cell constants to production of alpha-amylase per year. The question of whether a plant should or should not be built rests upon many assumed values. The best that can be done is to hope that the variables change to increase, not decrease profits. The main focus of this study was to calculate whether a continuous process using an airlift fermenter is an economic possibility.

It may be useful to plot cell or reactor constants versus an economic factor such as return on investment. The correlation between the science and economics is apparent. To plan an in depth economic study is not advisable without taking into account the factors for both the cell and reactor. The airlift fermenter model provides some approximations to the amount of reactor output, substrates used, and other information that is needed in the economic simulation. Although the results are only estimates they

indicate that this alpha-amylase plant has a good chance of working economically (i.e. having a reasonable ROI). using currently available technology. The reactor output studies show that product concentration based upon the assumed kinetic and stoichiometric constants is large enough to push the ROI above the 20-25% mark. Because of the vast differences in separation/purification methods which may be used, this study does not take into account the downstream processing losses. These must be taken into account when the final reactor calculation has been made.

## CONCLUSION

Determining whether a chemical plant should be built requires extensive research in many different areas. This process is even more difficult when the plant uses new technology and is configured differently than those previously constructed. In this case, a continuous process is used with a reactor, the airlift fermenter, that has not been previously used in this capacity. With this study, some of the parameters were found to be more important in reactor performance than others. The parameters that influenced the reactor output the greatest are the ones to be studied while the others can be neglected while in pursuit of the first group.

Of the various cell and reactor parameters studied, some were more important to the reactor output and conversion than others. The real system uses the Set 1 Monod constants. These were used in the models discussed below.

Each of the yield coefficients played a direct role in reactor performance and process economics. The yield coefficients, $Y_{x/o}$ (Figure 4) and $Y_{p/s}$ (Figure 5), have a point at which further increases would be of little use. For $Y_{x/o}$, this point occurs at 1.65, and for $Y_{p/s}$ this point occurs at 2.2. The model of the carbon substrate predicts that a maximum carbon substrate concentration of 9 - 10 g/L should be used in the inlet feed (Figure 6). At this concentration both reactor output and conversion are at a maximum. For

the oxygen substrate concentration, which is controlled by $K_La$ (Figure 8), the maximum $K_La$ factor should be 220 $hr^{-1}$. At this point, further increases will not aid production and would waste both capital and production costs. The dilution rate of the reactor can be optimized to a maximum conversion (Figure 15). This occurs at 0.4 $hr^{-1}$. Running the reactor at a lower dilution rate will drive the conversion higher, but the reactor output will be lower. It will have to be decided which is optimal by economic studies.

Both the number of tanks-in-series model (Figure 11) and the recycle rate model (Figure 13) showed that little or no effect on reactor output was caused by changing these parameters. Research should be moved towards other areas in this case.

The economics show that using previously purchased equipment has a dramatic effect upon both capital investment (Figure 17) and Return on Investment (Figure 16). When using older equipment, profits are obtained at a much lower level of reactor output. The reactor output sizes all of the equipment used in the process. It is from this concentration that all prices are then calculated. Therefore, the reactor output is the most important variable to optimize.

The largest production cost was the carbon substrate. This may be optimized by using a cheaper compatible carbon source or by optimizing the various reactor parameters for increased conversion. According to the mathematical

studies, this process has an excellent chance of surviving economically using currently available technology.

## RECOMMENDATIONS

The airlift simulation programs show the dependence of both conversion and product output upon the various cell constants studied. Future research in genetic engineering should take into account this dependence. Genetic research should be directed toward increasing the amount of product output per unit of substrate input. Both Figures 4 and 5 show that research in this area would increase the amount of production per unit of cell.

Recycle of both cell and substrate is another area where reactor output could be increased. Instead of separating the cells and substrate and subsequently sending them to waste treatment, they should be recycled back into the reactor. Increasing cell concentration in the reactor through recycle will allow higher dilution rates to be used. Recycling the substrate back into the reactor will save money spent on substrate and make the process more profitable. Of course, the costs of separation for the substrate and cells must be taken into account. Yet a process that uses this type of recycle would benefit greatly both production and profits.

