

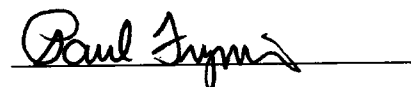
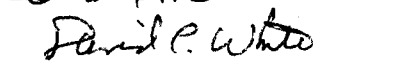
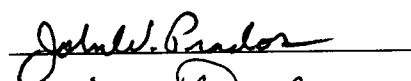
To the Graduate Council:

I am submitting herewith a dissertation written by May Yongmei Sun entitled "Process Development and Modeling of Fluidized Bed Reactor with Co-immobilized Biocatalyst" I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemical Engineering.



Paul R. Bienkowski, Major Professor

We have read this dissertation
and recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor and
Dean of The Graduate School

**Process Development and Modeling of
Fluidized Bed Reactor with Co-immobilized
Biocatalyst for Fuel Ethanol Production**

**A Dissertation
Presented for the
Doctor of Philosophy Degree
The University of Tennessee, Knoxville**

**May Yongmei Sun
December 1997**

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DEDICATION

This dissertation is dedicated to my parents

Mr. Zhe Sun and Mrs. Chenhui Li

孫哲先生和李成慧女士

Who have given me invaluable encouragement and support

ACKNOWLEDGMENTS

There are many people to whom I am grateful for making my time at the University of Tennessee so rewarding. I am particularly grateful to my dissertation committee at UT, Paul R. Bienkowski, John W. Prados, Paul D. Frymier, and D.C. White for their support and encouragement. They have all read a number of revisions of the dissertation and the merit of this work is due in no small part to their suggestions.

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ABSTRACT

This research focuses on two steps of commercial fuel ethanol production processes: the hydrolysis starch process and the fermentation process. The goal of this research is to evaluate the performance of co-immobilized biocatalysts in a fluidized bed reactor with emphasis on economic and engineering aspects and to develop a predictive mathematical model for this system.

The productivity of an FBR is higher than productivity of a traditional batch reactor or CSTR. Fluidized beds offer great advantages over packed beds for immobilized cells when small particles are used or when the reactant feed contains suspended solids. Plugging problems, excessive pressure drops (and thus attrition), or crushing risks may be avoided. No mechanical stirring is required as mixing occurs due to the natural turbulence in the fluidized process.

Both enzyme and microorganism are immobilized in one catalyst bead which is called co-immobilization. Inside this biocatalyst matrix, starch is hydrolyzed by the enzyme glucoamylase to form glucose and then converted to ethanol and carbon dioxide by microorganisms. Two biocatalysts were evaluated : (1) co-immobilized yeast strain *Saccharomyces cerevisiae* and glucoamylase. (2) co-immobilized *Zymomonas mobilis* and glucoamylase. A co-immobilized biocatalyst accomplishes the simultaneous saccharification and fermentation (SSF process). When compared to a two-step process involving separate saccharification and fermentation stages, the SSF process has productivity values twice that given by the pre-saccharified process when the time

required for pre-saccharification (15-25 h) was taken into account. The SSF process should also save capital cost.

The information about productivity, fermentation yield, concentration profiles along the bed, ethanol inhibition, et al., was obtained from the experimental data. For the yeast system, experimental results showed that: no apparent decrease of productivity occurred after two and half months, the productivity was 25-44g/L-hr (based on reactor volume), the average yield was 0.45 g ethanol/g starch, the biocatalyst retained physical integrity and contamination did not affect fermentation. For the *Z. mobilis* system the maximum volumetric productivity was 38 g ethanol/L-h, the average yield was 0.51 g ethanol/g starch and the FBR was successfully operated for almost one month.

In order to develop, scale-up and economically evaluate this system more efficiently, a predictive mathematical model that is based on fundamental principles was developed and verified. This model includes kinetics of reactions, transport phenomena of the reactant and product by diffusion within the biocatalyst bead, and the hydrodynamics of the three phase fluidized bed. The co-immobilized biocatalyst involves a consecutive reaction mechanism. The mathematical descriptions of the effectiveness factors of reactant and the intermediate product were developed. Hydrodynamic literature correlations were used to develop the dispersion coefficient and gas, liquid, and solid holdup. The solutions of coupled non-linear second order equations for biocatalyst bead and reactor together with the boundary conditions were solved numerically. This model gives considerable information about the system, such as concentration profiles inside both the beads and column, flow rate and feed

concentration influences on productivity and phase hold up, and the influence of enzyme and cell mass loading in the catalyst. This model is generic in nature such that it can be easily applied to a diverse set of applications and operating conditions.

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CHAPTER 1 BACKGROUND

1. 1 Introduction

Fermentative ethanol as fuel is becoming a more attractive option than gasoline fuels in many countries. The reason for this change is that ethanol can be produced from renewable resources, reduce the nation's dependence on foreign oil supplies, and improve air quality. Recent research advances promise to improve economics. In the U.S., ethanol plays a positive role in environmental, energy, trade, and economic policy.

Fuel ethanol protects and enhances environmental resources. An estimated 82% of the carbon monoxide, 43% of the reactive organic gases (precursors to ozone), and 57% of the nitrogen oxides in domestic cities are emitted from the use of petroleum-based transportation fuels. Ethanol is called a clean air renewable oxygenate because it provides significant reductions in the levels of exhaust like volatile organic compounds (VOC) and greenhouse gas emissions compared to gasoline. Because ethanol burns cleanly, it is an excellent method for reducing emissions from the transportation sector. In fact, ethanol is listed as a clean alternative fuel in both the 1990 Clean Air Act Amendments and the 1992 Energy Policy Act. According to the Renewable Oxygenate Requirement (ROR) proposed by the Environmental Protection Agency (EPA), renewable oxygenates such as ethanol will be required to be used in at least 30% of the Reformulated Gasoline Program (RFG). Furthermore, the carbon dioxide (CO₂) advantage of ethanol fuel is of particular importance. The threat of global climate

change resulting from buildup of CO₂ and other greenhouse gases may make it necessary for the world to move away from fossil fuels and towards energy forms, such as biomass, that result in little or no net additions of CO₂ to the atmosphere which is also called the CO₂ recycling effect . In fact, ethanol yields 27% less CO₂ than gasoline. According to the EPA, the market for ethanol resulting from the ROR was approximately 630 million gallons in 1995, and will grow as the RFG program expands (Bothast, 1994).

Further, fuel ethanol increases the nation's energy security. The transportation sector depends on petroleum for 97% of its energy needs. The largest contributor to the U.S. balance of trade/balance of payments problem is imported oil. In 1995, the oil trade deficit is expected to be \$66 billion. Nearly half of our oil is being imported, and the expectation is that this fraction will continue to increase in the future. Estimates by DOE indicate that by 2020, up to 74% of the oil will be supplied by imports. In that same time frame, the U.S. oil trade deficit is projected to be \$173 billion. Numerous studies have concluded that our heavy reliance on imported oil is becoming a serious energy security issue, and it is clear that this situation will get worse with time. Key among the reasons for rising oil imports is the limited domestic resource base of crude oil. Additionally, global oil resources are finite and not renewable, oil quality is deteriorating (becoming heavier and with more sulfur), while costs are expected to increase. An important way to stem the tide of imports is to substitute plentiful domestic energy resources, such as ethanol, for petroleum fuels in the transportation sector. The substitution of ethanol for imported energy will provide a net energy savings of more than 103 trillion BTUs -- or the equivalent of 17.7 million barrels of crude oil.

The development of fuel ethanol industry will also create jobs and new markets. These jobs include many domestic jobs in agribusiness, engineering, lending institutions, construction, fuel production and distribution. By using ethanol, consumers will obtain some economic benefits. In 1994, there were 16 states which offered ethanol fuel tax exemptions (Shapouri and Duffield, 1994). With this incentive, the price of ethanol is cheaper. Increasing available oxygenate supplies by 30 percent will reduce the consumer's cost of reformulated gasoline between \$310 and \$470 million each year. The economic benefit attributable to the ROR equates to more than \$265 per barrel of ethanol used (Bothast, 1994). Besides the cost effect, recent research on fermentative ethanol has indicated a potential for very favorable net energy yields (Sinor, 1993). Alexandrian and Schwalm also proved that at a high engine compression ratio, ethanol produced considerably more power than gasoline (Alexandrian and Schwalm, 1992).

Approximately 1.1 billion gallons of ethanol are produced from grain each year (Carlson, 1994). For the improvement of the competitiveness of ethanol as a fuel or fuel additive, technologies for lowering costs associated with ethanol production are in need of development. The goal of the ethanol fermentation industry is to lower the cost of ethanol. Much fundamental research has been conducted in the laboratory at bench or pilot scales and also some economic assessments have been completed. Technology changes will continue to reduce the cost of fuel-ethanol production. "As of 1992, industry analysts have begun to talk about future ethanol costs in the range of 29-35 cents (Canadian) per liter" (Ingledew, 1993).

1.2 Objectives

All commercial ethanol fermentation processes are composed of several steps (Keim, 1983):

- (1) preparation of the raw material;
- (2) hydrolysis, in the case of starches and cellulose;
- (3) fermentation;
- (4) recovery and concentration of alcohol;
- (5) recovery of by-products.

There are many different ways of performing each of these steps and variations within each alternative. Current processes need to be developed because of the increasing market demanding. The further improvements are necessary to maintain the ethanol economic compatibility. There are many opportunities for improvements in these process steps.

This research will focus on the hydrolysis starch process (step 2) and the fermentation process (step 3). The goal is evaluating the performance of the co-immobilized yeast-glucoamylase and *Zymomonas mobilis*-glucoamylase biocatalysts in the fluidized bed reactor with emphasis on economic and engineering aspects using both experimental and modeling. The biocatalyst will accomplish the simultaneous saccharification and fermentation. The objectives include the following parts:

- Experiments

Bench scale fluidized beds with two kinds of co-immobilized biocatalysts were operated at Oak Ridge National Laboratory (ORNL). The two biocatalysts are :

- (1) co-immobilized yeast strain *Saccharomyces cerevisiae* and enzyme glucoamylase
- (2) co-immobilized *Zymomonas mobilis* strain NRRL - B - 14023 and glucoamylase.

The raw material is soluble starch. The information about productivity, fermentation yield, concentration profiles along the bed, etc., were obtained from the experimental data.

- Mathematical modeling

The mathematical model for the co-immobilized biocatalyst fluidized bed reactor was developed and refined. This model includes kinetics of reactions, transport phenomena of the reactant and product by diffusion within the biocatalyst bead, and the hydrodynamics of the three phase fluidized bed. These coupled nonlinear second order equations together with the boundary conditions were solved numerically. The comparison of the predictive solution of the model with experimental data will be discussed.

CHAPTER 2 LITERATURE REVIEW

2.1 Raw Material

The majority of the ethanol cost comes from its feedstock. Raw material costs typically make up 55-75% of the final alcohol selling price (Maiorella, B. L. 1985). According to Esquivel et al. (1994), each \$10 per ton in raw material cost is roughly equivalent to \$0.10 per gallon in the required ethanol selling price. The great challenge of the future is determining how to use raw materials more effectively and what materials have the potential for adding the most quality to life while optimizing energy security and minimizing environmental impact (Schilling, 1994).

Raw materials for fermentative ethanol production can be divided into three classifications by carbohydrate type: saccharine materials, starchy materials, and lignocellulosics (Maiorella, 1985). The process of fermentation requires a fermentable sugar form such as simple hexose and some disaccharides. Saccharine materials can be converted to ethanol easily because they have available sugar in a fermentable form. Saccharine materials require the least extensive preparation, but are generally the most expensive to obtain. As an alternative, cellulose is available as cheap waste residue, but requires the most extensive and costly preparation process to convert to the fermentable form. Although new techniques for reducing cellulose preparation costs have been studied for years, no full-scale production plant dependent on cellulose now exists (Ingledew, 1993). Compared to saccharine

materials and cellulose, the starches are the most profitable fermentation material in U.S.. Starch materials are cheaper to obtain than saccharine materials and the techniques to convert the starch to fermentable sugars are economically acceptable.

Starchy raw materials include cereal grains, starch root plants, and some cacti. Corn is the major grain used for alcoholic fermentation. About 95% percent of the ethanol produced in the U.S. is corn based (Shapouri, 1994). Fractionation of the corn kernel, or milling, is a very important process. Traditional grain processing was via batch methods, but these are now often replaced by fully continuous processes (Wilkie and Prochaska, 1943; Stark, 1954). Currently, there are two main methods used to produce ethanol from corn. The first is known as the non-fractionation process, in which the whole kernels are ground and the ground corn, without any separation of components, is sent to the fermentor. The second method is to mill the corn to achieve a fractionation of the corn kernel into starch, protein (gluten meal), fiber (gluten feed), and oil. The starch is used as the primary energy source in ethanol fermentation, with the remaining fractions sold as by-products (Eckhoff, 1991). There are two milling methods that have currently been used, wet milling and dry milling. In the U.S., about 60 percent of ethanol is made from the wet-milling process and 40 percent by the dry-milling (Shapouri and Duffield, 1994). The details of these two process are given by Venkatasubramanian (1985).

2.2 Microorganism and Enzyme Used for Ethanol Fermentation

2.2.1 Microorganisms for Ethanol Fermentation

Many microorganisms have been tested for ethanol fermentation. Two microorganisms have been recognized as the most effective: yeasts and *Zymomonas mobilis*. Yeasts are without doubt a very important group of microorganisms commercially exploited by man. There are centuries of experience of using yeast to produce ethanol, although the process of ethanol production and the regulation of these processes are still not entirely clear. At present, only *Saccharomyces cerevisiae* and related species are of major industrial importance. *Saccharomyces cerevisiae* has the ability to take up and ferment a wide range of sugars, for example, sucrose, glucose, galactose, maltose, and maltotriose. On the other hand, the use of bacteria for the production of industrial ethanol has only recently been receiving attention. For example, it has been shown that *Zymomonas mobilis* can convert glucose to ethanol with higher specific rates of glucose uptake than *S. cerevisiae*, and higher yield. Its potential use for industrial fermentations still needs further study.

Yeast

Yeasts are the primary organism used for ethanol fermentation and can be purchased commercially. Yeast metabolizes glucose via the Embden-Meyerhof-Parnase (EMP) pathway. Yeast has a tight coupling between the energy-demanding and energy-

producing processes. Maiorella (1985) listed seven advantages by using yeasts as an ethanol fermentation microorganism:

1. High growth and fermentation rate allows the use of smaller fermentation equipment;
2. High ethanol yield gives high efficiency of ethanol production ;
3. High ethanol and glucose tolerance allows the conversion of concentrated feeds to concentrated products, reducing energy requirements for distillation and stillage handling.
4. Osmotolerance allows the handling of relatively 'dirty' raw materials, such as blackstrap molasses with its high salt content. Osmotolerance also allows the recycling of a large portion of stillage liquids, thus reducing stillage handling costs.
5. Low pH fermentation optimum combats contamination by competing organisms.
6. High fermentation optimum temperature increases the temperature tolerance which simplifies fermentor cooling.
7. General hardiness under physical and chemical stress allows yeast to survive both the ordinary stress of handling (such as centrifugation) as well as the stresses arising from a plant upset.

Yeast requires necessary nutrients to grow, and to stimulate fermentation (Ingledew et al., 1985). For many years, researchers have carefully studied yeast nutrition and given recommendations (Hodge and Hildebrandt, 1954; Agenbach, 1977; Vos et al., 1979, 1980; Monk, 1982; Ingledew and Kunkee, 1985, Ingledew, 1987; Cartwright et al., 1989). However, the optimum and cost-effective dosage levels are not easily determined. Among food for yeast, yeast extract is available to the laboratory

worker. U.S. Pharmacopoeia XVIII described yeast extract as a peptone-like substance derived from yeast cells. In industry, light steep water and corn steep liquid are used instead of yeast extract.

During the yeast fermentation process, there are several factors which in some way affect the productivity of ethanol: sugar concentration, ethanol concentration, oxygen, pH, and temperature. Hexose sugar (glucose, fructose, galactose or maltose) is the primary reactant in the yeast metabolism. At very low substrate concentrations (below about 3 g/L), the yeast is starved and productivity decreases (Levenspiel, 1980; Ciftci, 1983). At higher concentrations, a saturation limit is reached so that the rate of ethanol production per cell is essentially at its maximum at 150 g/L sugar concentration. Above 150 g/L, catabolic (sugar) inhibition of enzymes in the fermentative pathway becomes important, and the conversion rate is slowed (Holzer, 1986; Wang et al., 1979).

High ethanol tolerance is a an advantage for industrial strain yeast because ethanol is toxic to yeast. The inhibitory effect of ethanol is generally negligible at low alcohol concentrations (less than 20 g/L) but increases rapidly at higher concentrations. For most strains, ethanol production and cell growth are inhibited completely at above 105 -115 g ethanol/L (Novak et al., 1981, Luong, 1985). Ethanol inhibition is linked to the maximum intracellular ethanol concentration, which is related to the inhibition and denaturation of important glycolytic enzymes, as well as to modification of the cell membrane.

Although the high degree of aerobic metabolism, which utilizes sugar substrate but produces no ethanol, should be avoided, it has been found that trace amounts of oxygen may greatly stimulate yeast fermentation. Oxygen is required for yeast growth as a building block for the biosynthesis of polyunsaturated fats and lipids required in mitochondria and the plasma membrane (Haukeli and Lie, 1971). Trace amounts of oxygen are adequate and do not promote aerobic metabolism (Cysewski and Wilke, 1976a)

Yeasts show a broad pH optimum from at least pH 4-6 (Cysewski and Wilke, 1976b). Yeasts have a growth optimum temperature of 30-35°C (Gray et al, 1942). The optimum fermentation temperature at low alcohol concentrations is often up to 38°C (Temperatures between 40 and 42.5 °C for maximum specific alcohol productivity have been reported (Ciftci, 1983), but alcohol tolerance is improved at reduced temperatures (Hayashida and Ohta, 1981). Yeast can be stored inactive at low temperatures (above 0°C) and is readily revived (Stark, 1954).

***Zymomonas mobilis* versus Yeast**

The Gram-negative bacterium *Zymomonas mobilis* was discovered in the late 1970s as the fastest and most efficient ethanol producer. The original isolate of *Zymomonas mobilis* has been attributed to Lindner (1928) who isolated it from Mexican pulque and called it *Thermobacterium mobile*. It utilizes sucrose, glucose, or fructose as its sole carbon and energy source by the Entner-Doudoroff (ED) pathway. Although it

is classified as an anaerobic microorganism, *Zymomonas mobilis* grows under aerobic conditions (Doelle, 1993).

Zymomonas mobilis is unique in possessing the Entner-Doudoroff glycolytic pathway for glucose catabolism. This is of particular interest because this pathway for the breakdown of sugar is different from that of yeast (Embden-Meyerhof pathway) and yields only 50% of the energy associated with the conversion of glucose to ethanol in yeast. Consequently, *Zymomonas* is obliged to ferment twice as much sugar in order to obtain an equivalent amount of energy (energy is conserved as adenosine triphosphate or ATP) (Lawford, 1986).

Whereas yeast has a tight coupling between the energy-demanding and energy-producing processes, *Zymomonas mobilis* appears to have a less rigid control allowing the efficiency of energy utilization to vary between the conditions of "energetic uncoupled" and "energetic coupled". The ratio of biomass formed to substrate consumed is low in the energetic uncoupled condition while this ratio is high under energetic coupled growth. It is recognized that the "energetic uncoupled" can be exploited to maximize ethanol formation. In other words, yeast ethanol production is a growth-associated process, therefore high cell concentrations are required for high ethanol production rates. In contrast, *Zymomonas mobilis* shows no such dependence as the diagram of Lawford demonstrates (Figure 2.2.1). More recently, the relationship between uncoupled growth, glucose uptake rates, and ethanol production have been investigated in more detail (Lawford, 1986). There appear to

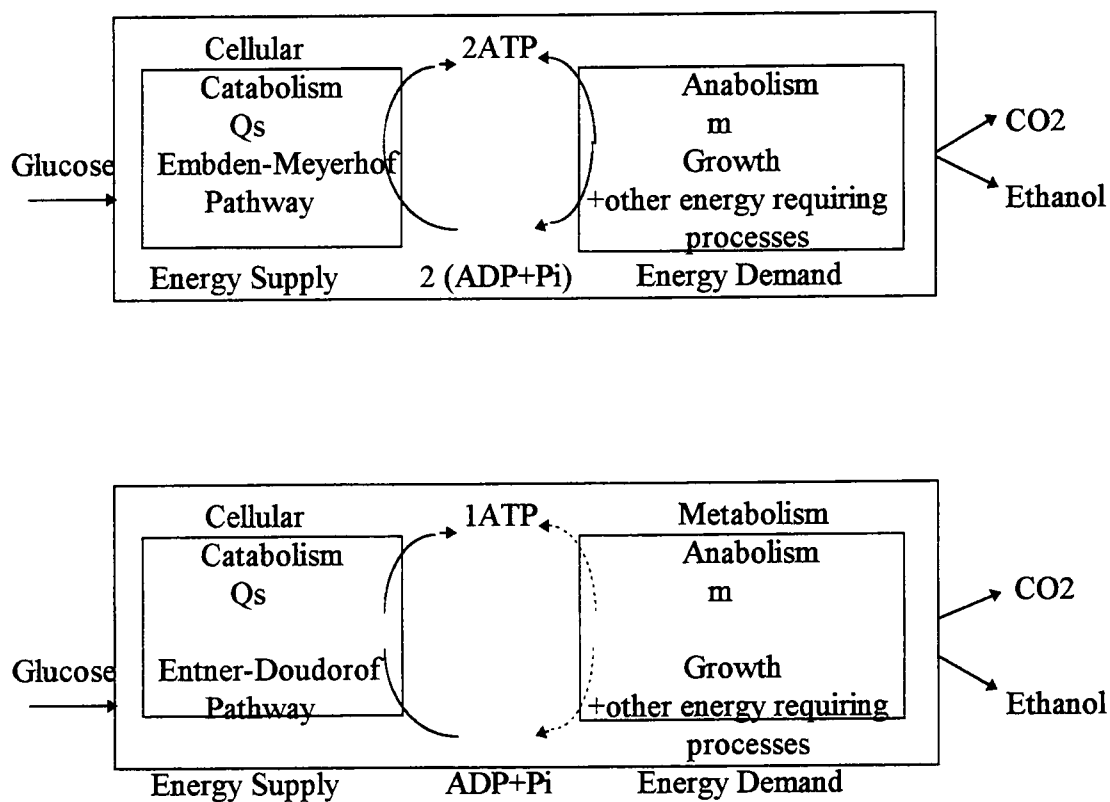


Figure 2.2.1 Diagrammatic representation of the relationship in yeast and *Zymomonas* between the metabolic cellular processes of energy supply and demand (Lawford, H.G. 1986)

be two general forms of uncoupled growth: first, an energetic uncoupling, where much of the energy generated is diverted away from growth caused by high maintenance energy requirements, that is, the nongrowth-associated component of metabolism [maintaining a proton gradient, macromolecule turnover, etc]. For instance, uncoupled growth in a batch culture is observed as a decrease in biomass yield, and a rise in specific substrate uptake rate; and second, the diversion of substrate carbon away from cellular macromolecule formation to endproduct formation. It cannot be assumed that the two forms of uncoupled growth occur independently and thus can be manipulated separately.

The optimum operating pH range and temperature for growth and ethanol production of *Zymomonas* were found to be 5.0-6.0 and 30-35°C (Benschoter, 1986) respectively. Nutritional requirements of *Zymomonas* are similar to yeast.

The advantages and disadvantages associated with *Zymomonas* are listed in the following paragraph (Lawford, 1986).

Advantages:

1. Energy uncoupled growth course, less cell mass produced, higher product yield and faster specific rate of alcohol production.
2. By-product formation is lower.
3. Flocculent strains are available.
4. Small amounts of oxygen are tolerable.
5. There is resistance toward many antibiotics.
6. Genetic manipulations are easier than in yeast.

Disadvantages:

1. The most serious disadvantage to using *Zymomonas* instead of yeast relates to its very restricted list of fermentable sugar substrates. Unlike yeasts which are known to be capable of producing ethanol from a relatively wide spectrum of feedstocks, *Zymomonas* is limited to glucose, fructose and sucrose (not maltose, lactose, galactose, cellobiose or pintos). In SSF processes, conditions need to be established so that the saccharification process (glucoamylase catalyzed conversion from starch to glucose) does not present a rate limitation, thereby realizing the potential for improved fermentation kinetics afforded by *Z. mobilis*.
2. Using *Zymomonas* to produce fuel ethanol is a new technology. *Zymomonas* is not produced commercially as a starter culture and results of fermentation trials are proprietary.