The use of pure sugars within the reactor is expensive. Various carbon sources on the lab side should be studied to see if a less expensive alternative can be found. *B. stearothermophilus* can use various carbon sources and doing so will drop the minimum production concentration output needed for profit even further.

The most expensive part of the capital investment and a large portion of the production costs involves separation of the alpha-amylase from the broth. This study suggests that ultra-filtration be used because of its wide acceptance throughout the chemical industry, and also the various size and cost factors were easily found. It does not necessarily make it the best method. A new method, using ion exchange gels which selectively bind to the alpha-amylase, are filtered and can then be easily unbound, has been discovered.[93] Although not implemented in industry as of yet, this method could replace the ultra-filtration step with a much less expensive filtration or centrifugation step.

Future research in the area of cell immobilization shows promise in increasing enzyme production. Chevalier[91] et. al. presents some interesting research in the cell immobilization of *B. subtilis*. This microorganism was suspended in carrageenan gel. The growth of the cell and the production of the alpha-amylase enzyme were studied in a simulated airlift fermenter. Results of 40-70% increase of alpha-amylase production and doubling of the bacterial density were reported with the cell immobilization.

There are many other research projects that attempt to increase the production of alpha-amylase or the growth of *B. stearothermophilus*. One example, Srivastava[92] et. al., studied the effects of different carbon substrates on alpha-amylase production and the growth of *B. stearothermophilus*. Starch was

found to induce alpha-amylase production, while glucose and maltose repressed the production. Different chemicals added to the broth either increased or decreased the production of alpha-amylase.

Further work must be completed in the lab to confirm the computer simulations. Both lab scale and pilot plant scale processes must be set up using the airlift fermenter in a continuous process.

# APPENDICES

# APPENDIX A

## APPENDIX A

### Derivation of Yield Coefficient, $Y_{p/o}$

The literature references were used for all constants in the mathematical models except for $Y_{p/o}$. Since this yield coefficient was unavailable, an electron balance and other calculations were performed which gave an estimate that was used in the computer simulations.

An electron balance allows the determination of the number of electrons reacted with cells or enzymes. Using this data it is possible to solve for the unknown yield coefficient. Since each atom has a certain number of reactive electrons, an electron content can be found for any compound. For biological compounds, there are four dominant atoms. Hydrogen and carbon have positive potentials having the ability to donate one and four electrons, respectively. Oxygen and nitrogen can accept from another atom two and three electrons, respectively. Those atoms that accept electrons have, by convention, negative numbers for their electronic content.

There are two methods of finding the electron content of a compound. The first involves solving the respiration equation for the unknown:

Glucose + Oxygen + Nitrogen --> Cell mass + Enzyme Mass + Carbon Dioxide + Water

$$\alpha C_6H_{12}O_6 + \beta O_2 + \Gamma NH_3 \rightarrow \pi CH_xO_yN_z + \sigma CH_aO_bN_c + \mu CO_2 + \tau H_2O$$

If the coefficients are known and either the cell or enzyme composition is known then it is possible to solve for the electron content of the other compound. In this case the cell composition is known to be:

$$CH_2O_{0.5}N_{0.25}$$

which is the composition of an "average" bacteria.[50] However, the coefficients of the chemical equation are unknown. If they were known then it would be possible to solve for the electron content of the enzyme.

To solve for the electron content of the enzyme, it was assumed that the enzyme had a similar composition to that of the *E. coli* protein. Both are large proteins. Using the amino acid composition structure of the *E. coli* bacteria along with the frequency of each amino acid, the electron content for the protein was found. The average electron content was found by:

1) Calculating the electron content for each amino acid found in *E. coli*.
2) Multiplying this specific electron content by the frequency of the amino acid in the protein then dividing by the total number of amino acids

This average electronic potential was found to be 3.87.

A specific molecular weight was found for each of the amino acids based upon the compound having only one carbon atom. The average molecular weight of the protein was found by following the same method used above and was calculated to be 25.5.

Equations were derived for the yield coefficients based upon the above calculated numbers. Three yield coefficients can define all others. An equation was derived which used the above calculations and two other yield coefficients to derive the third. (Note: All yield coefficients are in g/g units).