2.2.2 Enzyme Used for Ethanol Fermentation

Yeast only converts simple hexose sugar monomers (e.g. glucose and fructose) and some disaccharides (e.g. maltose and sucrose) to ethanol. *Zymomonas mobilis* even converts fewer sugar than yeast. No single yeast exists or bacteria exists which is able to utilize all possible substrates (Korhola, 1987). Neither *Saccharomyces* nor *Zymomonas* have the genes for amylase, cellulose, nor for degrading pentose. Native starch contains two types of macromolecules composed of glucose units (polysaccharides). One of the molecules, amylose, is linear and consists exclusively of α -D-(1-4) linked glucose units.

The second type of molecule, amylopectin (also the major component), is highly branched and contains α -D-(1-4) linked as well as α -D-(1-6) linked glucose units. (See Figure 2.2.2). The amylose is readily hydrolyzed to maltose, which can be converted to ethanol by *Saccharomyces* and *Zymomonas*. Amylopectin is broken down partly to maltose, but primary to branched nonfermentable dextrin. This dextrin cannot be directly converted to ethanol. The further breakdown of dextrin to maltose during fermentation will improve the fermentation efficiency. For conventional grain fermentation this final dextrin conversion, not the ethanol fermentation itself, becomes the rate limiting step in fermentation (Maiorella, 1985). There are two way to degrade the starch, enzyme and acid hydrolysis. Traditionally, starch was, and still is, hydrolyzed

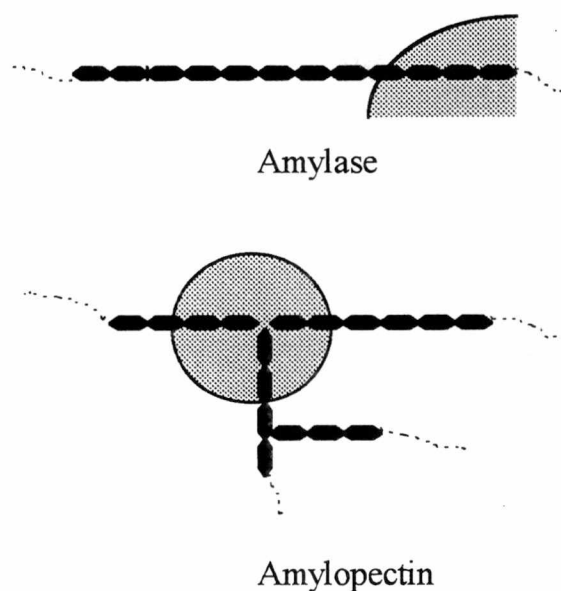


Figure 2.2.2 Structure of the amylose and amylopectin components of starch.

to low molecular-weight dextrans and glucose using acid, but enzymes have several advantages. First, the specificity of enzymes allows the production of sugar syrups with defined physical and chemical properties. Second, the reaction conditions of enzymatic hydrolysis are much milder. These mild reaction conditions result in few side reactions and less yield loss from sugar degradation (Nigam and Singh, 1995). Norman (1978) used submerged culture fungal amylase production to provide the highest activity glucoamylase enzyme solutions, which were added to the alcohol fermentation to promote dextrin conversion. Glucoamylase enzyme is also produced commercially from either *Aspergillus niger* (Pandey and Radhakrishnan, 1992) or *Rhizopus spp.* (Lyons, 1981). This enzyme is used for saccharification, resulting in a syrup of high glucose content (92 - 96%). In fact, the glucoamylase is capable of hydrolyzing the α -(1 $\rightarrow$ 6) bonds of amylopectin as well as the α -(1 $\rightarrow$ 4) bonds in both amylase and amylopectin. The disadvantage of most enzymatic grain-starch conversions in microorganism and enzyme co-immobilization systems is a prolonged fermentation time because of the slow hydrolysis of starch at temperatures tolerated by microorganism. Glucoamylase is most active near 60° C, and between pH 4 and 5; most yeasts and bacteria should be kept below 35°C to prevent thermal death.

Interestingly, several names have been used for glucoamylase, and this has created many misunderstandings in the past. Table 1 (shown below) is based on information from "Enzyme Nomenclature". This table includes the synonyms used in identifying glucoamylase.

Table 1. Glucoamylase Nomenclature

Name used in this thesis:	glucoamylase
Recommended name:	exo -1,4 - α - D-glucosidase
("Enzyme Nomenclature")	
E.C. number:	3.2.1.3
Systematic name:	1,4 - α - D-Glucan glucohydrolase
Other names:	Glucoamylase, Amyloglucosidase, γ - Amylase, Lysosomal, α - glucosidase, Acid maltase, Exo-1,4- α - glucosidase,

2.2.3 Immobilized cells and enzymes

Immobilized cells and enzymes have many practical applications. Ethanol fermentation is one of these applications using immobilization technique. Immobilized yeast are widely used in fermentations (Gencer, 1983; Furusaki, 1983; Garcia, 1989; Bravo, 1991; Norton 1995). The purpose of immobilization in fermentation is to retain high concentration of cells during continuous or semicontinuous operation and limit cell washout; in addition, it provides easy separation of the product and cell. Commercialization of the immobilized cell fermentation process should result in reduced sizing of fermentation tankage, economical reuse of the cells, elimination of the need for centrifuges, and higher rates of glucose conversion to ethanol. Immobilized enzymes have many applications, such as converting starch to glucose (Trevan, 1980).

In the ethanol fermentation process, increased fermentation capabilities have been demonstrated by immobilized microbes used in a continuous system. The cell immobilization technique results in increased activity because the higher cell density increases the rate of conversion of substrate to product. This also may result from a reduction in the ethanol concentration in the immediate environment, which would reduce the effect of ethanol intolerance. A number of reviews have been written on immobilized yeasts and the resultant continuous-fermentation technology (Kosaric, 1983; Linko 1984; Margaritis and Merchant, 1987; Linko, 1987). Results demonstrated a significant increase of yeast survival against ethanol for immobilized cells as compared to free cells. In contrast, no marked difference in heat resistance has been observed

between free and immobilized cells (Norton, 1995). Immobilized *Zymomonas* also had good performance in ethanol production. For instance, an ethanol productivity of 60 - 120 g ethanol/L/hr and an average yield of 0.49 g ethanol /g glucose or > 97% of theoretical was achieved (Davison, 1988). 99% of theoretical has also been reported (Grote, 1980; Scott, 1987).

The relatively high concentrations of enzyme in immobilized systems also permit the use of continuous reactors at relatively low temperatures with reasonably sized vessels. This results in the prolonged use of the enzyme, which decreases the overall cost of enzyme per unit weight of product. Significant and favorable considerations in the application of immobilized enzymes also include greater stability of the bound enzyme, enzymatic activity over broad ranges of pH and temperature, and adaptability of immobilized enzymes to a variety of specific process.

The two major factors of concern in immobilization of enzymes and cells are the choice of immobilization methods and the effect of immobilization on enzyme and cell activity. The methods available for immobilization can be grouped into three main classes: 1. adsorption on inert supports or ion-exchange resins, 2. gel entrapment, 3. covalent bonding to inert packing materials. Choice is governed by a number of factors, some of which will not be apparent until the procedure is tried (Trevan, 1980; Wingard 1976). The immobilization might affect the cell metabolism and enzyme kinetics. The polymer matrix provides the cells and enzymes with a microenvironment that may differ in many respects (e.g. pH, substrate concentration) from the solvent in which the immobilized cell and enzyme are suspended. To distinguish it from the

microenvironment of the cell and enzyme, the solvent outside the immobilization matrix is referred to as the "bulk phase," "bulk solution," or "macroenvironment". Of course substrate concentration is measured in the bulk phase. Some discussion of the immobilization effects on the microorganism and enzyme kinetics will be described in the mathematical model section.

There have been some studies on immobilized yeast, immobilized *Zymomonas* and immobilized glucoamylase with fluidized bed reactors. However, they were all independent single catalyst investigations. In other words, there is not a fermentation system using co-immobilized biobeads that includes both microorganism and glucoamylase in a fluidized bed reactor. Although suspended yeast - glucoamylase systems and immobilized yeast with suspended glucoamylase systems have been investigated, immobilized yeast-glucoamylase has not been reported. Lee and Woodward (1983) immobilized β -D-Glucosidase with *Z. mobilis* in calcium alginate which was used to convert cellobiose to ethanol. However, the use of a fluidized bed reactor with the *Zymomonas*- glucoamylase system is a new topic. The study of the co-immobilization with a fluidized bed reactor system is the main focus of this research. The co-immobilization system belongs to the simultaneous saccharification and fermentation (SSF) process. When compared to a two-step process involving separate saccharification and fermentation stages, the SSF process appears to be superior to the pre-saccharified process when the time required for pre-saccharification (15-25 hr) was taken into account, with productivity values twice those given by the two-step process. SSF process should save the pre-saccharification capital cost. Thus, the SSF process

would appear to offer significant commercial advantages over the pre-saccharification process for the production of alcohol by microorganism from starch. It is quite obvious that co-immobilization system will have more advantages over the system in which only cells are immobilized because of free enzyme loss if continuous runs of fermentation system are taken into account. In the separately immobilized system, glucose converted from starch in one matrix entrapping glucoamylase (GA) has to diffuse through another matrix where cells are immobilized and ethanol is produced. This would give the system more diffusion resistance than in the co-immobilized system (Kim, Lee, Abidin, 1988).

2.3 Fermentor Systems

Depending on design, fermentation makes up 10-25% of the total fixed capital cost of an ethanol plant. The fermentation process is central to the overall plant design. Traditionally, ethanol fermentation processes have been operated batchwise. Batch fermentation has been preferred over the years because it has been easy to operate. In addition, it was possible to use relatively unskilled labor, reduce capital costs, and utilize relatively simple processing technology. The disadvantage of batch fermentors is the cyclic operation, in which the cell culture experiences all the phases of the growth cycle. The inhibitory effect of ethanol on the yeast growth rate is apparent at a relatively low ethanol concentration. Hence, the cell concentration in a typical fermentation is very low, and consequently the ethanol productivity is as low as 2.2 g ethanol/L/ hr (Cysewski and Wilke, 1978).

Continuous fermentation, however, eliminates much of the unproductive downtime seen in batch processes, increases the productivity, provides stable steady state fermentation characteristics, and reduces labor and reduced cleaning expenses. However, some problems exist in the continuous process. Continuous stirred tank reactors (CSTRs) improve the apparent kinetics, but they continuously lose cells through the effluent. CSTRs operate at the final product and substrate concentration - in this case ethanol, which is inhibitory. Therefore, these fermentors must be operated with dilute substrate levels so as to maintain the ethanol concentration at a level which maximizes the ethanol productivity. In addition, the fermentation rates are low due to low bulk substrate glucose concentration and low cell density. Consequently, large fermentor volumes must be employed to produce large amounts of ethanol within a given time. Because of these disadvantages, true continuous fermentation is not yet practiced widely (Keim, 1983), even though Cysewski and Wilke (1978) and others have noted marked improvements in the productivity of single-stage continuous fermentation (82 g /L/ hr) when compared to batch fermentation (1-4 g/L/hr). Lyons (1981a), Kosaric et. al. (1983), Maiorella (1985) , and Margaritis and Merchant (1987) have described the various types of continuous systems, and they have indicated a number of advantages and disadvantages. Murtagh (1986) has indicated that only four major producers of ethanol in the USA have utilized semicontinuous fermentation for production of ethanol. All are maize wet-milling-based plants and are able to obtain a mash free from solid.

Fluidized bed reactors are attracting more and more people's attention. In order to overcome the continuous loss of cells through the effluent, the immobilization

technique is used in the fluidized bed process. The support matrix containing the immobilized cells is loosely packed into a column. The substrate stream is then passed into the bottom of the column at a sufficiently high flow rate. This lifts and mixes the immobilized cell particles within the column. Obviously the immobilized cell particles must have a higher density than the reaction medium, and the flow rate must not be so high that the particles are carried out of the top of the column. Fluidized beds offer great advantages over packed beds for immobilized cells when small particles are used or when the reactant feed contains suspended solids. Plugging problems, excessive pressure drops (and thus attrition), or crushing risks may be avoided. No stirring is required due to the natural turbulence in the fluidized process. Nguyen and Shieh (1992) used a fluidized bed reactor to conduct continuous ethanol fermentation by using immobilized yeast. The substrate was glucose. The yield they obtained was 0.36 g ethanol/ g glucose (71% of theoretical yield) at a dilution rate of 0.04/hr-0.14/hr. At a dilution rate of 0.15h, the ethanol yield declined steeply to 0.22 g-ethanol/g-glucose (44% of theoretical yield). Although the percentage of theoretical yield was low, they believe that the general performance of the system was stable and efficient over a period of days and high dilution rates. Davison (1988) conducted ethanol fermentation using *Zymomonas* in a fluidized bed with glucose as the raw material. Volumetric productivities of 50-120 g ethanol/L/hr and 97% of the theoretical yield have been achieved. Due to this and factors such as ease of maintenance, absence of sterilization requirements, and cell reuse, fluidized bed reactors using immobilized cells represent a potentially useful technology

for industrial ethanol fermentation. The table 2 list some information about ethanol productivity in various bioreactor.

Table 2 Ethanol Productivity in Various Bioreactor

Reactor	Productivity
Batch	1.8-2.5
CSTR	6-8
Batch with cell recycle	6-7
CSTR's in series	10-12
CSTR with cell recycle	10-18
Hollow fiber	15-30
Immobilized-cell CSTR	10-20
Immobilized-cell packed reactor	10-50
Immobilized-cell fluidized bed reactor	20-100

CHAPTER 3. EXPERIMENTS

For the reasons described in the previous chapters and for testing co-immobilization systems, soluble starch was used as substrate in the feed instead of glucose in the bench scale experiments. Glucoamylase was used to saccharify starch to the fermentable sugar - glucose. The experiment includes two parts. In experimental part I, co-immobilized yeast (*Saccharomyces cerevisiae*) and glucoamylase catalyst was used. This biocatalyst was supplied by Genencor International. The purpose was to demonstrate the performance of yeast-glucoamylase biocatalyst in the fluidized bed reactor. Experimental part II, based on the information obtained from experimental part I, used co-immobilized *Zymomonas mobilis* and glucoamylase. In part II, the biocatalyst beads were made at Oak Ridge National Laboratory. The co-immobilized catalyst in the fluidized - bed reactor was evaluated and the experimental results were addressed.

3.1 Experiment Part I: Co-immobilized *Saccharomyces cerevisiae* and Glucoamylase Biocatalyst in Fluidized Bed Reactor

3.1.1 Material And Apparatus

Biocatalyst Beads

The yeast strain *Saccharomyces cerevisiae* is immobilized in covalently crosslinked gelatin (6 wt%) and Chytosan (0.25 wt%) with enzyme glucoamylase (GA).

Crosslinking was accomplished by glutaraldehyde. These biocatalyst beads were supplied by Genencor International. These beads were stored in 50 % glycerol to keep them inactive and alive. The particle diameters ranged between 1.2 mm to 2.8 mm.

Feed Stock Solutions

The feed stock solutions consisted of various concentrations of StarDri 100 starch (A. E. Staley, Mfg. Co., Decatur, IL), 5 g/L Tastetone 900 AG yeast extract (Red Star, Juneau, WI), 0.1 % w/v Antifoam B (Dow Corning, Midland, Michigan) in tap water. Sterile media was prepared in an autoclave at 121°C for 3 hours.

Bioreactor

The reactor depicted in Fig. 3.1.1 was constructed of a 30 cm inlet section which expanded from 1.27-2.5 cm in ID, three 30 cm sections of 2.5 cm ID jacketed glass pipe, and a 10 cm disengagement section of 9 cm ID with a screened sidearm for disengagement of beads from the reactor effluent. Temperature was controlled at 34 °C by a Haake A82 (Berlin, Germany) recirculating water bath. The feed was introduced at the reactor bottom using a model 7550-60 Masterflex peristaltic pump (Cole Parmer, Niles, IL). The pump was calibrated daily. The reactor was open to the atmosphere for gas-liquid-biocatalyst disengagement at the outlet.

Sample Taking and Analytical Methods

Starch, glucose, and ethanol concentrations were measured using a Shimadzu high performance liquid chromatograph (HPLC) consisting of a RID6A refractive index detector, a SIL 10A auto injector, a LC10AD pump, CTD10A column oven, and a CR501 integrator. An Aminex HPX-87H (BioRad Laboratories, Hercules, CA) column

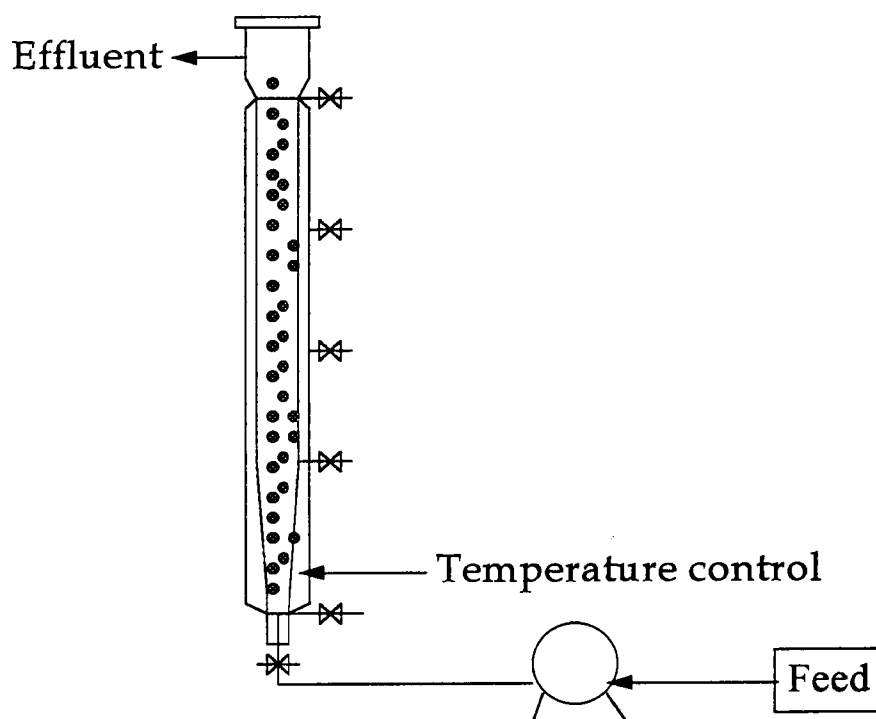


Figure 3.1.1. Fluidized bed reactor schematic. The reactor consisted of an expanded 30 cm inlet section (1.27 to 2.5 cm in ID), three 30 cm sections of 2.5 cm ID jacketed glass pipe, and a 10 cm disengagement section of 9 cm ID with a screened sidearm for disengagement of beads from the reactor effluent.

with a 5 mM H₂SO₄ mobile phase separated analytes.

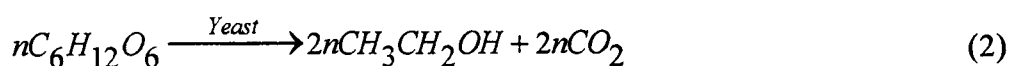
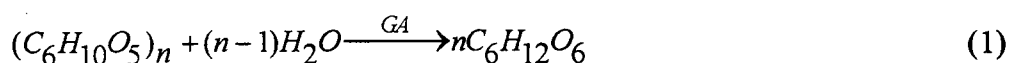
Minimal procedures were used for mitigation of contaminant growth. These included (1) changing feed lines with each new charge of feedstocks, (2) replacement of feed containers with each charge of fresh feed, and (3) sterilization of the medium by autoclave prior to use as significant amounts of contaminants existed in the yeast extract. Previous investigations demonstrated that sterile operation is not necessary; however, the feed must be kept free of high levels of contamination (e.g., contamination > 10⁸ cells/mL). The reactor was not sterilized or cleaned after the 10 week experiment began.

3.1.2 Results and Discussions

The operability of the reactor was good throughout the experiment. The reactor system generally operated without operator intervention or attendance. The pH within the reactor was not controlled and ranged from approximately 6.5 at the inlet to about 3.5 at the column outlet. The temperature was maintained at 34°C throughout the experiment. The biocatalyst was used continuously for 10 weeks in the FBR without recharging. There was no noticeable loss of biocatalyst from the reactor. There were no obvious signs of physical degradation of the biocatalyst except for a very small number beads that demonstrated significant diameter increases.

The fluid dynamics and reaction kinetics are coupled and are a complex function of reaction rate, starch and glucose concentration, solids loading, and gas-liquid-solid properties (Petersen and Davison, 1995; Webb, Davison, and Scott, 1996). The fluidization of the bed changes rapidly with axial position due to significant changes in

fluid flow rates and physical properties. Fluidization of the reactor can be thought of as occurring in three zones that may be distinguished visually. The first zone, located at the bed entrance, is fluidized by liquid. The second zone, fluidized by gas product, starts within the expansion section and encompasses most of the bed. The third zone, termed the disengagement section, is characterized by high gas holdup (e.g., 5 to 20%) and significant mixing. The axial reaction rate in the FBR is a strong function of reactor biocatalyst concentration. Gas and liquid holdup, and dispersion which become larger in the upper parts of the reactor reduce the biocatalyst concentration. Inside the biocatalyst matrix, starch is hydrolyzed by glucoamylase (GA) to form glucose and is then converted to ethanol and carbon dioxide by yeast:



The biocatalyst was loaded with sufficient GA to convert low concentrations of starch at significant rates. The GA reaction rate was thus expected to be rapid at higher starch concentrations.

Figure 3.1.2 depicts an example concentration profile for low-starch reactor loading. Starch concentration decreased with reactor position as GA conversion proceeded. Glucose is an intermediate between the GA and yeast reactions. Hence,

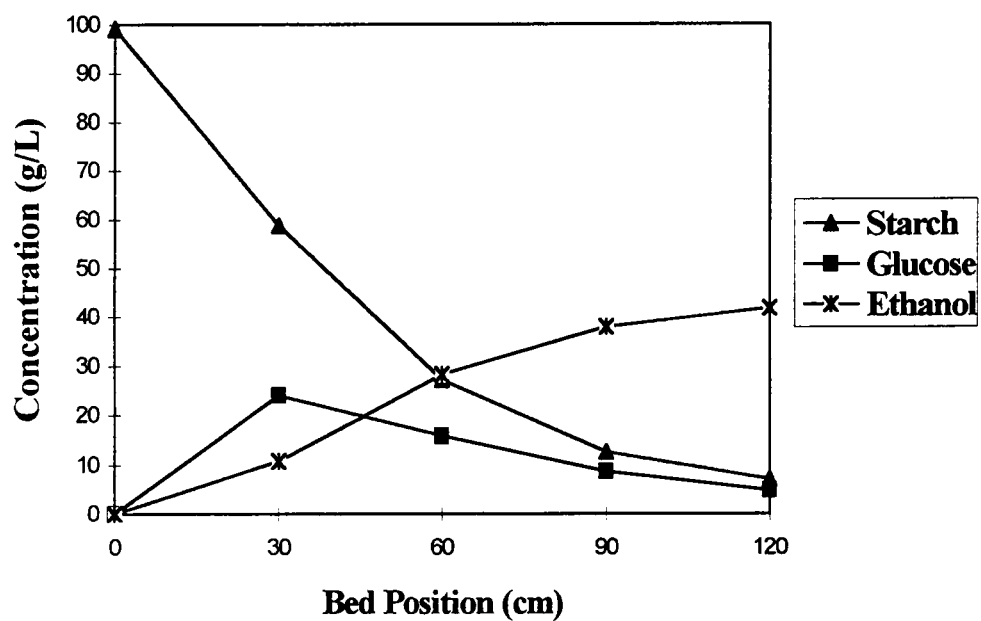


Figure 3.1.2 Example concentration profile. Starch concentration decreased with reactor position as AG conversion proceeded. Glucose is an intermediate between the AG and yeast reactions initially increased and then decreased. Ethanol concentration increased as the glucose was converted.

axial glucose concentration will be a function of both GA and yeast reaction rates.

Glucose accumulates in the first reactor section because of high GA conversion rate (high starch concentration). The ethanol concentration increases as glucose is made available by the GA and is then converted to ethanol by the yeast.

Figure 3.1.3 illustrates the conversion and/or accumulation of starch, glucose, and ethanol within individual reactor sections. Glucose accumulation in the first section indicates that the yeast reaction is slower than the GA reaction because of high bulk starch concentration. The conversion of starch to glucose drops in later sections as the available starch is reduced by prior reaction. The reduction of bulk glucose concentration after the first section was due to the reduction of the bulk starch concentration. With low bulk starch concentration, there was low glucose conversion rate inside the catalyst bead. Therefore, bulk glucose diffuses into the catalyst bead which causes the bulk glucose concentration to decrease. Product may inhibit yeast in later sections of the reactor.

Figure 3.1.4 allows visualization of the effect of mass loading on reactor performance. The data clearly demonstrate that, as mass loading increased, glucose accumulation also increased. Reactor position was also important as a variable to indicate the effect of contact time between substrates and the GA and yeast. Thus, as the contact time increased, the total conversion of starch and glucose also increased. The case depicted in Figure 3.1.2 (previously discussed) is shown in Figure 3.1.4 as the first data series closest to the bed position axis. In this case, glucose initially accumulated; however as the starch supply rapidly decreased via GA conversion, almost

complete conversion of substrates was achieved. The GA reaction is influenced greatly by starch concentration and to a minor extent by glucose concentration. As ethanol is formed, the yeast become inhibited. The onset of ethanol inhibition begins at concentrations of approximately 20 g/L (Maiorella, Blanch, Willee, 1985). Figure 3.1.4 also indicates that the reaction was limited by yeast and not the GA. Under actual practice, the GA activity would ideally be balanced against the yeast activity to maintain a low glucose concentration throughout the reactor. Yeast are the primarily the greatest unknown in this system because their performance not only depends upon glucose and ethanol concentration but also on nutrition, yeast and biocatalyst age, and other factors.

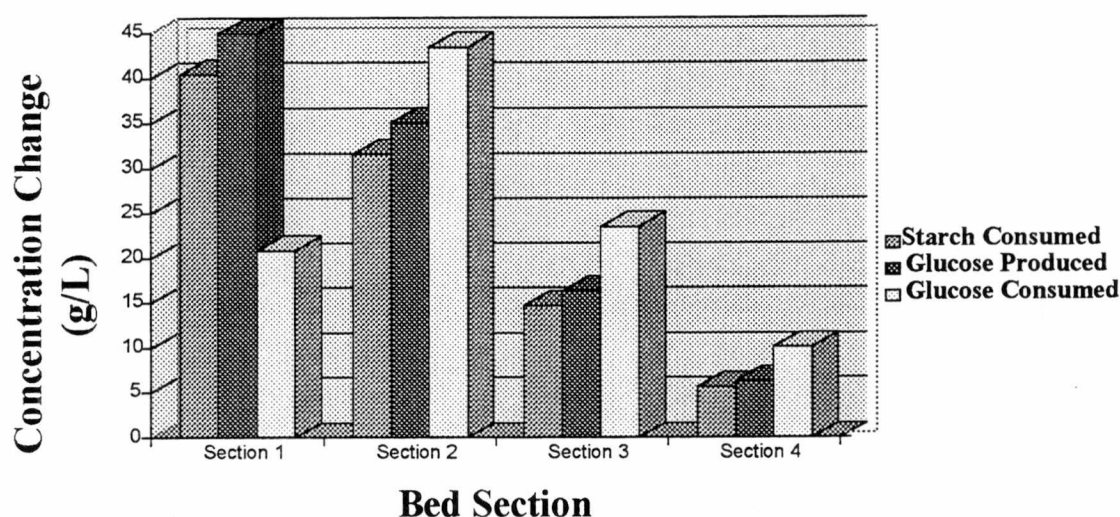


Figure 3.1.3. The conversion and/or accumulation of starch, glucose, and ethanol as change within individual reactor sections.

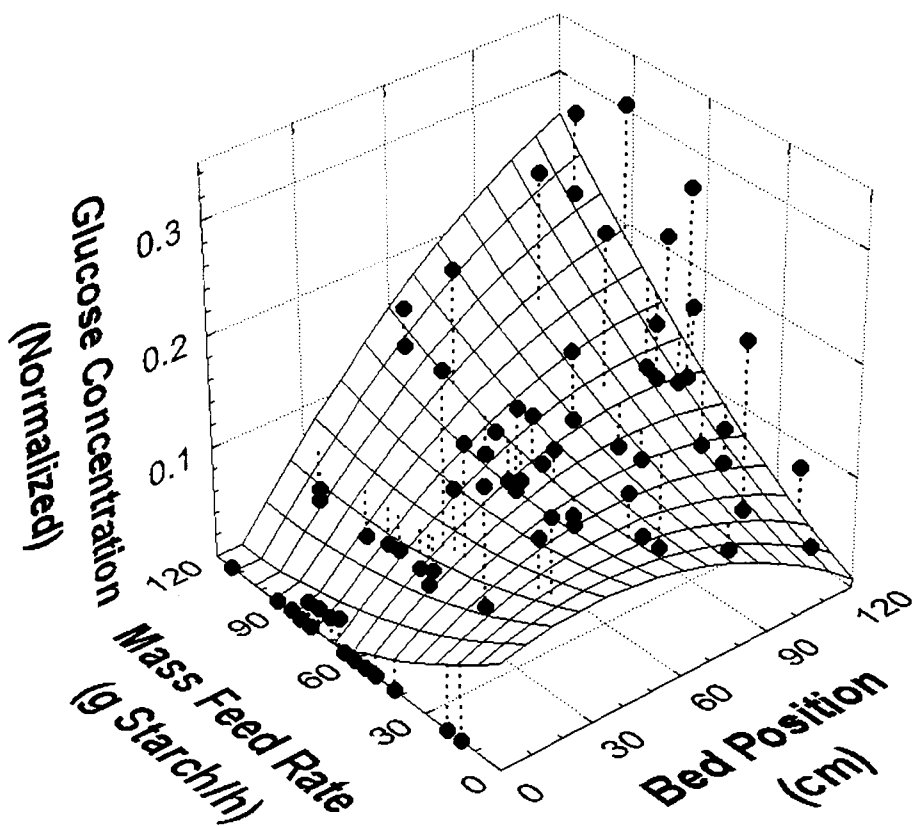


Figure 3.1.4 Mass loading influence. As mass loading increased, glucose accumulation also increased. Bed position was also important as a variable to indicate the effect of contact time between substrates and the AG and yeast. Glucose is normalized to glucose equivalents in the feed.

The surface of figure 3.1.4 was produced by fitting the data to a second order polynomial using position and mass loading as independent variables using the distance-weighted least squares method in STATISTICA (StatSoft, Inc., Tulsa, Oklahoma) similar to the method of McLain (1974). The surface in Figure 3.1.4 is qualitative because of the complex coupling of reactions (GA and yeast) and three phase hydrodynamics.

In a series of experiments, ethanol was added to the reactor feed solution for verifying the product inhibition and for reactor scale-up. Ethanol inhibition at the top of the reactor is masked by three phase hydrodynamic effects, gas hold-up in particular. Figure 3.1.5 depicts an example of ethanol inhibition. Glucose accumulation was significant because of ethanol in the feed. Ethanol concentration increased slowly relative to the case without ethanol in the feed.

As yeast ethanol inhibition increases, the difference in yeast rates and GA will increase as GA is not inhibited by ethanol. Thus, the ratio of glucose to starch concentrations at different points within the reactor was used as a general indicator of ethanol inhibition. Figure 3.1.6 allows visualization of the effects of ethanol inhibition on reactor performance. The data clearly show that as the ethanol in the feed increased, that rate differences between GA and yeast became more pronounced. The ratios also depend somewhat upon feed concentrations and liquid feed rates.

Figure 3.1.7 demonstrates that the mass loading effect was small compared to ethanol inhibition. There was a small increase in the glucose to starch mass ratio with bed position and with mass loading when ethanol was not added to the feed. The data

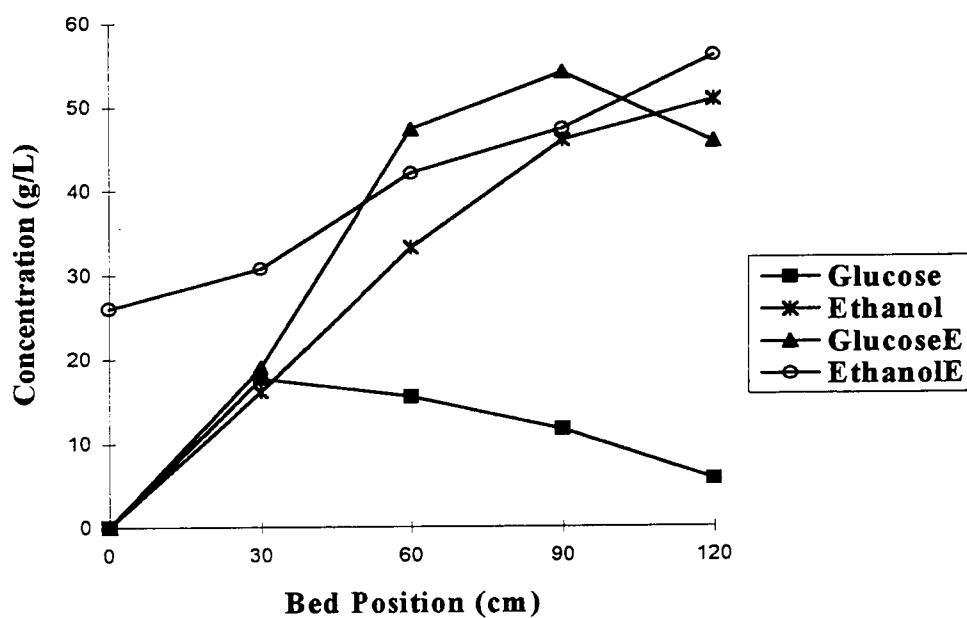


Figure 3.1.5 Ethanol inhibition example. The concentration profile of glucose in the reactor was a function of ethanol inhibition demonstrated by addition of ethanol to the feed.

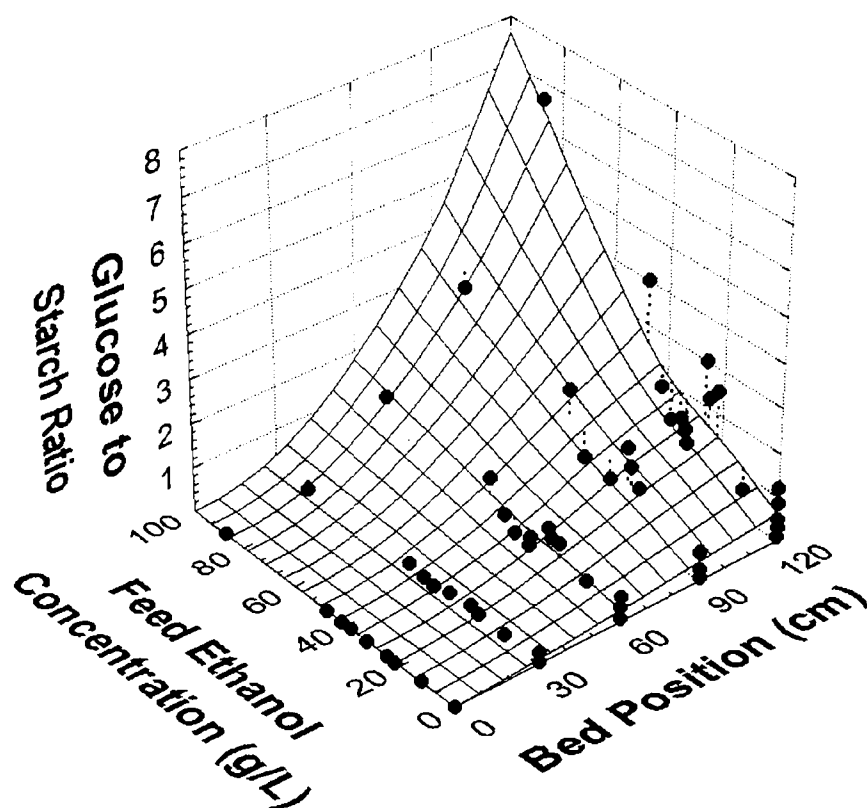


Figure 3.1.6 Visualization ethanol inhibition effects. As yeast ethanol inhibition increased, the difference in glucose and starch conversion rates increased because AG was not inhibited. The ratio of glucose to starch concentrations at different points within the reactor was used as a general indicator of ethanol inhibition.

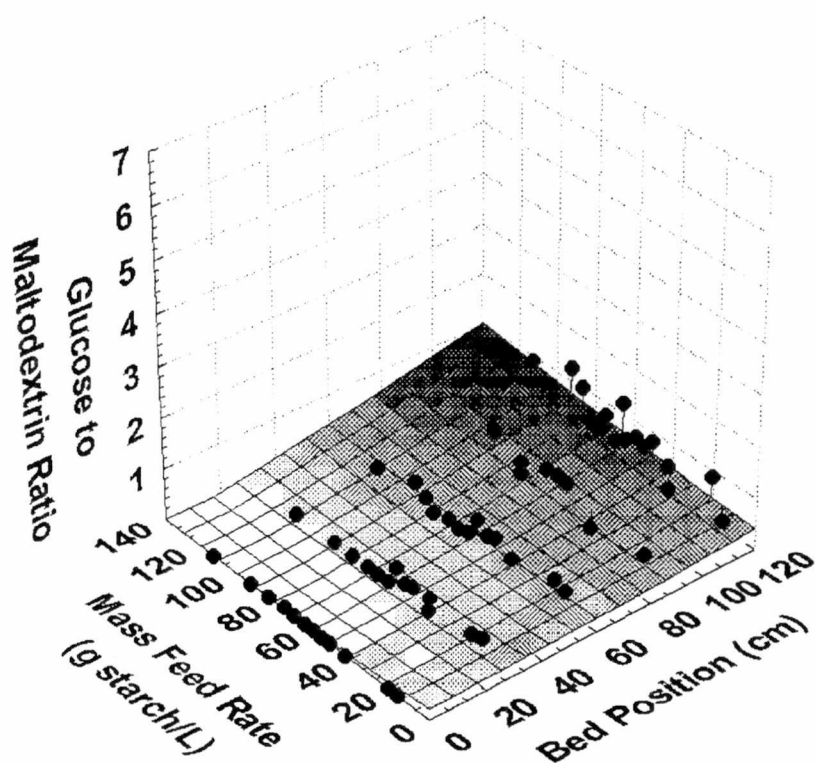


Figure 3.1.7 Influence of mass loading on dextrose conversion and accumulation. The ratio of glucose to starch is a function of yeast and AG reaction rates. There is a small affect on the ratio of glucose to starch within the bed with bed position and mass feed rate.

for Figure 3.1.6 and 3.1.7 were generated using the same ranges of starch feed concentrations and flow rates. STATISTICA generated the surfaces for visualization in Figures 3.1.6 and 3.1.7.

Figure 3.1.8 depicts productivity over the course of the last eight weeks of the ten week experiment. Two weeks were allowed for stabilization of yeast activity. Mass loading varied throughout the experiment by varying feed concentrations and slow rates. The data is grouped into two subsets: one subset containing feeds with ethanol and one subset containing feeds without ethanol. Most of the data with no ethanol in the feed was grouped in the range of 25 to 44 g ethanol/L/hr. Three points not falling in this group were due to very low mass loading of the reactor. When ethanol was included in the feed the range of productivities generally ranged between 5 and 25 g ethanol/L/hr. The differences in these productivities further demonstrate that ethanol inhibition had a significant effect on reactor performance. Also, there were no obvious declines in productivity in either set of data over time

The average yield was calculated by the slope of substrate conversion to ethanol production (Figure 3.1.9). The yield for these experiments ranged around 80% of theoretical. A yield of greater than 90% of theoretical has been demonstrated with stirred tank reactors and similar biocatalysts (Wallace, 1993). Possibly yeast nutrition played a significant role in this lower yield. In a batch reactor system, Cysewski (1976) demonstrated that ethanol yield was severely restricted if the concentration of yeast extract was 4.0g/L or 6.0 g/L. He suggested a yeast extract requirement of 8.5g/L. The physiology of immobilized yeast may differ from free cell physiology. Yeast extract was

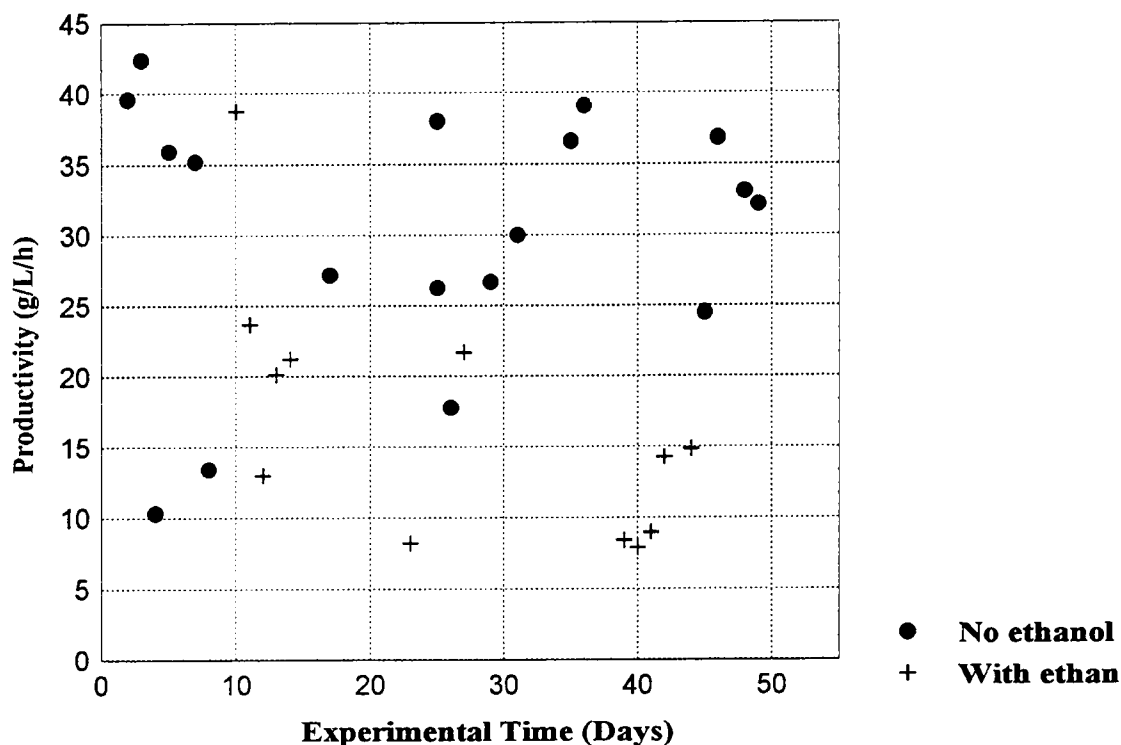


Figure 3.1.8 Productivity profile. The data is grouped into two subsets: one subset containing feeds with ethanol and one subset containing feeds without ethanol. Most of the data with no ethanol in the feed was grouped in the range of 25 to 44 g ethanol/L/hr. Low values were due to very low loading of the reactor. When ethanol was included in the feed, the range of productivities generally ranged between 5 and 25 g ethanol/L/hr. The differences in these productivity further demonstrate that ethanol inhibition had a significant effect on reactor performance. From both set of data, there were no obvious declines in productivity.

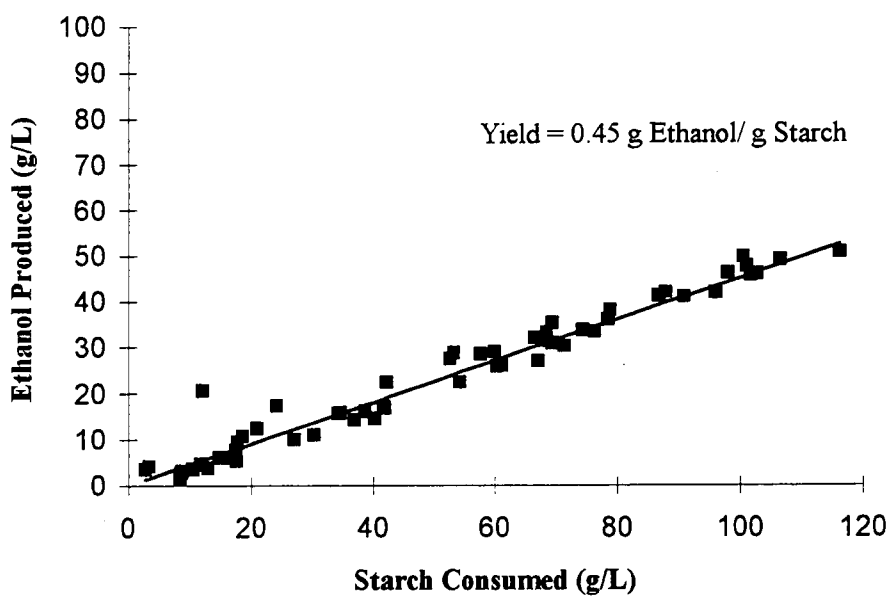


Figure 3.1.9 Average yield. The yield for these experiments ranged around 80% of theoretical as measured by the slope of substrate converted to ethanol produced. Possibly, yeast nutrition limited yield.

used at 5 g/L concentration levels for direct comparison with previous work with *Z. mobilis*. Under these conditions, *Z. mobilis* demonstrated yields of 97 and 96% of theoretical for experiments at the batch and pilot scale (Davison, and Scott, 1988; Webb, Scott, Davison, and Scott, 1995). This data suggests that yeast has higher nutritional requirement than *Z. mobilis* when immobilized.

3.1.3 Conclusions

Co-immobilized yeast and glucoamylase were used to effectively convert soluble starch to ethanol in one reactor. This research demonstrates that yeast is very hardy and produces ethanol over a very long period of time. The biocatalyst performed well and demonstrated no significant loss of activity or physical integrity during 10 weeks of continuous operation. The reactor was easily operated and required no pH control. No operational problems were encountered from bacterial contaminants even though the reactor was operated under non-sterile conditions over the entire course of experiments. Ethanol inhibition is an important factor. Productivities ranged significantly above industrial benchmarks. Ethanol yields appear to be somewhat lower due to low nutrient supplements (e.g. yeast extract). The Co-immobilized biocatalyst continues to demonstrate the potential for fluidized - bed reactor use.

3.2 Experiment Part II: Co-immobilized *Zymomonas mobilis* **and Glucoamylase Biocatalyst in Fluidized Bed Reactor**

Experiment Part I showed that the fluidized bed reactor with co-immobilized biocatalyst system has potential value for application. Glucoamylase hydrolyzes starch to the fermentable sugar glucose, and microorganisms then convert the glucose to ethanol. Saccharification and fermentation happened simultaneously in this system (SSF). From an economic aspect, the key performance parameter is the yield. In the literature review, it has been described that immobilized *Zymomonas mobilis* has much higher ethanol yield such as 97 and 96% of theoretical for experiments at the batch and pilot scale (Davison, and Scott, 1988; Webb, Scott, Davison, and Scott, 1995). Fermentation studies with various strains of *Zymomonas mobilis* have been shown that these microorganisms have many other advantages for ethanol production when compared with yeasts. These advantages include a faster specific rate of ethanol production and enhanced ethanol tolerance (Rogers, Lee, and Tribe, 1980; Lee, Skotnicki, Tribe, and Rogers, 1980). In order to improve the SSF system and obtain a higher yield, *Zymomonas mobilis* was used as fermentation microorganism in the experiment part II. This part of the experiment includes making co-immobilized *Zymomonas mobilis* and glucoamylase biocatalyst beads, evaluating the performance of this catalyst in a fluidized bed reactor, and reducing the feed cost by using light steep water instead of the yeast extract in the feed.