Deriving $Y_{x/s}$:

$$Y_{x/s} = \frac{\text{g cells produced}}{\text{g substrate used}} = \frac{\text{carbon moles cells produced}}{\text{moles substrate used}} \cdot \frac{\frac{\text{moles substrate}}{180\text{ g glucose}}}{\frac{\text{C-mole cells}}{25.5\text{ g cells}}}$$

$$= \left(\frac{\pi}{\alpha}\right)(25.5/180) = .141\left(\frac{\pi}{\alpha}\right)$$

Molecular weight of 1 carbon mole of cell = $C_1H_2O_{.5}N_{.25}$

$= (1)(12) + (2)(1) + .5(16) + .25(14)$

$= 25.5$

Deriving $Y_{x/o}$:

$$Y_{x/o} = \frac{\text{g cells produced}}{\text{g } O_2 \text{ consumed}} = \frac{\text{carbon moles cells produced}}{\text{moles oxygen used}} \cdot \frac{\frac{\text{mole } O_2}{32\text{ g } O_2}}{\frac{\text{C mole cell}}{25.5\text{ g cell}}}$$

$$= \frac{\pi}{\beta}\left(\frac{25.5}{32}\right) = .791\left(\frac{\pi}{\beta}\right)$$

Molecular weight of 1 carbon mole enzyme = $(1)(12) + a(1) + b(16) + c(14)$

Deriving the unknown $Y_{p/o}$:

$$Y_{p/o} = \frac{\text{g enzyme produced}}{\text{g } O_2 \text{ consumed}} = \frac{\text{carbon moles product produced}}{\text{moles } O_2 \text{ used}} \cdot \frac{\frac{\text{mole } O_2}{32\text{ g } O_2}}{\frac{\text{C-mole enzyme}}{\text{M.W. enzyme}}}$$

$$= \left(\frac{\text{M.W. enzyme}}{32\text{ g}}\right)\left(\frac{\sigma}{\beta}\right)$$

Electron Balance can now be used to solve for coefficient of enzyme:

Glucose + Oxygen = cells + enzyme

$$24\alpha + (-4)\beta = 4.2\pi + \sigma(\xi)$$

Where $\xi$ = electron potential of enzyme

$$\sigma = \frac{24\alpha - 4\beta - 4.2\pi}{}$$

$$Y_{p/o} = \frac{\text{M.W. enzyme}}{32\,\xi}\left(24\frac{\alpha}{\beta} - 4.2\frac{\pi}{\beta} - 4\right)$$

M.W. enzyme and $\xi$ given previously

# APPENDIX B

## APPENDIX B

### Derivation of Oxygen and Carbon Substrate Mass Balances

Derivation of carbon substrate Balance:

$$D(S_F - S) - \frac{\mu}{Y_{x/s}} x = \phi \quad \text{where } \mu = \mu_{max}\left(\frac{S}{K_s + S}\right)\left(\frac{O}{K_o + O}\right)$$

$$D(S_F - S) - \frac{\mu_{max}}{Y_{x/s}}\left(\frac{S}{K_s + S}\right)\left(\frac{O}{K_o + O}\right)x = \phi \qquad \xi = \frac{\mu_{max}}{Y_{x/s}}\left(\frac{O}{K_o + O}\right)x$$

$$D(S_F - S) - \xi\left(\frac{S}{K_s + S}\right) = \phi$$

$$DS_F(K_s + S) - DS(K_s + S) - \xi S = \phi$$

$$-DS^2 + (DS_F - DK_s - \xi)S + DS_F K_s = \phi$$

$$A = -D; \quad B = DS_F - DK_s - Y_{s/x}\mu_{max}\left(\frac{O}{K_o + O}\right)x$$
$$C = DS_F K_s$$

$$\therefore S = \frac{-b \pm \sqrt{b^2 - 4AC}}{2a}$$

---

Derivation of Oxygen Balance:

$$D(O_F - O) = \frac{\mu}{Y_{NO}} x = \phi \quad \text{where } \mu = \mu_{max}\left(\frac{S}{K_s + S}\right)\left(\frac{O}{K_o + O}\right)$$

with <u>K la</u> effects $O_2$ input has 2 sources:

$$D(O_F - O) - \frac{1}{Y_{x/O}}\mu x + kla(O^* - O) = \phi$$

$$DO_F - DO - Y_{O/x}\mu x + kla\,O^* - kla\,O = \phi$$

$$D O_F - D_O - Y_{o/x}\mu_{max}\underbrace{\left(\frac{S}{K_s+S}\right)\left(\frac{O}{K_o+O}\right)}_{\xi} X + k_la\,O^* - k_la\,O = \phi$$

$$(K_o+O)D S_F - [(K_o+O)D_O - Y_{o/x}\mu_{max}\xi_O X + (K_o+O)k_la\,O^* - (K_o+O)k_laO = \phi$$

$$DK_oO_F + OO_FD - K_oDO - DO^2 - Y_{o/x}\mu_{max}\xi_O X + K_o k_la\,O^* + k_la\,OO^* -$$

$$K_o k_la\,O - k_la\,O^2 = \phi$$

$$-\underbrace{(O + k_la)}_{a}O^2 + \underbrace{[O_FD - K_oD - Y_{o/x}\mu_{max}\xi_X + k_la\,O^* - K_o\,k_la]}_{b}O$$

$$+ \underbrace{[DK_oO_F + K_o\,k_la\,O^*]}_{c} = \phi$$

"$[O] = \quad -b \pm \sqrt{b^2 - 4AC}$"

$$a = -(D + k_la)$$

$$b = [O_FD - K_oD - Y_{o/x}\mu_{max}\left(\frac{S}{K_s+S}\right)X + k_la\,O^* - K_ok_la]$$

$$c = (DK_oO_F + K_o\,k_la\,O^*)$$

$O^*$ = interfacial $O_2$ Conc.

All others are those stated previously.

# APPENDIX C

## APPENDIX C

### Price Equations for Capital Investment

Sterilization Section:

Main & Preheater: (Peters et. al. P.670)

$$\$ = 10^{(2.322 + 0.6835 \cdot \log(Area_{ft2}))}$$

Pipe: (Peters et. al. P. 532)

$$\$ = 120 * length_{ft}$$

Tanks: (Peters et al. P. 591)

$$\$ = 10^{(2.5944 + 0.51436 \cdot \log(Gallons))}$$

Agitators: (Peters et al. P.591)

$$\$ = 10^{(3.041 + 0.57 \cdot \log(HP))}$$

Pumps: (Peters et al. P.557)

$$\$ = 10^{(1.661 + 0.4056 \cdot \log(dP_{PSI}))}$$

Air compressor: (Peters et. al. P.559)

$$\$ = 10^{(1.661 + 0.7558 \cdot \log(ft^3/min))}$$

Fermentors: (Demain et. al P.373)

$$\$ = 10^{(4.9540 + 0.3539 \cdot \log(m^3))}$$

Centrifuge: (Demain et al. P.374)

$$\$ = 10^{(1.6722 + 0.76352 \cdot \log(ft^3/min))}$$

Ultrafilter: (Demain et. al. P.374)

$$\$ = 10^{(3.1987 + 0.87653 \cdot \log(ft^3/min))}$$

# APPENDIX D

## APPENDIX D

### Proof of Conversion Fall Off for Low Substrate Concentrations

Figure 7 Illustrates an important discovery. It shows that the conversion of the carbon substrate reaches a maximum near 3 g/L and drops to zero at very low concentrations. The importance of these findings have been previously explained. The following is a mathematical proof that the substrate conversion does go to zero as the concentration also goes to zero. Oxygen influence has been neglected for simplicity and because carbon is the limiting substrate at these low concentrations.

$-r_s = D\Delta S \propto \left(\frac{g}{L \cdot S}\right)$ · $r_s$ = substrate rate use

$-r_s = D(S_i - S_o)$

$= \mu max \left(\frac{s}{K_s + S}\right) \times Y_{s/x}$

$\therefore \Delta s = (S_i - S_o) = \frac{-r_s}{D}$

constant 'K'

$\Delta S = \frac{-r_s}{D} = \frac{\mu_{max} S}{D(S+K_s)} \left[ Y_{x/s} (S_F - DK_s / \mu max - D) \right]$

conversion : $\frac{S_{in} - S_{out}}{S_{in}} = \frac{\Delta S}{S_{in}}$ $\therefore$ conversion $\propto \Delta S$

Take:

$\lim_{S \to 0} \Delta S \longrightarrow \frac{\mu_{max} S}{D(S+K_s)} K = 0$

Since $\Delta S$ is proportional to conversion, as $\Delta S \to 0$ so does conversion and reaction output.