3.2.1 Material and Apparatus

Biocatalyst Beads Formation

Microorganism Culture Preparation:

Z. mobilis NRRL-B-14023 was used. The stock culture was maintained in 25% glycerol and kept at -70°C. To provide the cells for the preparation of the biocatalyst beads, *Z. mobilis* was grown in a 75-liter fermentor (New Brunswick Scientific Co., Edison, New Jersey). The inoculum was prepared in a three-liter fernbach containing two liters medium. The fernbach medium contained 50 g/L glucose, 5 g/L Tasterone 900AG yeast extract (Red Star, Juneau, Wisconsin), and 6 g/L KH_2PO_4 . The pH of the medium was adjusted to pH 5 with concentrated phosphoric acid, sterilized by autoclaving at 121°C for 20 minutes, and allowed to cool to ambient temperature prior to inoculation. One stock vial containing 1.7 mL of cells culture was thawed and used to inoculate the fernbach. The fernbach was incubated at 30°C with gentle mixing for 36 hours before its entire contents was used to inoculate the fermentor. The fermentor medium had the same composition as the fernbach medium. The fermentor was maintained at 30°C. The pH was maintained at 5.0 using 2N NaOH. The 75-liter fermentor consists of the following main elements:

1. Culture vessel with ports, headplate, agitators and bottom drive,
2. Piping module including valves for operationg and sterilization control, pipelines and electrical connections.

3. Instrumentation for 1) Sterilization, 2) Temperature Control, 3) Airflow Control, 4) Agitation Control, 5) pH Control.

After 20 hours, when 90% of the glucose in the medium had been consumed, the cells were recovered by centrifugation (Sharples Super-Centrifuge AS26 NF). The Sharples Super-Centrifuge is a machine for applying centrifugal force to a liquid. Centrifugal force is generated by the rapid rotation. The concentrated biomass were stored at 4°C until its immobilization was carried out.

Enzyme Glucoamylase:

GA immobilized on a solid matrix having average particle diameter of 1.0 to 1.5 mm was provided by Genencor International Indiana, Inc. Since the particle size was too large for the preparation of the co-immobilization of the enzyme with the *Z. mobilis* cells (see below), the immobilized GA was ground in a ceramic mortar placed in an ice bath until the particle diameter was less than 0.1 mm. After the grinding process was complete, a small sample centrifuged. The supernatant was tested for activity at 35°C using soluble starch at 80 g/L as substrate. In this test, no glucose was released which indicated that no enzyme was lost from the support during the grinding process. The activity of the ground immobilized GA was measured at 35°C and pH 5.0. One hundred milliliters of buffer (6 g/L KH₂PO₄ adjusted to pH 5.0) was used to wash 2 mL of ground GA into a 250 mL flask. The flask then was placed in a 35°C water-bath and its contents was mixed with a small stir bar. When the temperature in the flask was the same as the temperature of the water-bath, 100 mL of a 80 g/L soluble starch in the same buffer which had been kept at 35°C was quickly added to the flask. Samples were taken at intervals, quickly centrifuged to separate the immobilized enzyme, and the glucose concentration in the supernatant was measured with a YSI glucose analyzer

(Yellow Springs Instruments, Wisconsin). The specific activity of the ground immobilized GA was calculated as 1.16 g glucose/mL enzyme/hr.

Gel Solution:

To prepare the biocatalyst beads, 40 g κ -carrageenan (Type NSAL 798 from FMC Corporation) was slowly dissolved in about 700 mL de-ionized water kept in a waterbath at 70°C. Mixing was provided by a stir bar. Then the temperature of gel solution is reduced to 35°C and maintained with stirring in the 35 °C water bath. 40 g of *Z. mobilis* cell paste was added, followed by 150 mL ground immobilized GA. Finally deionized water was added to the volume of one liter to constitute the final biocatalyst mixture. In the original procedure for the preparation of immobilized *Z. mobilis* in κ -carrageenan, Fe₂O₃ was added to the mixture to increase the specific density of the biocatalyst beads to prevent them from floating (Davison, and Scott, 1988). In this work, the addition of Fe₂O₃ was not necessary and therefore omitted because the solid matrix onto which the GA was immobilized by the manufacturer already made the specific density of the beads sufficiently high to allow them to sink.

Bead Formation System:

The bead formation system consists of a stirred gel solution reservoir kept temperature at 35°C in a water bath , Masterflex peristaltic pump which delivered the gel solution to a small nozzle (100 mL pipet tip). Figure 3.2.1 is the experimental setup for production of biocatalyst beads. Vibration energy is introduced into the system by direct

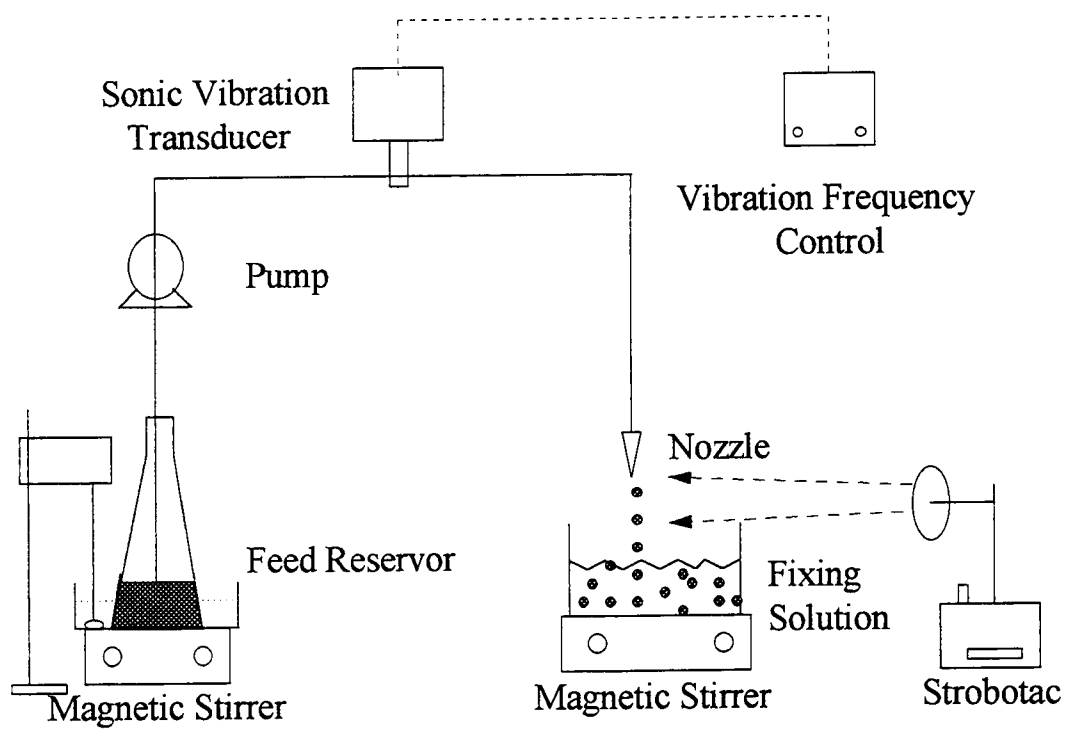


Figure 3.2.1 Experimental setup for production of biocatalyst beads

attachment of the vibrator transducer to the external nozzle surface or to the side of the flexible delivery tube (Scott. , 1987). The latter approach resulted in a simpler construction and an easier mode of operation. The resulting stream of gel material exited the nozzle as droplets that were collected in a reservoir containing the fixing 0.3 M KCl solution. A strobotac (General Radio Company, Concord Massachusetts) was used to observe the size of the bead. The solution is slowly stirred so that the impacting beads would not contact each other prior to surface interaction with the fixing cation. The gel beads were stored in the solution consisting 0.3 M KCl and 7.5 g/l KH_2PO_4 . The solution pH was adjusted to 5.0.

Fluidized Bed Reactor System

Feed media:

Feed solutions consisted of various desired concentration of StarDri 100 starch (A. E. Staley Co., Decatur, IL), 0.05 M KCl, 0.1 % w/v Antifoam B (Dow Corning, Midland, Michigan), 25% (v/v) light steep water (LSW) which was provided by A. E. Staley Co., from their ethanol production plant at Loudon, TN. Sterile media were prepared in an autoclave at 121°C for two hours.

Fluidized Bed Reactor:

The reactor is a jacketed glass column the same one as experimental part I except there is the pH probe in the section three for pH observing. Temperature was controlled at 35°C. Prior to the loading of the biocatalyst beads, the reactor was decontaminated

by rinsing with hot water and 75% ethanol. 350 mL of beads were then placed into the reactor. The rest of the reactor was reserved for expansion during its operation. Feeds were pumped into the reactor at flow rates varied from 10 to 37 mL/min. Minimal efforts were made to mitigate contaminant growth. In addition to sterilization of the feed solutions and the cleaning of the reactor prior to biocatalyst loading, the feed lines and containers were changed with each new charge of feedstocks. Previous experience demonstrated that sterile operation was not necessary. For each set of experimental conditions (substrate concentration and feed flow rate), at least six residence times were allowed before samples were taken for analyses of starch, glucose and ethanol.

Measuring method:

Starch, glucose, and ethanol concentrations were measured using a Waters high performance liquid chromatograph (HPLC) consisting of a 410 Differential Refractometer detector, a Waters 717plus Autosampler auto injector and Alltech 425 HPLC pump. The HPLC is interfaced with an PC which served as the data system. Waters Millenium software was installed in the computer to perform data acquisition, curve integration, calibration, peak detection and plotting. An Aminex HPX-87H (BioRad Laboratories, Hercules, CA) column with a 5 mM H₂SO₄ mobile phase separated samples.

3.2.2. Results and Discussion

The operability of the reactor was good throughout the course of the experiments. The biocatalyst beads were used continuously for 22 days in the FBR

without the need of recharging. There was no noticeable loss of biocatalyst from the reactor. There also were no obvious signs of structural failure of the beads.

Inside the biocatalyst matrix, starch was hydrolyzed by GA to glucose; the glucose produced then was converted to ethanol and CO₂ by *Z. mobilis*. Figure 3.2.2 shows an example of concentration profiles of starch, glucose, and ethanol along the reactor. Starch concentration decreased with vertical position in the reactor as GA conversion proceeded. In this particular experiment, 65% conversion of starch was obtained. Glucose was an intermediate of the series of two consecutive reactions, and therefore, its concentration was a function of both GA and *Z. mobilis* conversion rates. Glucose concentrations were extremely low (near zero) at all positions in the reactor. This indicated that the glucose production rate was much slower than its consumption rate. In other words, the hydrolysis of starch by GA was the rate-limiting step. Glucose was converted to ethanol and CO₂ immediately after it was produced and therefore did not accumulate in the reactor. These results were different from those obtained with the GA-*S. cerevisiae* system in the experiment II. In the GA-*S. cerevisiae* system, the starch conversion rate was much faster than the glucose consumption rate and glucose accumulated in the middle section of the reactor. Starch diffusion accounted for this difference. In the GA-*S. cerevisiae* system, GA was in its free form before it was co-immobilized with the yeast. In the current system, the enzyme was immobilized in a solid matrix before it was co-immobilized with *Z. mobilis*. This "double" immobilization increased the restriction of starch diffusion into the active sites of the enzyme. Consequently, the concentrations of starch at these locations were low and therefore, its conversion rate to glucose was slower. There is a need of new enzyme immobilization technique for co-immobilization system to be developed, and this technique will improve enzyme rate-limiting situation.

Ethanol volumetric productivity was calculated for the three sections of the

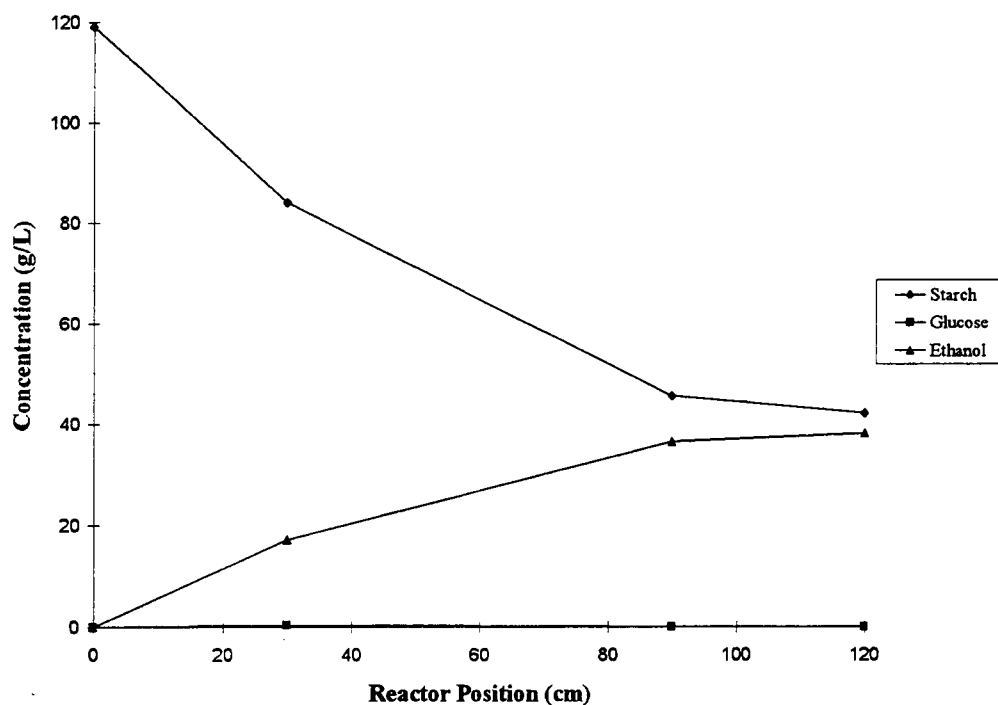


Figure 3.2.2 Example of concentration profiles of starch, glucose, and ethanol.

Starch concentration decreased with reactor position as GA conversion proceeded. Glucose was an intermediate between the GA and *Z. mobilis* reactions and its concentrations were very low in the reactor. Ethanol concentration increased as the glucose released was converted.

reactor. The results are shown in Figure 3.2.3. In the bottom section of the reactor, the ethanol volumetric productivity was 50 g/L/hr. The productivity decreased to 17 and 3 g/L/hr for the middle and the top sections, respectively. This decrease was related to fluid dynamics, phase hold-up, and reaction kinetics in the reactor. The fluidization of the biocatalyst bed changed rapidly with the axial position due to significant changes in fluid flow rates and physical properties. At the entrance to the reactor, the biocatalyst beads were fluidized by the liquid and in the middle section by both liquid and the CO₂ gas. The upper most section was characterized by gas hold-up. The reaction rates in the reactor were a strong function of the biocatalyst concentration. This concentration was reduced in the upper part of the reactor by larger gas hold-up and liquid dispersion. The bottom section had the highest biocatalyst concentration and bulk starch concentration, and consequently had the highest productivity.

An example of the pH profile along the reactor is shown in Figure 3.2.4. The pH gradually decreased from 5.1 at the reactor entrance to 4.1 at the exit. The production of ethanol also generated CO₂, some of which dissolved in the liquid and decreased the pH. Since the feed solutions were not buffered, the decrease of the liquid pH along the reactor was directly proportional to the quantities of CO₂ dissolved in it. In the bottom section of the reactor, where the rates of ethanol and CO₂ production were highest, the decreasing rate of pH also was fastest. For the same reason, the decreasing rate of pH in the middle section was faster than that in the top section. Although the pH dropped more than one unit at the exit in the reactor and was not in the optimum range (5-6) for *Z. mobilis* production, the pH still was within the range that allows ethanol production by *Z. mobilis* (Doelle, Kirk, Crittenden, and Toh, 1993). Besides, pH 4-5 is the optimum range for enzymatic reaction. Since enzyme reaction is the limiting step in this

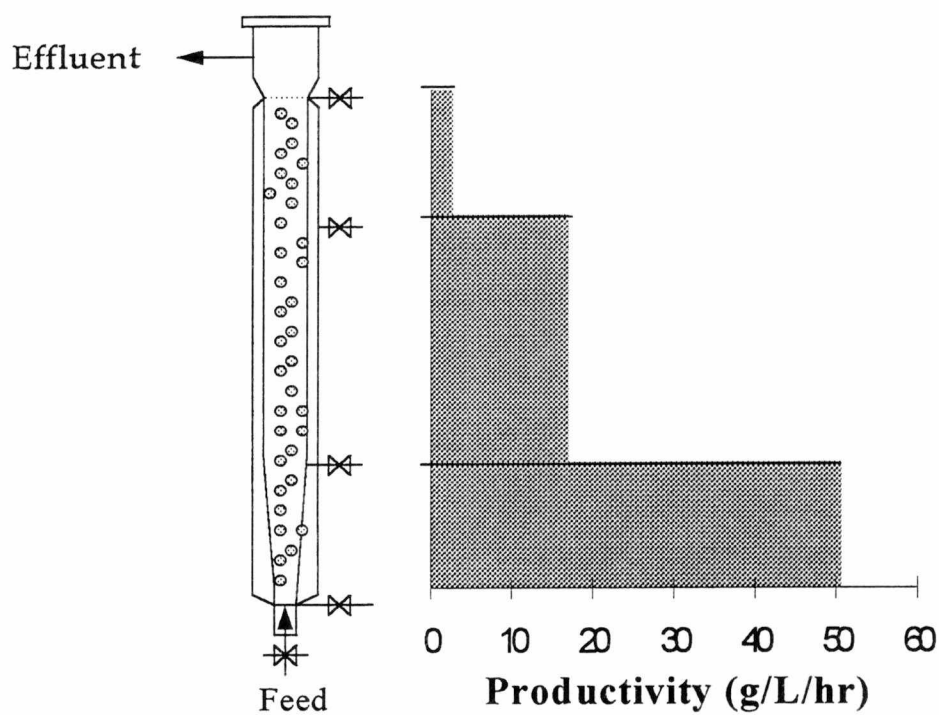


Figure 3.2.3 Ethanol volumetric productivity at different sections of the reactor. The first section(1-30 cm) of the reactor had highest productivity. The third section had lowest productivity.

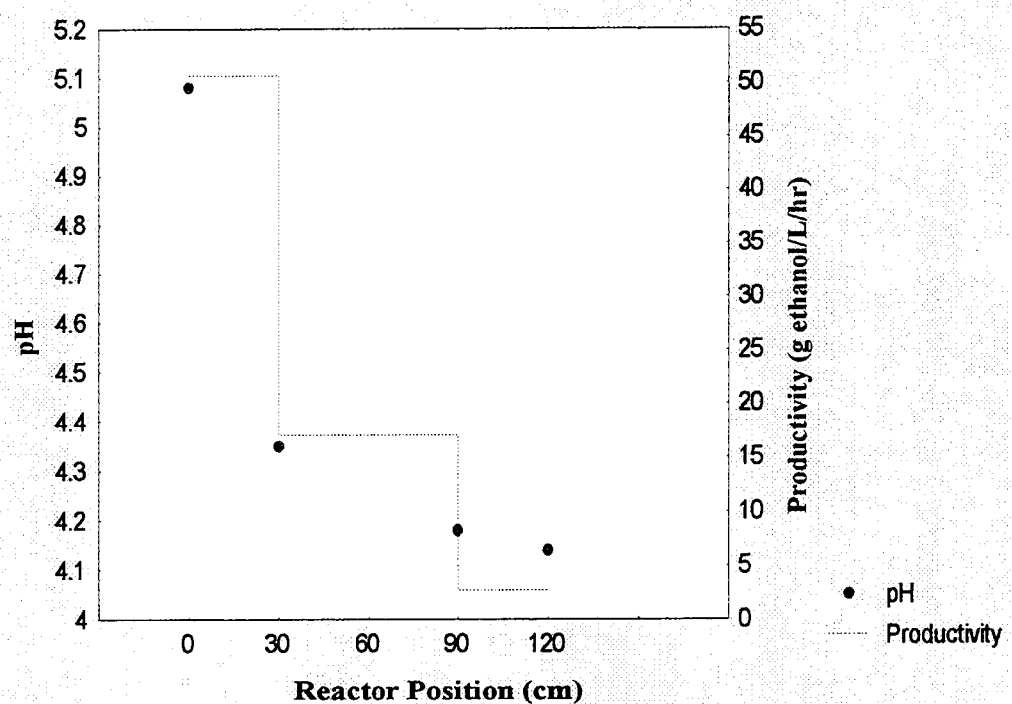


Figure 3.2.4 pH and productivity changes along the reactor position. In the first section of the reactor, the pH decreased faster than in other sections. The rate of pH decrease was directly related to the rates of production of ethanol and CO_2 .

system, this pH range was the most suitable range for this system operation.

Figure 3.2.5 illustrates the effects of starch mass loading and operation time on the reactor performance. The surface was generated with the STATISTICA software(StatSoft, Inc., Tulsa, Oklahoma). The results showed that the ethanol volumetric productivity decreased with time after 10 days operation. For example, the ethanol volumetric productivity obtained on day 4 at a mass flow rate of 71.4 g/h was 38.5 g/L-h; it dropped to 17.3 g/L-h on day 16 at a similar mass flow rate of 66 g/h. During the course of the experiments, no significant loss of the biocatalyst beads was observed. Therefore, the decrease of productivity could only be due to lower activities of the beads. In all of the experiments, glucose concentrations stayed very close to zero in the reactor. Therefore, under the conditions of decreasing volumetric productivity, starch hydrolysis still was the rate limiting step. The reason of this decreasing with time might caused by the experimental uncertainty. For example, the water quality was uncertain and changed with the time. There may have been some unknown toxic component for the enzyme such as heavy metal or other kinds of the compounds. Similar situations had happened twice during author's experiments and also happened in other researcher's fermentation system (personal contact with Dr. John Nghiem)

The highest volumetric productivity of ethanol obtained was 38.5 g/L/hr. Since 150 mL ground immobilized GA having a specific activity of 1.16 g glucose/mL-h was used to prepare one liter of biocatalyst beads, and 350 mL of beads was placed in the reactor having a working volume of 0.6 L, the maximum ethanol volumetric productivity which could be expected in the reactor would be 51.8 g/L/hr. This was calculated assuming a yield of 0.51 g ethanol/g glucose consumed and that the co-immobilization of the ground immobilized GA with *Z. mobilis* did not increase restriction to starch

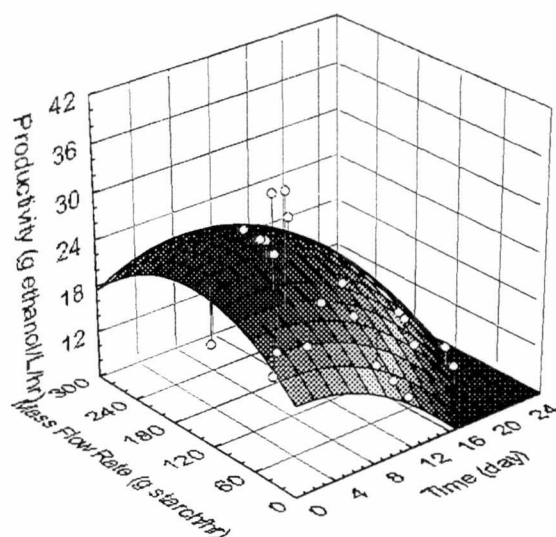


Figure 3.2.5 The influence of starch mass feed rate and time of operation on productivity. Productivity decreased with time.

diffusion into the biocatalyst beads. The highest ethanol volumetric productivity obtained experimentally therefore was 74% of the calculated value. This highest volumetric productivity obtained was lower than the productivity obtained for co-immobilized *GA-Z. mobilis* in Na-alginate in a PBR which is 45 g/L/hr, but significantly higher than the productivity obtained with the similar system which is 7.5 g/L/hr. Figure 3.2.6 is a contour plot of the starch mass feed rate, operation time and ethanol volumetric productivity. It can be seen that the optimum mass feed rate was between 120 and 180 g starch/hr. Figure 3.2.7 shows a plot of ethanol production versus starch consumption. The slope of the best-fit line gives an average yield of 0.51 g ethanol/g starch consumed. The average yield obtained therefore was 90% of the theoretical value.

This was slightly lower than the yields obtained in other studies using immobilized *Z. mobilis* with glucose as substrate in an FBR (96% of the theoretical value) (Davison, and Scott, 1988). However, the yield of *Z. mobilis* co-immobilization system is still higher than the conventional reactor system (see section 2.3) and the yeast co-immobilization system which is 80% of the theoretical value.

3.2.3 Conclusions

It has been demonstrated that co-immobilized GA and *Z. mobilis* could be used for ethanol production from starch in a single reactor. The fluidized-bed reactor used was easy to operate and required no pH control. Under relaxed sterile conditions, no contamination was observed. The biocatalyst beads were quite structurally stable over the 22-day period of operation. Between the two consecutive conversion steps, starch hydrolysis was shown to be the rate-limiting one. The application of the FBR technology with co-immobilized GA-*Z. mobilis* will be extended to the use of real industrial feedstocks such as hydrolyzed dry-milled corn flour. With further research and evaluation at ORNL, this technology will be soon demonstrated on the industrial set.

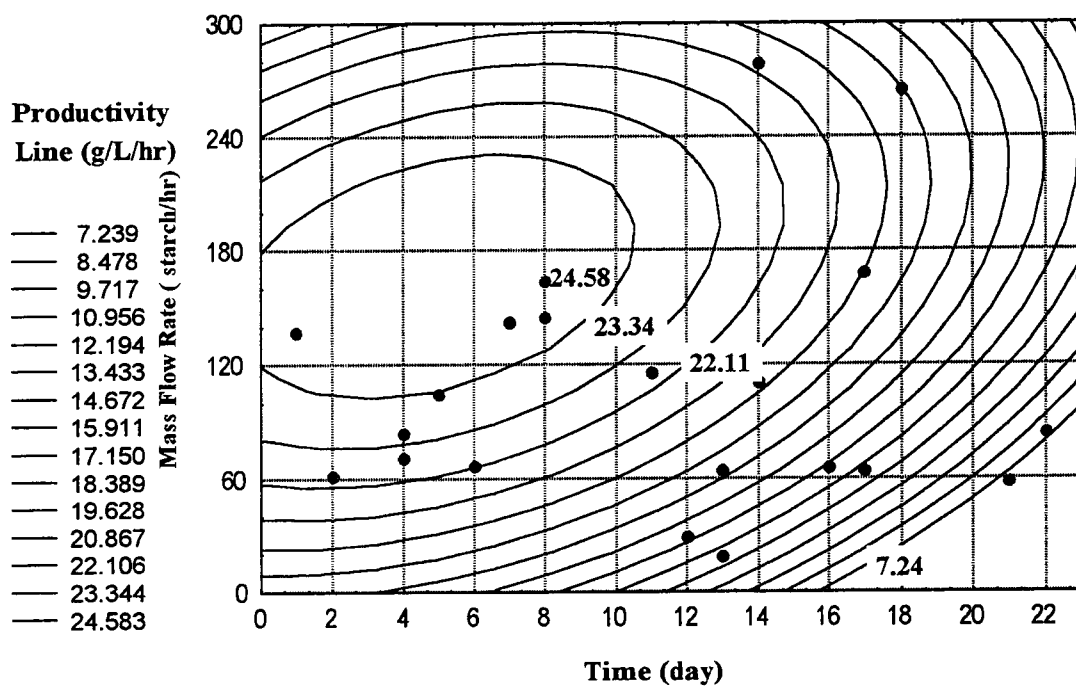


Figure 3.2.6 The contour plot of productivity vs. starch mass feed rate and time.

The optimum mass feed rate was 120-180 g starch/hr.

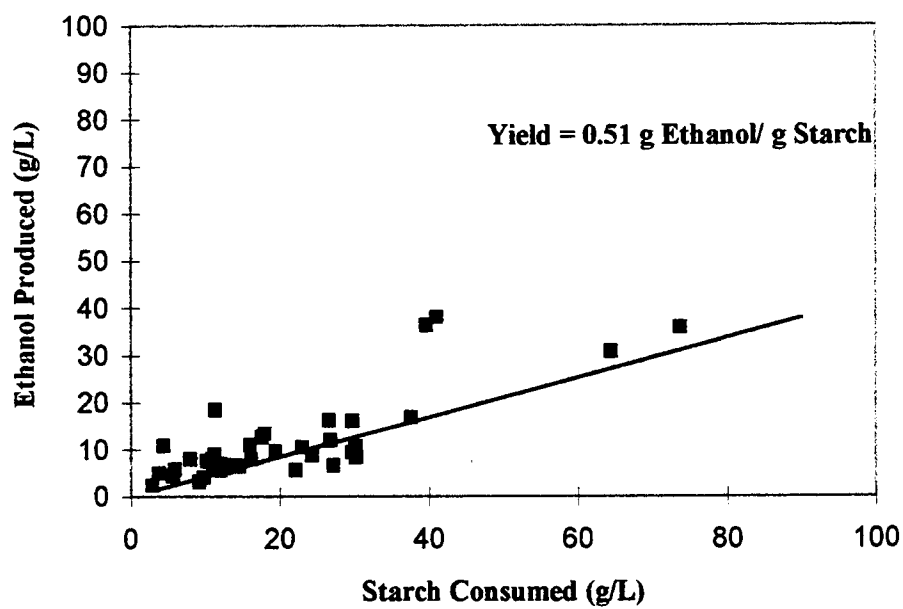


Figure 3.2.7 Determination of average ethanol yield of GA-Z. mobilis system.

CHAPTER 4 MATHEMATICAL MODEL DEVELOPMENT

In the past, most bioreactors, including fluidized-bed bioreactors, have been developed and scaled by trial and error. However, it is important to be able to design such systems more accurately. To be able to understand these reactors, predictive mathematical descriptions that are based on fundamental principles and accepted correlations that describe the important physical phenomena must be developed and verified. These models must be generic in nature such that they can be easily applied to a diverse set of applications and operating conditions. People have attempted to develop such predictive models for three phase fluidized bed reactor with single reaction system (Park, 1984; Hamamci, 1987; Godia, 1987; Wisecarver, 1989, Petersen and Davison, 1991; Weuster-Botz, 1993). However, these models had different limitations such as: plug flow, without CO₂ effect, constant axial dispersion coefficient, constant solids holdup, etc.. Petersen and Davison (1995) developed a model which describes multiphase fluidized-bed reactor used for continuous ethanol production by *Zymomonas mobilis* with glucose as substrate. The predictions of this model were compared with different scale experimental data (Davison and Scott, 1988; Webb and Davison, 1996). In this thesis experimental system, in order to use starch as substrate, the biocatalyst will have the ability of simultaneous saccharification and fermentation. The co-immobilized microorganism and enzyme biocatalyst involve a consecutive reaction mechanism. The reactions couple to each other and make the modeling more difficult. The solution of

the coupled non-linear second order equations together with the boundary conditions requires a significant numerical effort.

In order to explicitly describe the model for an ethanol fluidized fermentor system, several main aspects must be addressed. Figure 4.1 shows the mechanism inside the reactor. There are two key parts in the model: biocatalyst and reactor. The co-immobilized biocatalyst model includes fermentation kinetics of the *Zymomonas mobilis*, saccharification kinetics of glucoamylase, transport phenomena of the reactant and product by diffusion within the biocatalyst bead. In the fluidized bed reactor model, the dispersion coefficient and the fraction of the bed's cross section that is occupied by the gas, liquid, and solid phases is a function of the CO₂ and liquid feed flow rates through that portion of the bed. All these factors affected the species concentration at each axial point in the bed. In the following sections, the mathematical models for each of these topics will be addressed.

4.1 Fermentation kinetics of the *Zymomonas mobilis*

The first assumption concerning *Z. mobilis* kinetics is that the immobilized cell employs the same mechanisms as the free cell. Therefore, the kinetics developed for free cells can also be used to describe the immobilized *Z. mobilis* reaction in this system.

Mathematical modeling of fermentation kinetics can provide a detailed understanding of a process. Model has been developed for ethanol production by *Z.*

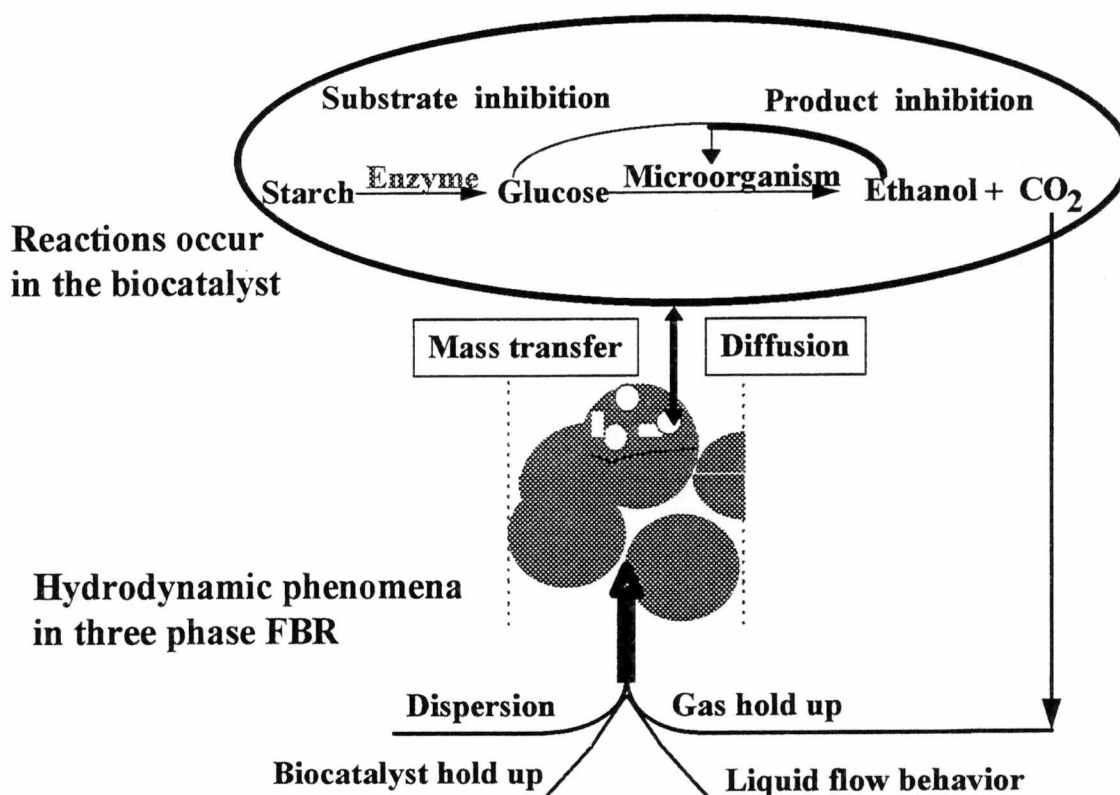


Figure 4.1 The mechanism inside the reactor

mobilis. The model has been based on the assumptions that the predominating factors influencing the kinetics of *Z. mobilis* are substrate limitation, product inhibition and substrate inhibition at high substrate levels. The general equations for microbial kinetics which include these three effects are as follows: the rate of product production is given by

$$\gamma_p = (q_p)_m \frac{C_g}{K_s + C_g} \frac{K_p}{K_p + P} \frac{K_i}{K_i + C_g} X \quad (4.1.1)$$

and the rate of substrate uptake by

$$\gamma_g = -\frac{1}{Y_{p/g}} \frac{dP}{dt} \quad (4.1.2)$$

where,

γ_p = product production rate (g/L(fluid phase) /hr)

γ_g = glucose consumption rate (g/L(fluid phase) /hr)

C_g = glucose concentration (g/L (fluid phase))

P = the ethanol concentration (g/L (fluid phase))

K_s = Substrate limitation constant for ethanol production

K_i = Substrate inhibition constant for ethanol production

K_p = Product inhibition constant for ethanol production

X = Biomass concentration

$Y_{p/s}$ = Ethanol yield

These general types of equations have been proposed and evaluated previously by several researchers (Aiba, Humphrey, and Millis, 1973; Rogers, Bramall, and McDonald,

1978)

In developing a detailed model for *Z. mobilis* fermentation, Lee and Rogers (1983) used the above basic structure initially and then modified it to be consistent with the observed *Z. mobilis* kinetic behavior. They reported that the rate of glucose consumption rate is given by:

$$\gamma_g = \frac{(q_p)_m}{Y_{p/g}} \frac{C_g}{K_s + C_g} \frac{K_i}{K_i + C_g} f_p X \quad (4.1.3)$$

where,

$$f_p = \begin{cases} 0 & \text{for } P \geq P_m \\ 1 - \frac{P - P_i}{P_m - P_i} & \text{for } P_i \leq P \leq P_m \\ 1 & \text{for } P \leq P_i \end{cases} \quad (4.1.4)$$

The other parameters are defined and numerical values given by Lee and Rogers (1983) are provided in Table 3.

4.2 Kinetic Model for Glucoamylase

The glucoamylase (GA) is capable of hydrolyzing the α -(1 $\rightarrow$ 6) bonds of amylopectin as well as the α -(1 $\rightarrow$ 4) bonds in both amylose and amylopectin. GA plays a very essential role in industrial ethanol fermentation processes. Several kinetic studies of the enzymatic hydrolysis of starch by glucoamylase have been carried out. Two

Table 3 Parameter Values for *Z. mobilis* Kinetic Model

Parameter	Description	Value
P_i	Threshold ethanol concentration for ethanol production	55 g/L
P_m	Maximum ethanol concentration for ethanol production	127 g/L
$(q_p)_m$	Maximum specific ethanol production rate	5.0 g/g/hr
K_s	Substrate limitation constant for ethanol production	0.5 g/L
K_i	Substrate inhibition constant for ethanol production	500 - 2000 g/L
$Y_{p/s}$	Ethanol yield	0.48 g/g

models will be discussed in the following sections for the better understanding of the glucoamylase kinetics.

Model 1.

The simplest equation to express the reaction kinetics of glucoamylase is the classical Michaelis-Menten kinetic model.

$$v_s = \frac{v_{sm} C_s}{K_m + C_s} \quad (4.2.1)$$

where,

v_s = glucose production rate

v_{sm} = the maximum glucose production rate

K_m = Michaelis-Menten constant

C_s = substrate starch concentration (g/L fluid phase)

Hamdane et al.(1988) used the differential gradientless reactor to determine the parameters in this kinetic model.

$$v_m = 0.38 \text{ mmol (glucose)/min.g (immobilized enzyme weight)}$$

$$K_m = 2.8 \text{ g /l}$$

In Hamdane's experiment, the glucoamylase enzyme was covalently bound to a vegetal support: crushed corn stover. The experiments were carried out with particles of diameter 200 μm . The immobilized enzyme had a maximum activity of 220 Unit/g . The substrate was a soluble starch solution. This complete model had already included

internal diffusion in the particle. Therefore, the Hamdane model is for the whole immobilized enzyme, but not for free enzyme. Although this model did not account for product inhibition, it worked very well in Hamdane's model for the fluidized bed reactor of starch hydrolysis system.

Compared to other systems used to evaluate glucoamylase kinetic parameters (Miranda, 1991, Fujii 1985), Hamdane's system is more similar to the research which is carried out in this thesis. First, Miranda and other researchers used liquid free enzyme. In this research, the enzyme was immobilized in carrigenioe beads with microorganism cell. Therefore the Hamdane's parameters are able to better to describe immobilized enzyme situation. Second, most researchers used batch reactors for free enzyme study. The maximum velocity of glucoamylase is g glucose/L(total enzyme solution volume)/min, which is not suitable for immobilized catalyst in fluidized bed reactor. Hamdane used the differential reactor for the determination of kinetic parameters which was made for simulate the performance of biocatalyst in fluidized bed reactor. The maximum velocity of glucoamylase is mmol/g(immobilized enzyme)/ min. Hamdane's kinetic model worked very well in his model for the fluidized bed reactor system.

The starch consumption rate γ_s was calculated by using Hamdane's glucose production rate model.

$$\gamma_s = \frac{v_s x_e}{Y_{g/s}}$$

where, γ_s = starch consumption rate(g/L catalyst/hr)

v_s = glucose production rate (g/L catalyst/hr)

x_i = enzyme loading (g immobilized enzyme/L catalyst)

$Y_{g/s}$ = glucose yield from starch (g/g)

$$v_s = \frac{v_{sm} C_s}{K_m + C_s}$$

where, v_s = glucose production rate (g/g/hr)

v_{sm} = the maximum glucose production rate (4.104 g/g/hr)

K_m = Michaelis-Menten constant (2.8 g/L)

C_s = substrate starch concentration (g/L total catalyst volume)

Model 2.

Immobilization will effect starch hydrolysis due to mass transfer resistance.

Substrate molecule size, substrate species concentration and biocatalyst size have effects on the pore diffusion limitation on starch hydrolysis using immobilized glucoamylase (Miyamoto, 1973; Swanson, 1978; Lee 1976; Lee 1980). Equations were set up for the irreversible hydrolysis of each linear maltooligosaccharide contained in the feed dextrin from C10 (which included all larger species) through C3 and the oligosaccharide containing one less glucose unit. They were the simplified Michaelis - Menten form:

$$v_i = \frac{v_{\max} C_i}{K_{mi} \left(1 + \sum_{j=1}^{10} C_j / K_{mj} \right)} \quad (4.2.3)$$

where,

C_i = concentration of species i (M/ L fluid phase)

K_{mi} = Michaelis constant of species i (M/L fluid phase)

This model recognized that the hydrolysis followed a multichain mechanism and the possibility of competition among oligosaccharide species for enzyme sites.

This model may yield more accurate predictions of the reaction rate in laboratory and plant settings. However, our experiment lacks some information which is needed in order to fully use this model, such as the identification of different species in the solution, concentration of each species, etc.. Even so, this model can help in understanding the complicated diffusion phenomena in immobilized glucoamylase starch hydrolysis system.

4.3 Mathematical Model for Biocatalyst Bead

Within a biocatalyst bead, the substrate and products are transported by molecular diffusion. In addition, it is here that the biocatalyst actually converts the substrate to products. Thus, the reactions that produce the desired products are not dependent on the bulk fluid concentrations, but rather on the local conditions within the biocatalyst bead. The theory of combined reaction and diffusion has been well developed by researchers in the petroleum industry and by academic people responsive to their problems, starting with the pioneering work of Thiele (1939) and the later contributions

of Aris (1957, 1969), Satterfield (1965, 1970), Petersen (1965), and others. Application to biochemical reactors has been made by Kobayashi (1973), Marsh (1973), Kasch (1974) and Toda (1975), Ramachandran (1976) and others.

In this research, starch pore diffusion affects the ethanol production rate. Starch is converted to glucose by glucoamylase in the biocatalysts. Glucose is then the substrate for the microorganism. It is assumed that the microorganism is distributed uniformly in the beads and the microorganism can obtain the glucose immediately after it is produced inside bead. The effectiveness factor is usually employed to account for the effect of diffusion on the reaction rate. Most effectiveness factor calculations are about the single immobilization in which only one step reaction is involved. In order to calculate the actual reaction rate of glucoamylase and microorganisms in the co-immobilized biocatalyst, the two effectiveness factors relating these two reactions must be calculated. Figure 4.3.1 shows the reactions and mechanism inside the co-immobilized glucoamylase (GA) and *Z. mobilis* biocatalyst.

The following assumptions were made to predict the effective glucoamylase *Z. mobilis* activity of the biocatalyst immobilization:

- 1) negligible resistance of external diffusion
- 2) steady state
- 3) spherical shaped biocatalyst beads of uniform size
- 4) homogeneous distribution of enzyme and microorganism inside the biocatalyst
- 5) constant diffusion coefficient through the biocatalyst

The first effectiveness factor of glucoamylase is defined as the ratio of the actual



The schematic of the co-immobilized microorganism - GA biocatalyst

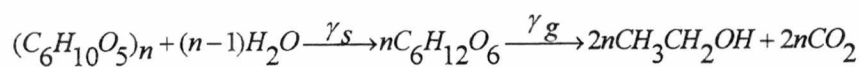
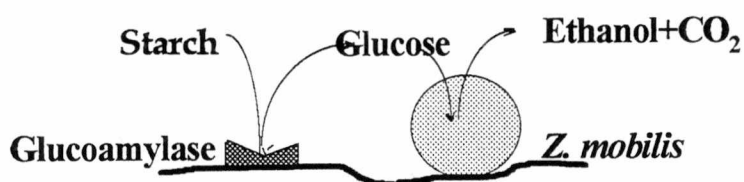


Figure 4.3.1. The reactions and mechanism inside the co-immobilized glucoamylase (GA) and *Z. mobilis* biocatalyst

starch consumption rate of enzyme within the biocatalyst particle to the consumption rate of starch if all the enzyme were exposed to the exterior bulk concentration, C_{sb} . At steady state, the actual starch consumption within a particle equals the total starch transfer to the particle, which is:

$$\frac{4}{3}\pi R^3 \bar{\gamma}_s = -4\pi R^2 \left[D_s \left(\frac{dC_s}{dr} \right)_{r=R} \right] \quad (4.3.1)$$

where,

C_s = concentration of the starch in the biocatalyst

R = radius of the biocatalyst

r = radial distance from the center of the bead

D_s = effective diffusivity of the starch

$\bar{\gamma}_s$ = actual starch consumed rate inside the catalyst

The effectiveness factor η_1 can then be expressed by:

$$\eta_1 = \frac{\bar{\gamma}_s}{\gamma_s} = \frac{4\pi R^2 \left[D_s \left(\frac{dC_s}{dr} \right)_{r=R} \right]}{\frac{4}{3}\pi R^3 (\nu_s)_{C_s=C_{sb}}} \quad (4.3.2)$$

Section 4.1 already discussed the calculation of γ_s . To calculate the theoretical effectiveness factor at each axial-bed bulk concentration, the concentration gradient of starch in the particle near its outer surface $\left(\frac{dC_s}{dr} \right)_{r=R}$ should be obtained. To solve for

$\left(\frac{dC_s}{dr}\right)_{r=R}$, one must solve the mass balance equation with the boundary conditions for

the biocatalyst beads. Using a shell balance, the mass balance equation can be expressed

as:

$$4\pi(r^2 N_{sr})_r - 4\pi(r^2 N_{sr})_{r+\Delta r} - \gamma_s \frac{4}{3}\pi[(r + \Delta r)^3 - r^3] = 0 \quad (4.3.3)$$

$$\begin{aligned} (r^2 N_{sr})_r - (r^2 N_{sr})_{r+\Delta r} - \gamma_s r^2 \Delta r &= 0 \\ -\frac{d(r^2 N_{sr})}{dr} - \gamma_s r^2 &= 0 \end{aligned} \quad (4.3.4)$$

Fick's first law gives:

$$N_{sr} = -D_s \frac{dC_s}{dr} = \text{mass flux of starch in the biocatalyst bead at distance } r$$

from the center of the bead.

Theoretical values of the effectiveness factor are calculated by numerical solution of the equation:

$$\frac{1}{r^2} \frac{d}{dr} \left(r^2 D_s \frac{dC_s}{dr} \right) = \gamma_s \quad (4.3.5)$$

This equation can be written in dimensionless form as:

$$\begin{aligned} \xi = \frac{r}{R}, \quad s_1 = \frac{C_s}{C_0} \\ \frac{1}{\xi^2} \frac{d}{d\xi} \left(\xi^2 \frac{ds_1}{d\xi} \right) = \frac{R^2}{C_0 D_s} \gamma_s \end{aligned} \quad (4.3.6)$$

where,

C_0 = the feed starch concentration

Assuming that the resistance to external mass transfer can be neglected because of mixing in the fluidized bed, then the associated boundary conditions are:

$$\frac{ds_1}{d\xi} = 0 \quad \text{at } \xi = 0 \quad (4.3.7)$$

$$s_1 = s_{1,bulk} = \frac{C_{sb}}{C_0} \quad \text{at } \xi = 1 \quad (4.3.8)$$

The effectiveness factor was calculated as:

$$\eta_1 = \frac{3D_s C_0 \left(\frac{ds_1}{d\xi} \right)_{\xi=1}}{R^2 (\gamma_s)_{\xi=1}} \quad (4.3.9)$$

In order to calculate the effectiveness factor for the microorganism, η_2 , a mass balance for glucose in the bead is needed and expressed by shell balance as:

$$4\pi(r^2 N_{gr})_r - 4\pi(r^2 N_{gr})_{r+\Delta r} + Y_{g/s} \gamma_s \frac{4}{3} \pi [(r + \Delta r)^3 - r^3] - \gamma_g \frac{4}{3} \pi [(r + \Delta r)^3 - r^3] = 0$$

$$-\frac{d(r^2 N_{gr})}{dr} + (Y_{g/s} \gamma_s - \gamma_g) r^2 = 0 \quad (4.3.10)$$

where,

$Y_{g/s}$ = glucose yield from starch

γ_g = glucose consumption rate

$N_{gr} = -D_g \frac{dC_g}{dr}$, mass flux of glucose in the biocatalyst at r .