# APPENDIX E

## APPENDIX E

## Base Case Studies

### Monod Constants: Set 1

| CSTR | SUBSTRATE | PRODUCT | CELL | OXYGEN |
|---|---|---|---|---|
| INLET | 7.22500 | 0.82140 | 0.91575 | .00333333 |
| 1 | 6.43546 | 1.05511 | 1.17630 | .00019671 |
| 2 | 5.64828 | 1.28813 | 1.43607 | .00012205 |
| 3 | 4.85681 | 1.52241 | 1.69724 | .00008954 |
| 4 | 4.06279 | 1.75745 | 1.95927 | .00007057 |
| 5 | 3.26710 | 1.99298 | 2.22185 | .00005818 |
| 6 | 2.47022 | 2.22886 | 2.48481 | .00004948 |
| 7 | 1.67247 | 2.46501 | 2.74807 | .00004305 |
| 8 | 1.67230 | 2.46506 | 2.74813 | .00000000 |
| 9 | 1.67230 | 2.46506 | 2.74813 | .00000000 |
| 10 | 1.67230 | 2.46506 | 2.74813 | .00000000 |

...INITIAL CONDITIONS...

| | | | | | |
|---|---|---|---|---|---|
| DRAFT REGION ENDS AFTER TANK NUMBER | 7 | | DRAFT/OVERALL VOL. RATIO | | .667 |
| DIL. RATE | 0.40 | | MU MAX | 2.10 | |
| SUBSTRATE FEED | 10 | | O2 FEED | .00500 | |
| Yxs | 0.330 | Ypo | 1.220 | Yxo | 1.360 |
| DRAFT KLA | 200.000 | | ANNULAR KLA | 0.000 | |
| Ks | .00250 | | Ko | .0001140 | |
| RECYCLE RATIO | .500 | | FINAL ITERATED CONVERSION % | | 83.24999 |
| OVERALL DRAFT CONVERSION % | 83.275 | | PRODUCTIVITY | 0.9860 | |

## Monod Constants: Set 2

| CSTR | SUBSTRATE | PRODUCT | CELL | OXYGEN |
|---|---|---|---|---|
| INLET | 8.84167 | 0.34287 | 0.38225 | .00333333 |
| 1 | 8.74837 | 0.37049 | 0.41304 | .00377290 |
| 2 | 8.64854 | 0.40004 | 0.44598 | .00367842 |
| 3 | 8.54232 | 0.43148 | 0.48103 | .00354274 |
| 4 | 8.42962 | 0.46484 | 0.51822 | .00340154 |
| 5 | 8.31035 | 0.50014 | 0.55758 | .00325813 |
| 6 | 8.18445 | 0.53741 | 0.59913 | .00311367 |
| 7 | 8.05194 | 0.57663 | 0.64286 | .00296923 |
| 8 | 7.59380 | 0.71225 | 0.79404 | .00252824 |
| 9 | 7.08129 | 0.86396 | 0.96317 | .00213113 |
| 10 | 6.52282 | 1.02927 | 1.14747 | .00179475 |

...INITIAL CONDITIONS...

| | | | | | |
|---|---|---|---|---|---|
| DRAFT REGION ENDS AFTER TANK NUMBER 7 | | | | DRAFT/OVERALL VOL. RATIO | .667 |
| DIL. RATE | 1.20 | | MU MAX | 2.10 | |
| SUBSTRATE FEED | 10 | | O2 FEED | .00500 | |
| Yxs | 0.330 | Ypo | 1.220 | Yxo | 1.360 |
| DRAFT KLA | 200.000 | | ANNULAR KLA | 200.000 | |
| Ks | .20000 | | Ko | .0020000 | |
| RECYCLE RATIO | .500 | | FINAL ITERATED CONVERSION % | | 34.75000 |
| OVERALL DRAFT CONVERSION % | | 19.481 | PRODUCTIVITY | | 0.6920 |

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