Equation 4.3.10 can be written in dimensionless form as::

$$\xi = \frac{r}{R}, \quad s_2 = \frac{C_g}{C_0}$$

$$\frac{1}{\xi^2} \frac{d}{d\xi} \left(\xi^2 \frac{ds_2}{d\xi} \right) = \frac{R^2}{C_0 D_g} (\gamma_g - Y_{g/s} \gamma_s) \quad (4.3.11)$$

$$\text{B.C.} \quad \frac{ds_2}{d\xi} = 0 \quad \text{at } \xi = 0 \quad (4.3.12)$$

$$s_2 = s_{2,bulk} = \frac{C_{gb}}{C_0} \quad \text{at } \xi = 1 \quad (4.3.13)$$

where,

C_{gb} = glucose concentration in the bulk phase

D_g = effective diffusivity of the glucose

γ_g = glucose consumption rate

The calculation of the effectiveness factor, η_2 , for the conversion of glucose to ethanol by microorganism is more complicated than the calculation of η_1 . In order to calculate the effectiveness factor η_2 , a conservation of glucose for the whole bead is needed. Figure 4.3.2 shows the two possible mass flow situations. The diffusion direction of glucose totally depends on the glucose concentration gradient in the particle near its outer surface. The glucose inside the bead could diffuse out or the bulk glucose could diffuse into the beads, which depends on the glucose concentration inside the catalyst and the glucose concentration in the bulk solution. This can be expressed as follow equations.

$$\begin{aligned} \text{Total glucose produced from starch} &= \text{Reacted glucose in the bead} \\ &+ \text{glucose diffused out from the bead} \end{aligned}$$

or

$$\begin{aligned} \text{Total glucose produced from starch} &= \text{Reacted glucose in the bead} \\ &- \text{glucose diffused into bead from bulk} \end{aligned}$$

These two possible solutions can be expressed as single mathematical equation 4.3.13.

$$-Y_{g/s} * 4\pi R^2 \left[-D_s \left(\frac{dC_s}{dr} \right)_{r=R} \right] = \frac{4}{3} \pi R^3 \bar{\gamma}_g + 4\pi R^2 \left[-D_g \left(\frac{dC_g}{dr} \right)_{r=R} \right] \quad (4.3.13)$$

The sign of $\left(\frac{dC_g}{dr} \right)_{r=R}$ determines that glucose diffused into the bead or diffuse out the

bead. This equation can be rearranged as:

$$\frac{4}{3} \pi R^3 \bar{\gamma}_g = Y_{g/s} * 4\pi R^2 \left[D_s \left(\frac{dC_s}{dr} \right)_{r=R} \right] - 4\pi R^2 \left[-D_g \left(\frac{dC_g}{dr} \right)_{r=R} \right] \quad (4.3.14)$$

$$\eta_2 = \frac{\bar{\gamma}_g}{(\gamma_g)_{r=R}} = \frac{Y_{g/s} * 4\pi R^2 \left[D_s \left(\frac{dC_s}{dr} \right)_{r=R} \right] - 4\pi R^2 \left[-D_g \left(\frac{dC_g}{dr} \right)_{r=R} \right]}{\frac{4}{3} \pi R^3 (\gamma_g)_{r=R}} \quad (4.3.15)$$

This equation can be written in dimensionless form as:

$$\eta_2 = \frac{3C_0 \left[Y_{g/s} D_s \left(\frac{ds_1}{d\xi} \right)_{\xi=1} + D_g \left(\frac{ds_2}{d\xi} \right)_{\xi=1} \right]}{R^2 (\gamma_g)_{\xi=1}} \quad (4.3.16)$$

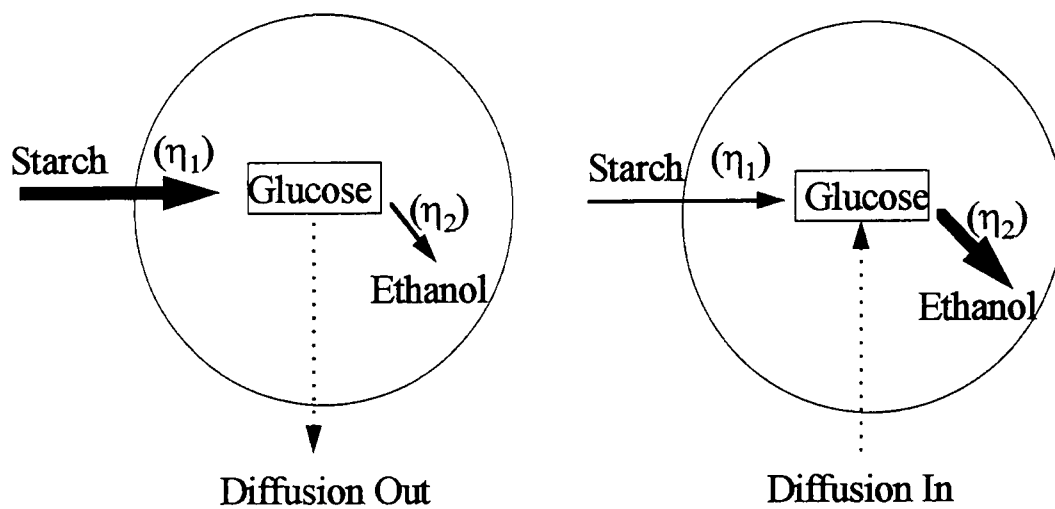


Figure 4.3.2. Mass Flow and two effectiveness factors of the co-immobilized catalyst

The two effectiveness factors are very important in the system modeling. They will be used to calculate the effective reaction rate of glucoamylase and the *Z. mobilis*.

A mass balance for product ethanol (P) gives the following equation:

$$\frac{1}{\xi^2} \frac{d}{d\xi} \left(\xi^2 \frac{dP}{d\xi} \right) = - \frac{R^2 Y_{p/g}}{C_0 D_p} \gamma_g \quad (4.3.17)$$

By using the invariant method, this equation together with starch equation 4.3.6 and glucose equation (4.3.11) obtain the expression of product concentration :

$$P = P_b + Y_{g/s} Y_{p/g} \frac{D_s}{D_p} (s_{1b} - s_1) + Y_{p/g} \frac{D_g}{D_p} (s_{2b} - s_2) \quad (4.3.18)$$

where,

- P = product concentration in the bead
- P_b = bulk product concentration in the reactor
- s_{1b} = starch concentration in the bulk phase
- s₁ = starch concentration in the bead
- s_{2b} = glucose concentration in the bulk phase
- s₂ = glucose concentration in the bead
- Y_{g/s} = glucose yield from starch
- Y_{p/g} = product ethanol yield from glucose

4.4 Model for the Fluidized Bed Reactor

A rectangular coordinate system was first chosen to set up the equations for the cylindrical fluidized bed reactor because these coordinates are much easier to visualize.

Then the rectangular coordinate will be switched to cylindrical coordinates. The mathematical model developed for process simulation starts with the law of conservation of mass for starch (S) in a stationary volume element $\Delta x \Delta y \Delta z$ (see Figure 4.4.1) through which the fluid is flowing. Assuming the system is isothermal.

$$\left\{ \begin{array}{l} \text{time rate of change} \\ \text{of mass of S in the} \\ \text{volume element} \end{array} \right\} = \left\{ \begin{array}{l} \text{rate of mass} \\ \text{of S in} \end{array} \right\} - \left\{ \begin{array}{l} \text{rate of mass} \\ \text{of S out} \end{array} \right\} - \left\{ \begin{array}{l} \text{rate of production of} \\ \text{S by chemical reaction} \end{array} \right\}$$

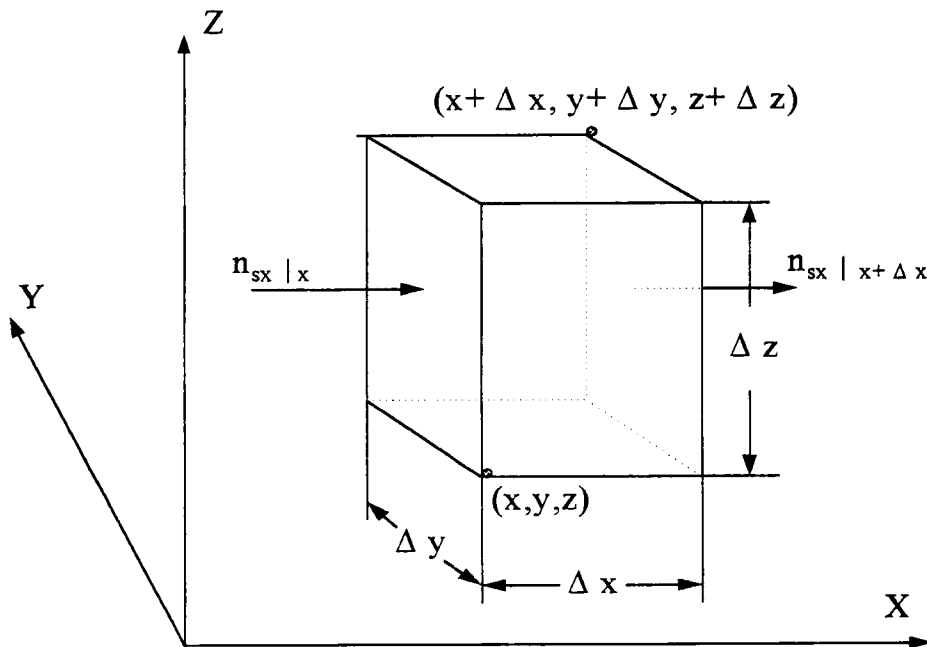


Figure 4.4.1 Stationary volume element $\Delta x \Delta y \Delta z$ through which the fluid is flowing

The various contributions to the mass balance are:

1. time rate of change of mass of S in the volume element

$$\frac{\partial C_s}{\partial t} \Delta x \Delta y \Delta z \quad (4.4.1)$$

where, C_s = starch concentration

t = time

2. rate of production of S by chemical reaction

$$\gamma_s \eta \varepsilon_s \Delta x \Delta y \Delta z \quad (4.4.2)$$

where, γ_s = reaction rate

η = effectiveness factor with C_s as the bulk concentration

ε_s = solid fraction in the reactor

3. input of S across face at x

$$n_{sx} \big|_x \Delta y \Delta z \quad (4.4.3)$$

where, n_{sx} = mass flux

4. output of S across face at $x + \Delta x$

$$n_{sx} \big|_{x+\Delta x} \Delta y \Delta z \quad (4.4.4)$$

Similar expressions may be written for the other two pairs of faces. After dividing by Δx

$\Delta y \Delta z$ and taking the limit as these dimension approach zero, the mass balance then

becomes

$$\frac{\partial C_s}{\partial t} + \left(\frac{\partial n_{sx}}{\partial x} + \frac{\partial n_{sy}}{\partial y} + \frac{\partial n_{sz}}{\partial z} \right) = \gamma_s \eta \varepsilon_s \quad (4.4.5)$$

Thus far the rectangular coordinates x, y, z were used to develop the equation.

For the experimental system, reactor is a cylindrical column. The rectangular coordinates need to be transformed to cylindrical coordinate (Bird, Stewart, Lightfoot, 1960). The derivative operators are readily found to be related as follows:

$$\frac{\partial}{\partial x} = \cos\theta \frac{\partial}{\partial r} + \left(-\frac{\sin\theta}{r}\right) \frac{\partial}{\partial \theta} \quad (4.4.6)$$

$$\frac{\partial}{\partial y} = \sin\theta \frac{\partial}{\partial r} + \left(-\frac{\cos\theta}{r}\right) \frac{\partial}{\partial \theta} \quad (4.4.7)$$

$$\frac{\partial}{\partial z} = \frac{\partial}{\partial z} \quad (4.4.8)$$

Substitution of equations 4.4.6-4.4.8 into equation (4.4.5) gives

$$\frac{\partial C_{st}}{\partial t} + \left(\frac{1}{r} \frac{\partial}{\partial r} (r n_{sr}) + \frac{1}{r} \frac{\partial n_{s\theta}}{\partial \theta} + \frac{\partial n_{sz}}{\partial z} \right) = \gamma_s \eta \varepsilon_s \quad (4.4.9)$$

This is the equation of continuity of starch in the cylindrical reactor. This equation is a general mathematical model for a cylindrical reactor which could be modified according to the system which is going to be simulated. Based on this initial mathematical structure, different assumptions for different systems could be made. For the fluidized bed reactor system in this thesis, the following assumptions are valid:

1) steady state $\left(\frac{\partial C_{st}}{\partial t} = 0 \right)$

2) no radial dispersion $\left(\frac{1}{r} \frac{\partial}{\partial r} (r n_{sr}) = 0 \right)$

3) uniform distribution of substrate, product, and biocatalyst

at cross-sectional area $\left(\frac{1}{r} \frac{\partial n_{s\theta}}{\partial \theta} = 0\right)$

With these assumptions, equation 4.4.9 becomes

$$\frac{\partial n_{sz}}{\partial z} = \gamma_s \eta \varepsilon_s \quad (4.4.10)$$

Rate of mass in or out includes two parts: bulk flow and dispersion.

$$An_{sz} = (V_f C_s)_z - (V_f C_s)_{z+\Delta z} + (AN_s)_z - (AN_s)_{z+\Delta z} \quad (4.4.11)$$

Then 4.4.10 becomes

$$\frac{d(V_f C_s)}{dz} + \frac{d(AN_s)}{dz} + A \eta \varepsilon_s \gamma_s = 0 \quad (4.4.12)$$

where,

$A =$ reactor cross-sectional area

$V_f =$ volume flow rate

A is constant for a cylindrical reactor, but varies with the height for a tapered column reactor (see Figure 4.4.2) .

$$A = \pi r^2 = \pi(r_0 + z \tan \phi)^2 \quad (4.4.13)$$

Another assumption for this system is:

4) The volume flow rate (V_f) is constant along the reactor. Equation (4.4.12) becomes

$$V_f \frac{dC_s}{dz} + A \frac{dN_s}{dz} + N_s \frac{dA}{dz} + A \eta \varepsilon_s \gamma_s = 0 \quad (4.4.14)$$

$$V_f = AU_1 \quad (4.4.15)$$

Where, $U_1 =$ liquid superficial velocity

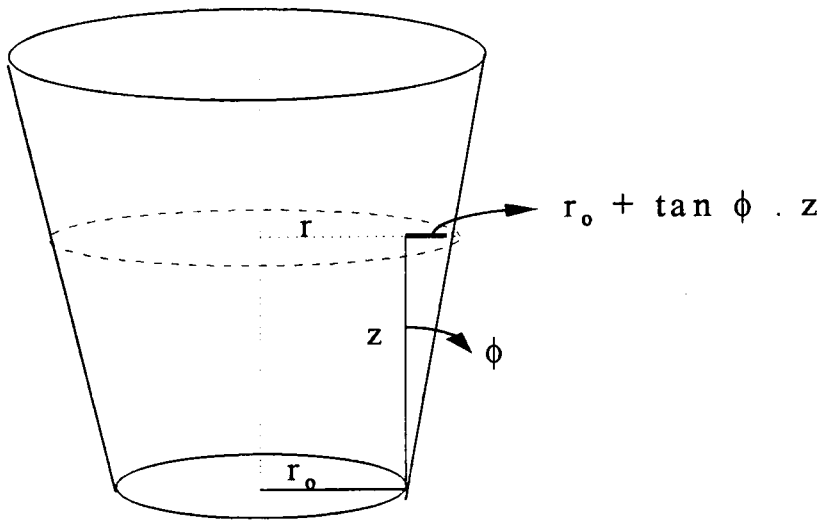


Figure 4.4.2 The cross-sectional area of a tapered column reactor

$$N_s = -D_z \frac{dC_s}{dz}$$

And, D_z = dispersion coefficient

Substitute A, V_f into the equation:

$$\frac{d^2 C_s}{dz^2} + \frac{dC_s}{dz} \left(\frac{2}{r} \tan \phi - \frac{U_l}{D_z} + \frac{1}{D_z} \frac{dD_z}{dz} \right) - \frac{\gamma_s \eta \varepsilon_s}{D_z} = 0 \quad (4.4.16)$$

Make the equation dimensionless by:

$$s_1 = \frac{C_s}{C_0}, \quad z' = \frac{z}{L} \quad (4.4.17)$$

The equation (4.4.16) becomes:

$$\frac{d^2 s_1}{dz'^2} + \frac{ds_1}{dz'} \left(\frac{2L}{r} \tan \phi - \frac{LU_1}{D_z} + \frac{1}{D_z} \frac{dD_z}{dz'} \right) - \frac{\gamma_s \eta_1 \varepsilon_s L^2}{D_z C_0} = 0 \quad (4.4.18)$$

There were two possible boundary conditions for this system: (1) the plug flow boundary condition (simplified boundary condition) assume that the starch flows in a plug flow fashion at the inlet of the reactor ($z'=0$); (2) the Danckwerts boundary condition expresses the fact that the rate at which starch is fed to the reactor is equal to the rate at which it crosses plane $z' = 0$ by combined flow and dispersion (Danckwerts, 1953). The mathematical expressions for these two boundary conditions are given by:

Plug flow boundary condition:

$$\begin{aligned} S_1 - 1 &= 0; & z' &= 0 \\ \frac{dS_1}{dz'} &= 0; & z' &= 1 \end{aligned} \quad (4.4.19)$$

Danckwerts boundary condition:

$$\begin{aligned} S_1 - 1 - \left(\frac{\frac{D_z}{L}}{-\frac{2D_z}{r} \tan \phi + U_1 - \frac{1}{L} \frac{dD_z}{dz'}} \right) \frac{dS_1}{dz'} &= 0; & z' &= 0 \\ \frac{dS_1}{dz'} &= 0; & z' &= 1 \end{aligned} \quad (4.4.20)$$

The solving of the Danckwerts boundary condition needs much more numerical effort than plug flow boundary condition. Therefore, if the difference of the results with respect to these two different boundary conditions is not significant, the plug flow

boundary condition will be used in the model calculation. The comparison of these two boundary conditions will be addressed in Chapter 5.

The same procedure was taken to obtain the equation for glucose

$$\frac{d^2 s_2}{dz'^2} + \frac{ds_2}{dz'} \left(\frac{2L}{r} \tan \phi - \frac{LU_1}{D_z} + \frac{1}{D_z} \frac{dD_z}{dz'} \right) - \frac{(\eta_2 \gamma_s - Y_{g/s} \gamma_s \eta_1) \varepsilon_s L^2}{D_z C_0} = 0 \quad (4.4.21)$$

The boundary conditions for the glucose mass balance equation are:

$$\begin{aligned} s_2 &= 0; & z' &= 0 \\ \frac{ds_2}{dz'} &= 0; & z' &= 1 \end{aligned}$$

Now the problem is to derive an expression for $\frac{dD_z}{dz'}$. Axial dispersion

coefficients of three phase fluidized beds have been well studied by Kim and Han (1992). The Peclet number based on particle diameter in three phase fluidized beds is well represented (Kim and Han, 1992) by:

$$Pe_z = \left(\frac{d_p U_1}{D_z} \right) = \alpha \left(\frac{U_1}{U_1 + U_g} \right)^{1.03} \left(\frac{d_p}{d_c} \right)^{1.66} \quad (4.4.22)$$

where, d_p = particle diameter

d_c = column diameter

The parameter α is a constant whose value is 11.96 and 20.72 for the bubble coalescing and bubble disintegrating regimes respectively.

From equation (4.4.22), the expression for D_z was obtained.

$$D_z = \frac{U_l}{\alpha \left(\frac{U_l}{U_l + U_g} \right)^{1.03} \left(\frac{d_p}{d_c} \right)^{1.66}} \quad (4.4.23)$$

where, $U_l = \frac{V_f}{\pi r_c^2}$ (4.4.24)

r_c = column radius

U_g = gas superficial velocity and assume the ideal gas law is applicable.

$$pV = nRT = \frac{M_{co2}}{mw} RT \quad (4.4.25)$$

where, M_{co2} = the mass of CO₂

mw = CO₂ molecular weight

$$U_g = \frac{M_{co2} RT}{(mw) p \pi r_c^2} \quad (4.4.26)$$

Substitution of equation (4.4.24) and (4.4.25) into equation (4.4.23) gives

$$D_z = \frac{2 \left(V_f + \frac{M_{co2} RT}{(mw) p} \right)^{1.03}}{\alpha \pi r_c^{0.34} V_f^{0.03} r_p^{0.66}} \quad (4.4.27)$$

The hydrostatic head of the liquid is used to calculate the pressure at any given axial position.

$$p = k(L - z) + p_0 \quad (4.4.28)$$

where, p_0 is the pressure at the top of the column. The factor $k(L-z)$ accounts for the static head of the liquid. Substitution of p into equation 4.4.27 and taking the first derivative of D_z with respect to z' :

$$\frac{dD_z}{dz'} = LD_z \left[\frac{1.03RT}{(mw)Q} \left(\frac{kM_{co2}}{p^2} + \frac{1}{Lp} \frac{dM_{co2}}{dz'} \right) - \frac{0.34 \tan \phi}{r_c} \right] \quad (4.4.29)$$

$$Q = V_f + \frac{M_{co2}RT}{p} \quad (4.4.30)$$

It is necessary to define $\frac{dM_{co2}}{dz'}$. Assume the gas is generated by the reaction and that it

flows in a plug flow fashion through the reactor in the gas phase.

$$(M_{co2})_z - (M_{co2})_{z+\Delta z} = \varepsilon_s \Delta z A \gamma_g \eta_g Y_{co2/g} \quad (4.4.31)$$

where $A = \frac{V_f}{U_l}$

$$\frac{dM_{co2}}{dz'} = L \varepsilon_s \gamma_g \eta_g Y_{co2/g} \frac{V_f}{U_l} \quad (4.4.32)$$

The vast majority of the CO₂ produced from fermentation will move into the gas phase and produce bubbles. These bubbles will then occupy some of the reactor volume, reducing the amount of volume that is available for the biocatalyst. Several researchers have shown that the solid holdup is a function of both the gas and liquid superficial velocities within a fluidized bed. Thus, as CO₂ is produced and the gas velocity increases, not only the gas but also the solid holdup will be affected. To predict the gas, liquid, and solid holdup, the widely accepted correlation of Begovich and Watson (1978) was used. Their expressions can be written as

$$\varepsilon_g = \alpha U_g^\beta d_p^\gamma D_c^\delta \quad (4.4.33)$$

$$\varepsilon_l = \sigma U_l^\zeta U_g^\eta (\rho_s - \rho_l)^\theta d_p^l \mu_l^\kappa D_c^\lambda - \varepsilon_g \quad (4.4.34)$$

$$\varepsilon_s = 1 - \varepsilon_g - \varepsilon_l \quad (4.4.35)$$

Previously, Davison (1990) had measured the various phase holdups within advanced fluidized bed reactors such as those employed in this study. Based upon the 39 hydrodynamic experiments reported by Davison, the parameters of the Begovich and Watson correlation for low density catalyst bead are given in Table 4.

4.5 Computer Code Block Diagram

Based upon the modeling equations, a computer program will be developed to perform the desired calculations. In this program, the FORTRAN software package COLSYS (Ascher, Christiansen and Russel; 1979, 1981, 1981) was used to solve the mixed- order system of boundary value problems in ordinary differential equations. The method used was spline collocation at Gaussian points by using piecewise polynomial functions within an adjustable number of finite elements. COLSYS adaptively adjusts the location of the elements to achieve the desired solution accuracy. The inputs to COLSYS are:

SUBROUTINE COLSYS (NCOMP, M, ALEFT, ARIGHT, ZETA, IPAR, LTOL, TOL, FIXPNT, ISPACE, FSPACE, IFLAG, FSUB, DFSUB, GSUB, DGSUB, SOLUTN)

Among these variables, FSUB, DFSUB, GSUB, DGSUB, and SOLUTN are user supplied external subroutines. In order to use this software properly, there is a need to change the modeling equations into the software defined format.

Table 4 **Begovich and Watson Parameters Used to Describe Data of Davison**

Parameter	Value
α	0.058
β	0.692
γ	0.107
δ	-0.037
σ	0.388
ζ	0.260
η	0.036
θ	-0.327
I	-0.278
κ	0.047
λ	-0.022

COLSYS problem definition:

The mixed-order system of d nonlinear differential equations of orders $1 \leq m_1 \leq m_2 \leq \dots \leq m_d \leq 4$ (F function),

$$\mu_n^{(m_n)}(x) = F_n(x; z(u)), \quad a < x < b \quad n = 1, \dots, d \quad (4.5.1)$$

where the desired solution $u(x) = (\mu_1(x), \dots, \mu_d(x))$ is an isolated solution vector and

$z(u) = (u_1, u_1', \dots, u^{(m_1-1)}, u_2, \dots, u_d, \dots, u_d^{(m_d-1)})$ is the vector of unknowns that would result

from converting the F function (4.5.1) to a first-order system. The system is subject to m^*

$= \sum_{n=1}^d m_n$ nonlinear multipoint separated boundary conditions (G function)

$$G_j(\zeta_j; z(u)) = 0, \quad j = 1, \dots, m^*$$

where ζ_j is the location of the j th boundary (or side) condition, $a \leq \zeta_1 \leq \zeta_2 \leq \dots \leq \zeta_m \leq b$.

In this research, the biocatalyst and fluidized bed reactor modeling equations which were discussed in chapter 4.3 and 4.4 will be changed into the F function format in subroutine FSUB1 and FSUB2 respectively. Boundary condition equations will be changed into the G function format in subroutine GSUB1 and GSUB2. Also the JACOBIAN subroutines, DFSUB1, DFSUB2, DGSUB1, DGSUB2 are made for FSUB1, FSUB2, GSUB1 and GSUB2 respectively. The JACOBIAN can be defined as the partial derivative of the differential equation with respect to each of the dependent variables.

Other input variables include :

NCOMP - number of differential equations (ncomp .le. 20)

M(j) - order of the j -th differential equation

ALEFT - left end of interval

ARIGHT - right end of interval

ZETA(j) - j-th side condition point (boundary point).

IPAR - an integer array dimensioned at least 11.

LTOL - an array of dimension ipar(4). ltol(j) = 1 specifies that the j-th tolerance in tol controls the error in the l-th component of z(u).

TOL - an array of dimension ipar(4). tol(j) is the error tolerance on the ltol(j) -th component of z(u).

FIXPNT - an array of dimension ipar(11). it contains the points, other than aleft and aright, which are to be included in every mesh.

ISPACE - an integer work array of dimension ipar(6). its size provides a constraint on nmax, the maximum number of subintervals.

FSPACE - a real work array of dimension ipar(5). its size provides a constraint on nmax.

IFLAG - the mode of return from colsys.

Outputs from COLSYS will depend on the user. The user may produce the solution vector $z(u(x))$ at a point x , $aleft \leq \xi \leq aright$ by calling : CALL APPSLN (x, z, fspace, ispace)

Once subroutine and input parameters have been set, the biocatalyst equations will be solved first by using COLSYS, which includes mass balance equations for starch (4.3.6) and glucose (4.3.11). The biocatalyst bead radius is the independent variable. Since the reactions happen simultaneously, the equations will be solved simultaneously. The bulk concentrations of starch and glucose will be given at certain intervals as a

constant. Two tables of first and second effectiveness factors will be developed. In each table, the effectiveness factors with respect to different bulk concentrations will be calculated. The bulk concentrations cover the permissible ranges of the starch and glucose bulk concentrations of the actual experimental system. The functions of first and second effectiveness factors will be made by using an interpolation method. By using these functions, the effectiveness factors corresponding to certain bulk concentration, which is in the concentration range of the effectiveness factor table, can be obtained. This method will reduce the degree of complication which would be caused if the effectiveness factors were calculated in every step of the reactor mathematical model solving loop. The table of the glucoamylase reaction effectiveness factor (η_1) verses the starch bulk concentration (C_{1b}) is two dimensional. The interpolation process is:

$$\eta_1^* = \eta_1^n + \frac{(C_{1b}^* - C_{1b}^n)(\eta_1^{n+1} - \eta_1^n)}{C_{1b}^{n+1} - C_{1b}^n}, \quad C_{1b}^n \leq C_{1b}^* \leq C_{1b}^{n+1}$$

where, η_1^n corresponds to C_{1b}^n in the effectiveness factor table.

η_1^{n+1} corresponds to C_{1b}^{n+1} in the effectiveness factor table.

C_{1b}^* is the bulk concentration between C_{1b}^n and C_{1b}^{n+1} .

η_1^* is the desired effectiveness factor corresponds to C_{1b}^* .

On the other hand, the table of the *Z. mobilis* reaction effectiveness factor η_2 verses the starch bulk concentration (C_{1b}) and glucose bulk concentration (C_{2b}) is three dimensional and a one dimension interpolation will be conducted three times to obtain η_2^*

(see Figure 4.5.1). In the first step, η_2^{i*1} is calculated, and in the second step, η_2^{i*2} is calculated. Finally η_2^* can be obtained.

When solving the reactor modeling equations, the reactor length is the independent variable. Mass balance equations for starch and glucose in the fluidized bed and the CO_2 equation will be solved simultaneously with reactor boundary condition equations by COLSYS. The effectiveness factor will be obtained from the effectiveness factor table which was built in the biocatalyst part. An outline of the computer program sequencing appears as Figure 4.5.2

It is important to realize that a sufficiently good start for the nonlinear iteration must sometimes consist of both a good initial solution profile and an adequate initial mesh. Failure to have one of these good initial guesses may cause the code to repeatedly halve the mesh without obtaining convergence of the nonlinear iteration until storage limitations are reached.

	C_{2b}^1	$\dots$	C_{2b}^i	C_{2b}^*	C_{2b}^{i+1}	$\dots$
C_{1b}^1	η_2	$\dots$	$\dots$	$\dots$	$\dots$	η_2
$\vdots$	$\vdots$	$\ddots$				$\vdots$
C_{1b}^k	$\vdots$		η_2^{ik}	η_2^{i*1}	$\eta_2^{(i+1)k}$	$\vdots$
C_{1b}^*	$\vdots$			$\begin{bmatrix} \eta_2^* \end{bmatrix}$		$\vdots$
C_{1b}^{k+1}	$\vdots$		$\eta_2^{i(k+1)}$	η_2^{i*2}	$\eta_2^{(i+1)(k+1)}$	$\vdots$
$\vdots$	η_2	$\dots$	$\dots$	$\dots$	$\dots$	η_2

Figure 4.5.1 Effectiveness factor for microorganism reaction

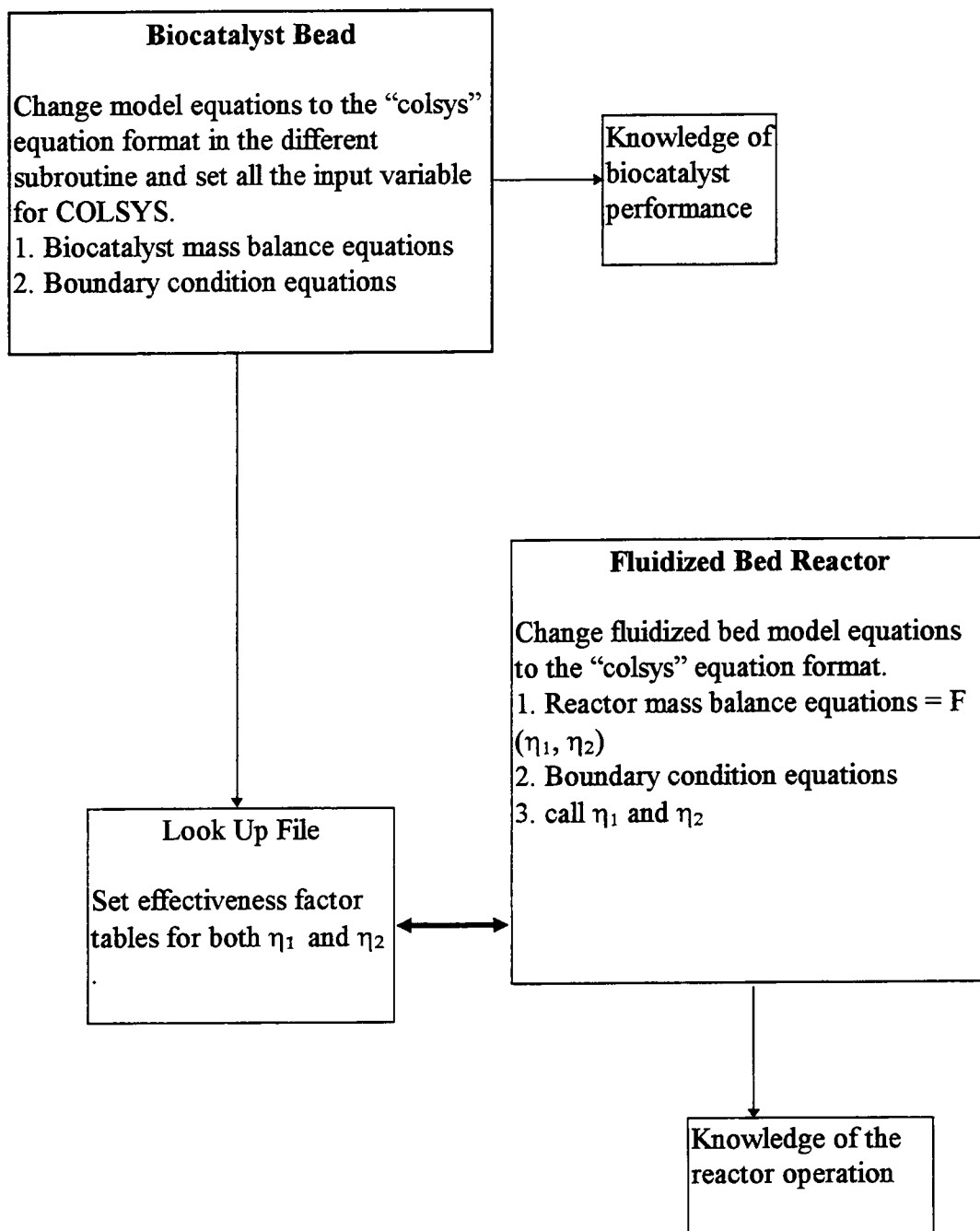


Figure 4.5.2 Computer code block diagram

CHAPTER 5 RESULTS AND DISCUSSIONS

The results of the mathematical model for fluidized-bed reactor with co-immobilized catalyst will be compared with the experimental data. The general applications of the model will be addressed in this chapter. Base case analysis represents the results of the model under the experimental conditions. The general case analysis discusses the generic and predictive applications of the model under different conditions.

5.1 Base Case Study

5.1.1 The generation of the effectiveness factor table

Before the calculation of the reactor mathematical model equations, the first step is to generate effectiveness factor tables. As discussed in section 4.3 of chapter 4, in the entrapped co-immobilization system, the gel does introduce an additional mass transfer resistance. An analysis of the catalyst performance show that diffusion of substrate plays an important role in the immobilized catalyst system. The bulk fluid concentrations are very different from the concentration inside the catalyst bead. The reactions occurring within the catalyst are not dependent on the bulk fluid condition, but rather on the local conditions within the biocatalyst bead. However, the experimentally measurable concentrations are bulk concentrations. The kinetic model used in the simulation employs bulk concentrations. Effectiveness factors are employed to account for the effect of diffusion on the reaction rate and correct for the fact that the actual concentration is different from the bulk. Two effectiveness factors for two reactions, the

enzymatic reaction and the microorganism reaction, are calculated using equations derived in section 4.3 respectively. When the computer program solves the biocatalyst mathematical model equations, it also generates the values of $\left(\frac{dS_1}{d\xi}\right)_{\xi=1}$ and

$\left(\frac{dS_2}{d\xi}\right)_{\xi=1}$ which are included in the effectiveness factor mathematical expression.

For the *Zymomonas mobilis* - GA system (Section 3.2), the computer generated the two effectiveness factors. The first effectiveness factor table is a matrix of 2 by 101 which means that there are 101 η_1 corresponding to 101 different bulk starch concentrations. Figure 5.1.1 and Figure 5.1.2 are the first effectiveness factor (η_1) charts which give values of η_1 for different bulk starch concentrations. As discussed before, the starch concentration inside the bead is not affected by glucose. Consequently, the η_1 chart is a two dimensional figure (η_1 versus bulk starch concentration). The first effectiveness factor will be used to modify the enzyme reaction rate which is calculated by the starch bulk concentration. All the values of η_1 are smaller than 1.0. In Figure 5.1.1, only the Y axis is a logarithmic scale and helps to visualize η_1 at high bulk concentration. It is obvious that η_1 is essentially unity until the bulk starch concentration is reduced to a value slightly below 100 g/L. Figure 5.1.2 uses a logarithmic scale for both the X and Y axis to show η_1 at low bulk starch concentration. In the range between 100 and 0.5 g/L, the effectiveness factor decreases as a smaller portion of each bead is used for the reaction. Figure 5.1.2 shows that when

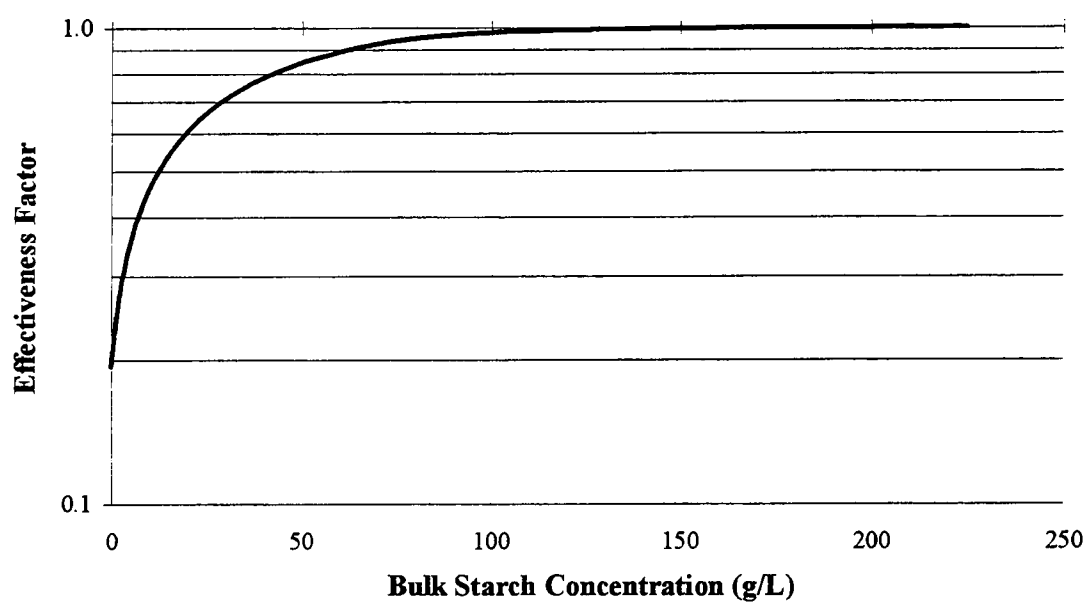


Figure 5.1.1 The first effectiveness factor table

(η_1 vs. bulk starch concentration) with linear Y axis for GA level of 150 ml

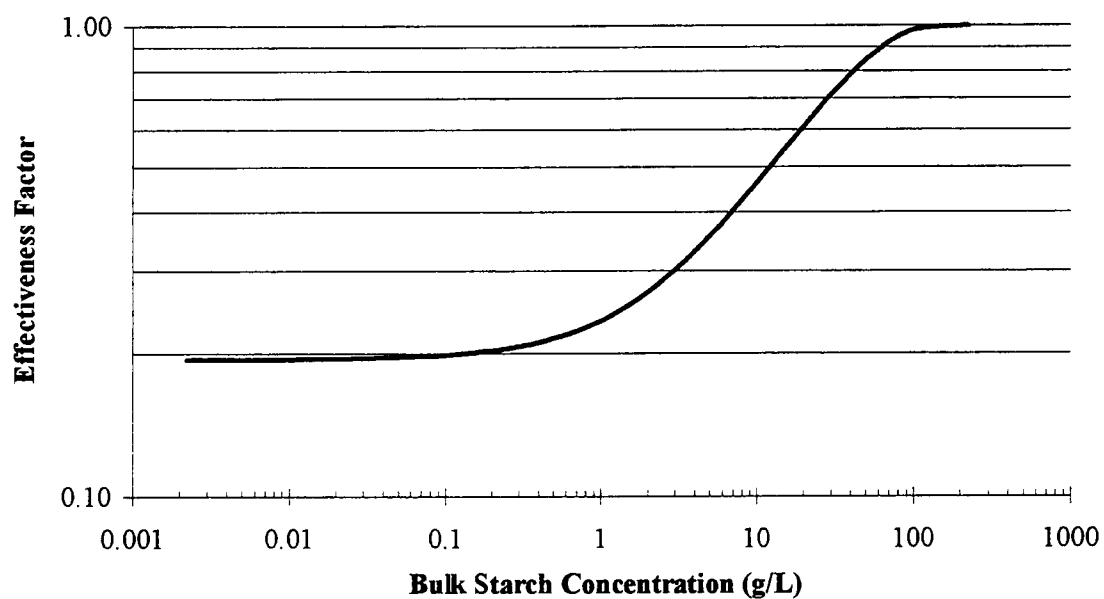


Figure 5.1.2 The first effectiveness factor table

(η_1 vs. bulk starch concentration) with logarithmic Y axis for GA level of 150 ml

the bulk concentration is below about 0.5 g/L, η_1 is relatively constant at a small value.

The reason of this is that only a small ring on the outside of each bead would be used for reaction at very low bulk concentration. The starch concentration profile inside the bead with different bulk starch concentrations is described in detail in Section 5.1.2.

The difference between the first effectiveness factor and the second effectiveness factor is that η_1 only relates to bulk starch concentration, but η_2 relates to both bulk starch concentration and bulk glucose concentration. The second effectiveness factor table is a matrix 101 by 101 which means that there are 101 η_2 corresponding to 101 different bulk starch concentrations and 101 different bulk glucose concentrations. The effectiveness factor chart for η_2 is three dimensional (Figure 5.1.3).

In order to analyze the second effectiveness factor, a two dimensional plot is prepared (see Figure 5.1.4). The second effectiveness factor is plotted with the different bulk glucose concentrations at three different bulk starch concentrations. At higher bulk glucose concentrations, the second effectiveness factor exhibits a trend similar to the first effectiveness factor. However, when the bulk glucose concentration is lower than some value which depends on different bulk starch concentration, there are turning points at which η_2 suddenly increases and become far larger than one. The values of bulk glucose concentrations corresponding to the η_2 turning points depend on the bulk starch concentrations. At higher starch concentrations, the value of the bulk glucose concentration corresponding to the turning point is higher. The explanation for this

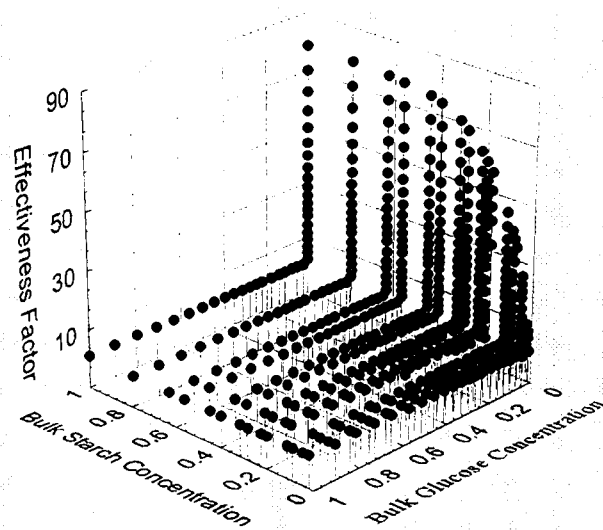


Figure 5.1.3 The second effectiveness factor (η_2) chart.

(η_2 vs. bulk starch concentration and bulk glucose concentration)

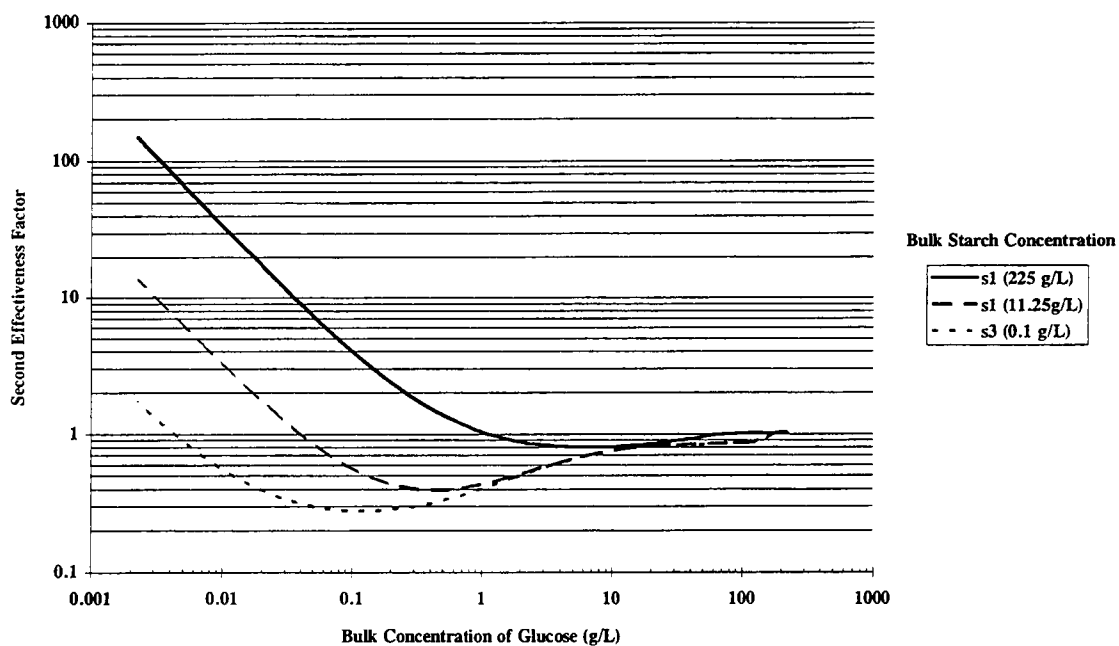


Figure 5.1.4 The second effectiveness factor on the two dimensional plot

phenomenon is that the starch diffusing into the catalyst converts to glucose. Therefore, at this certain point (turning point), the concentration of glucose inside the catalyst is higher than the bulk glucose concentration. In this situation, the real reaction rate inside the catalyst is higher than the reaction rate calculated using bulk glucose concentration. The second effectiveness factor is larger than one, which is used to multiply the calculated reaction rate to get the real reaction rate inside the catalyst. As a result, at very low bulk glucose concentration and high bulk starch concentration, the second effectiveness factor could be very large. This situation could happen at the bottom of the fluidized bed reactor. At the bottom of the reactor, the second reaction rate calculated by the bulk concentration could be very small because of the low bulk glucose concentration. However, high bulk starch concentration causes more starch to diffuse into the catalyst bead and this starch is converted into glucose. The concentration of glucose inside the bead is higher than the bulk concentration. The real second reaction rate is faster than the one calculated by the bulk concentration.

5.1.2 The performance of co-immobilized biocatalyst

Mathematical model equations for biocatalyst bead are solved numerically and the starch concentration profile inside the bead is generated using the solution of the differential equations. In Section 5.1.1, it has been discussed that the value of the effectiveness factor depends on the starch concentration in the bulk. Figure 5.1.5 represents the starch concentration inside the bead along the radial distance from the

center of the bead at the different bulk starch concentrations and helps in understanding the effectiveness factor profile in Section 5.1.1. Figure 5.1.5 shows that the higher the bulk starch concentration is, the more starch diffuses into the beads. When the bulk starch concentration is above 100 g/L, starch penetrates throughout the bead. At this condition, the catalyst is fully utilized. With the bulk starch concentration below 100 g/L, starch only diffuses into part of the bead volume. For example, at 45 g/L bulk starch concentration only 85% of the bead volume is used for reaction. While the bulk concentration is reduced, the used bead volume is reduced as well. At very low bulk starch concentrations such as below 0.5 g/L, starch only diffuses in the very thin ring of the bead and only a very small portion of each bead is used for reaction. This figure strongly proves the discussion of the effectiveness factors in Section 5.1.1.

In the fluidized bed reactor, the bulk starch concentration varies with the different axial positions. The catalyst can be used more efficiently at higher bulk substrate concentrations because of high substrate concentration inside the bead (Figure 5.1.5). In the fluidized - bed reactor, the starch concentration decreases along the axial position (this will be discussed in more detail in Section 5.1.3). Therefore, the biocatalyst beads located at the lower position of the reactor are used more efficiently than the beads at the higher position of the reactor. This explains the phenomenon observed during the experiment; the beads at the bottom always appear different from the beads at the top. At the bottom of the reactor, the starch concentration is higher and more starch diffuses into the beads. As a result, for the consecutive reactions, more glucose can be produced and there is more glucose available for microorganism growth

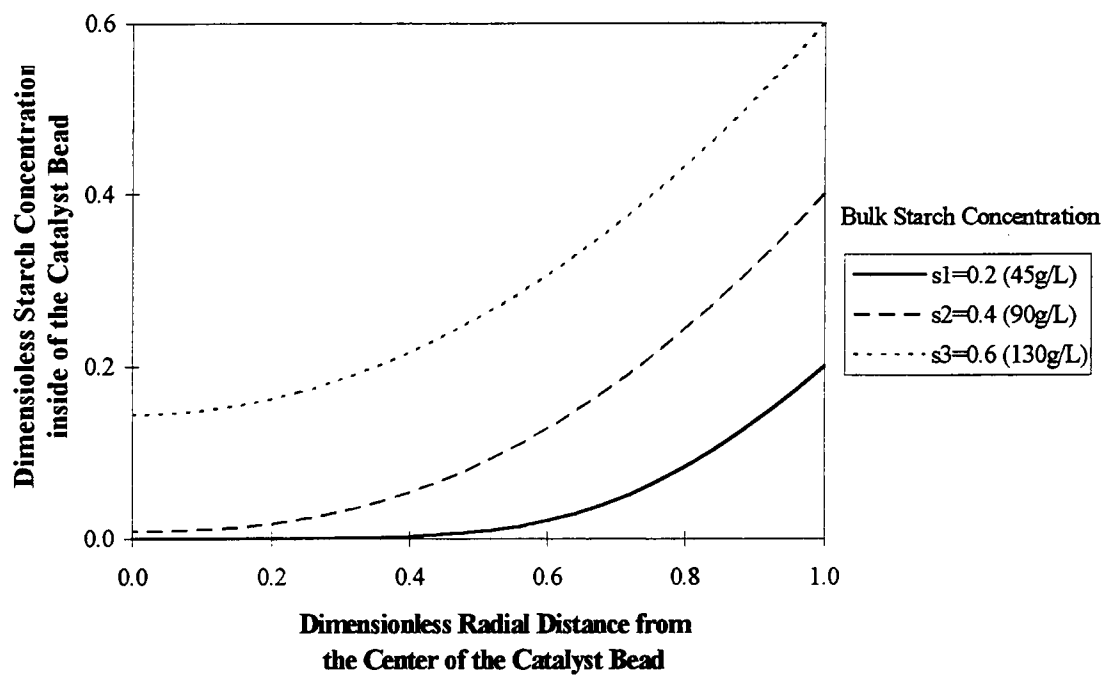


Figure 5.1.5 Starch concentration profile inside the bead along the radial distance from the center of the bead at the different bulk starch concentrations.

and the production product. These two process make the beads at the bottom of the reactor look different from those at the top . Generally they have a lighter color and produce more ethanol. Consequently, ethanol productivity of the bottom section of the reactor is higher than other sections. This further explains the experimental data in section 3.4. The experimental data shows that the volumetric productivity changes among the different sections of the reactor and the productivity of the bottom section is higher than other sections.

5.1.3 The results for fluidized - bed reactor

After the effectiveness factors are generated, the individual effectiveness factor which corresponds to a certain bulk concentration is then calculated through the linear interpolation methods from the exit effectiveness factor tables. The details of these methods have been described in Section 4.5. After the effectiveness factors are available, the real reaction rate can be calculated and the mathematical equations for the reactor can be numerically solved.

Boundary condition influence

The boundary conditions of the differential equations for the fluidized - bed reactor have been discussed in Section 4.4. Relative to the plug flow boundary condition, the Danckwerts boundary condition equation needs much more numerical effort to be solved. The necessity of the Danckwerts boundary condition for this system has been

tested under different conditions. Results using the plug flow boundary condition and Danckwerts boundary condition are compared in Figures 5.1.6 and 5.1.7 under various conditions. In Figure 5.1.6, the flow rate is 0.6 L/hr, the enzyme loading is 150 ml, and the catalyst bead diameter is 0.2 mm. In Figure 5.1.7, the flow rate is 2 L/hr, the enzyme loading is 375 ml, and the catalyst bead diameter is 0.4 mm. The numerical results from the model show no difference with two different boundary conditions. However, using the plug flow boundary condition reduces the numerical effort and calculation time. Therefore, in order to reduce the complexity of the calculation, the plug flow boundary condition can be used without introducing error.

Concentration profiles along the reactor

The concentration profiles under different operation conditions are generated and compared with the experimental data. The predicted starch, glucose, and ethanol concentrations are presented in Figures 5.1.8, and 5.1.9. These two experiments were conducted under a different flow rate and feed concentration. In general, the model predictions compared favorably with the experimental data. The model results show the correct concentration profile trends and behaviors such as decreasing starch concentration, increasing ethanol concentration, and very low glucose concentration along the axial position of the reactor.

The model results predict more conversion than actually occurred. The difference observed between the model predictions and the experimentally determined profiles may be due to a variety of factors. For example, some errors may be due to

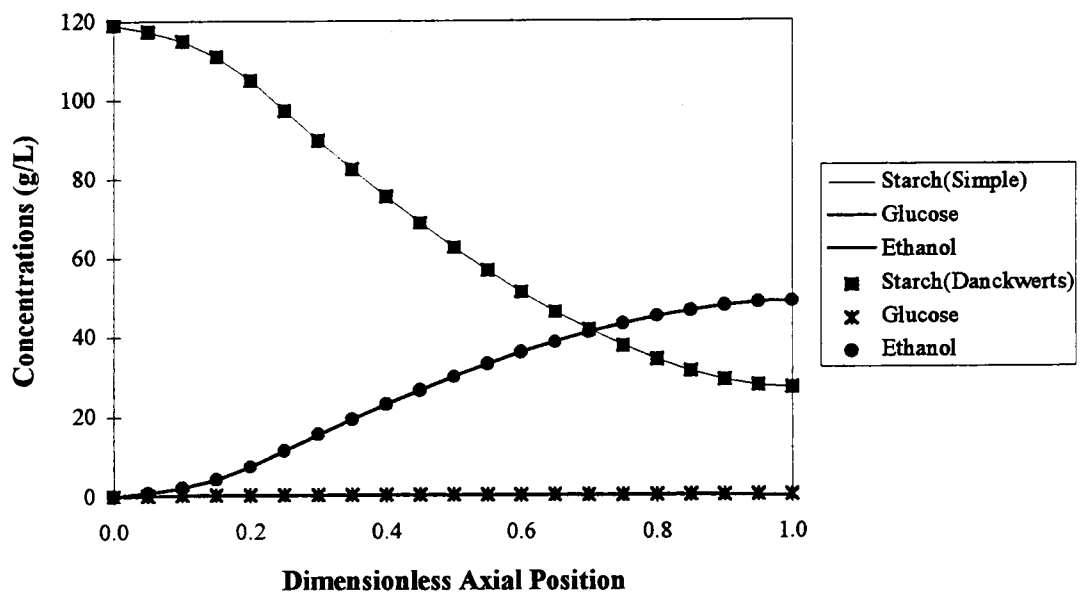


Figure 5.1.6 The comparison of the mathematical model results solved using the plug flow boundary condition (simple) and the Danckwerts boundary condition. (flow rate = 0.6 L/hr, enzyme loading = 150 ml, and catalyst bead diameter = 0.2 mm)

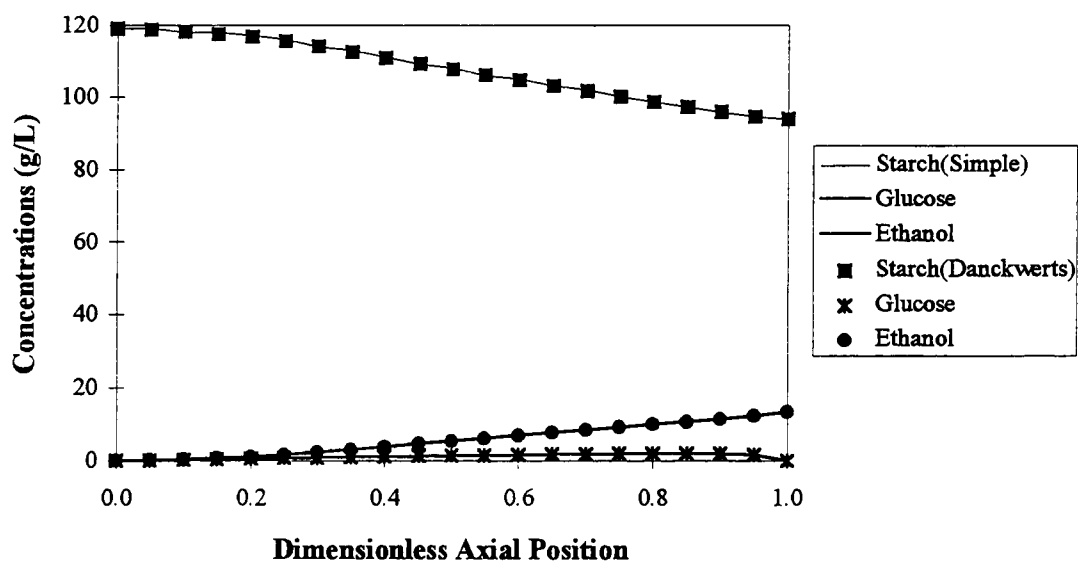


Figure 5.1.7 The comparison of the mathematical model results solved using the plug flow boundary condition (simple) and the Danckwerts boundary condition. (flow rate is 2 L/hr, enzyme loading = 375 ml, and catalyst bead diameter = 0.4 mm)

experimental uncertainty, such as uncertainty in the density of the beads, and in the amount of enzyme immobilized within each bead. The beads at the top of the reactor are generally less dense than the beads at the bottom of the reactor. This may be caused by reduction in enzyme particle loading. Reduction of enzyme causes less starch conversion and less ethanol production. Therefore, there are more differences between the model results and experimental data at the top of the reactor. Additional errors may be due to the mathematical model assumptions such as assuming all the beads are uniform geometrically and characteristically, such as having the same enzyme loading and the same composition in each bead.

Dispersion coefficient

The dispersion coefficient is predicted to be a function of axial position. The predictions are shown on Figure 5.1.10 of the two cases. The dispersion coefficient varies by more than an order of magnitude. Thus, the reactor can be considered to act as almost plug flow near the bottom of the column, and to behave in a manner similar to a continuous-stirred tank reactor (CSTR) near the top. For the higher feed flow rate, the dispersion coefficient at the bottom of the reactor is higher than the one at low feed flow rate reactor. This shows that higher flow rate causes more dispersion than low flow rate. However, the dispersion at the top of the reactor reaches almost the same level. This means that the dispersion coefficient changing along the reactor is larger for the low flow rate. The reason of this phenomenon is that at lower flow rate experiments, there is more CO_2 produced and CO_2 increases the dispersion in the reactor.

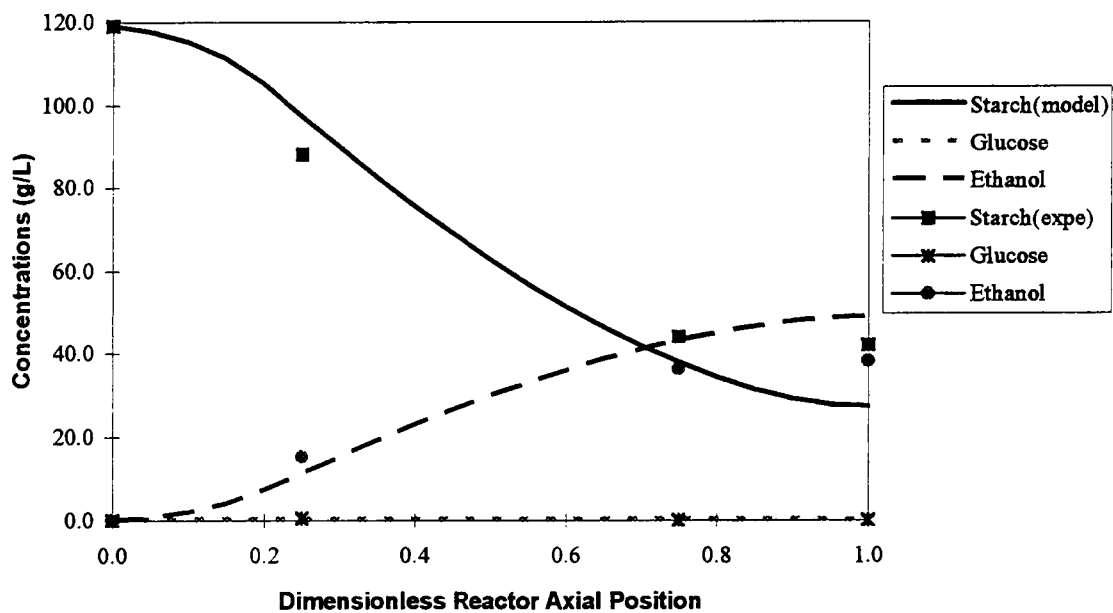


Figure 5.1.8 The starch concentration profile comparison between the model results and the experimental data. (experimental condition: flow rate = 0.6 L/hr, feed concentration = 119 g/L)

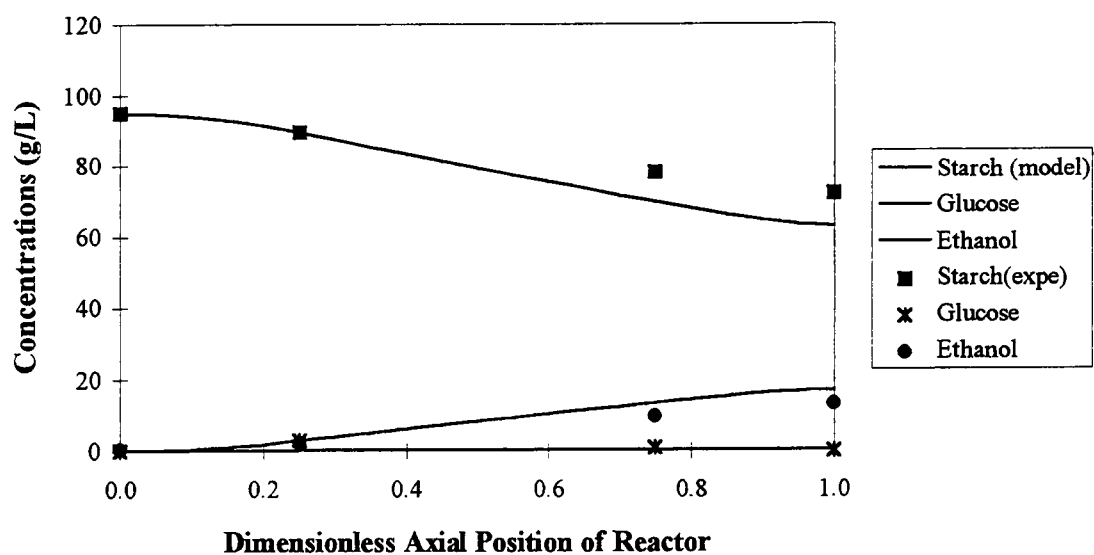


Figure 5.1.9 The starch concentration profile comparison between the model results and the experimental data. (Experiment condition: Flow rate = 2 L/hr, Feed concentration = 95 g/L)

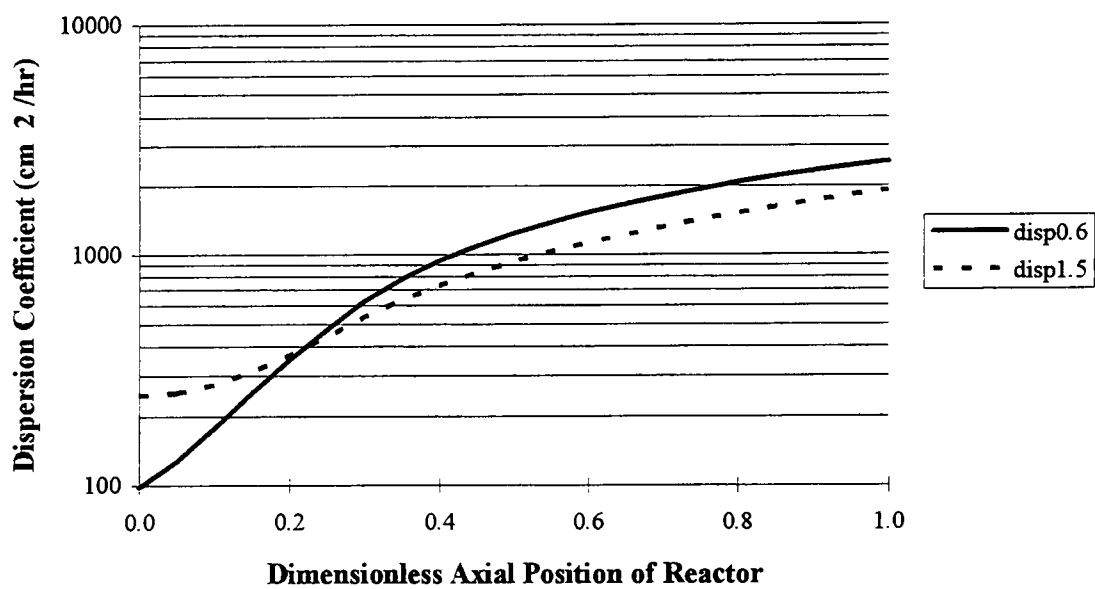


Figure 5.1.10 Dispersion coefficient as a function of axial position for the two experimental conditions: (1) flow rate = 0.6 L/hr, feed concentration = 119 g/L
(2) flow rate = 1.5 L/hr, feed concentration = 95 g/L

5.2 General Case Study

Mathematical models have been used on many occasions to describe bioreactor performance. Predictive results can be obtained for different experimental parameters, such as, enzyme loading, catalyst size, and feed flow rate, etc. These results give a better understanding of the system properties and help to design and optimize the experiments.

5.2.1 Analysis of Biocatalyst Performance Using Mathematical Model Solutions

There are several factors that affect the performance of the co-immobilized catalyst such as bulk starch concentration, bulk glucose concentration, catalyst bead size, and enzyme loading. The performance of the biocatalyst under different circumstances can be predicted using a mathematical model.

Figure 5.2.1 represents the starch concentration profile in the catalyst at different bulk starch concentrations. A 2 mm bead with an enzyme loading of 375 mL is used. The maximum dimensionless bulk concentration, $S_b = 1$, corresponds to 225 g/L of starch. Higher bulk starch concentrations result in higher diffusion rates into the beads. As a result, a greater concentration of the intermediate (glucose) occurs which is then available as a substrate for the microorganism. This has been discussed in Section 5.1.2. Figure 5.2.2 shows glucose concentration profiles corresponding to the starch

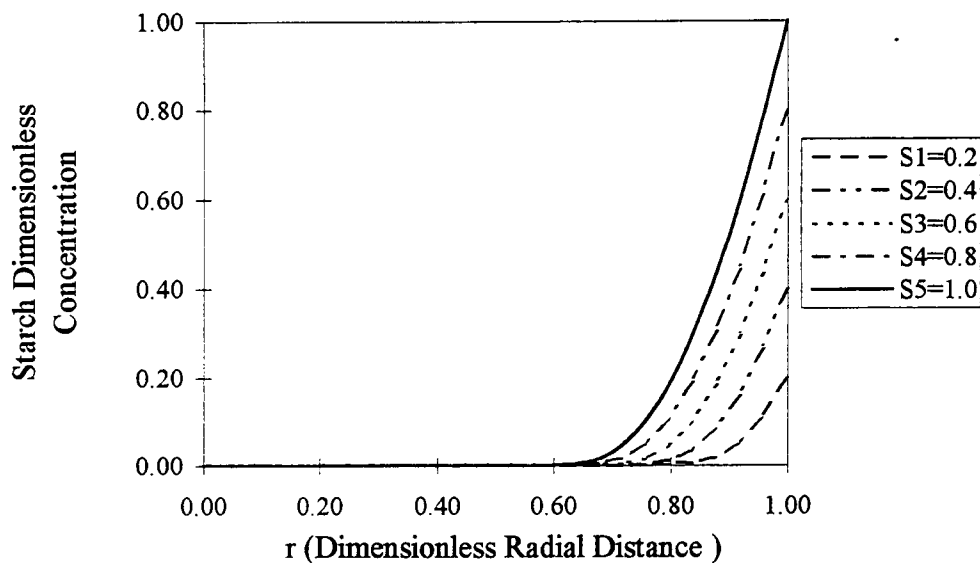


Figure 5.2.1. The starch concentration profile in the catalyst bead at different bulk starch concentrations.

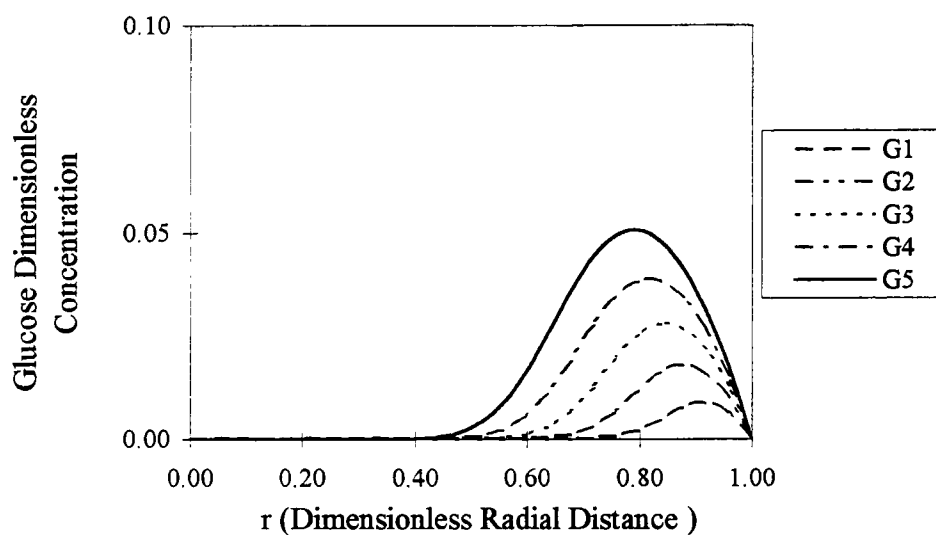


Figure 5.2.2. Glucose concentration profiles corresponding to the different starch bulk concentrations. Glucose concentration G1 corresponds to bulk starch concentration $S_b = 0.2$; G2 - $S_b=0.4$; G3 - $S_b=0.6$; G4 - $S_b=0.8$; G5 - $S_b=1.0$

concentrations in Figure 5.2.1. This figure shows that higher bulk starch concentrations causes higher glucose concentration in the bead.

Under some conditions, in the co-immobilized biocatalyst reactor system, the intermediate can accumulate in the reactor. For the ethanol fermentation, the intermediate is glucose. The mathematical model can predict both starch and glucose concentration profiles at different conditions. Figure 5.2.3A and 5.2.3B represent the concentration profiles in the bead at different bulk starch and bulk glucose concentrations. The effect of bulk starch concentration on the bead glucose concentration is shown in Figure 5.2.2.

Figure 5.2.1 shows that starch only partially diffuses into the bead. Even at the highest starch concentration, starch can only be observed at a radial distance of 0.65. This indicates that only 72% of the catalyst volume is used. Besides the bulk concentration influence, starch diffusivity has a significant impact on the concentration profile. Figure 5.2.4 depicts starch and glucose concentrations inside the beads at different starch diffusion coefficients. High starch diffusivity increases the starch concentration inside the bead and enlarges the volume of catalyst used. At the high starch diffusion coefficient ($D_s = 0.008 \text{ cm}^2/\text{hr}$), starch diffuses into the bead as far as $r = 0.4$. This means that about 93.6% of the catalyst volume is used. In this case, glucose can penetrate all the way to the center of the bead and this will increase the catalyst efficiency.

Another concern for the biocatalyst efficiency is the bead size. In cases where a low starch diffusion coefficient occurs, reducing the size of the bead could save

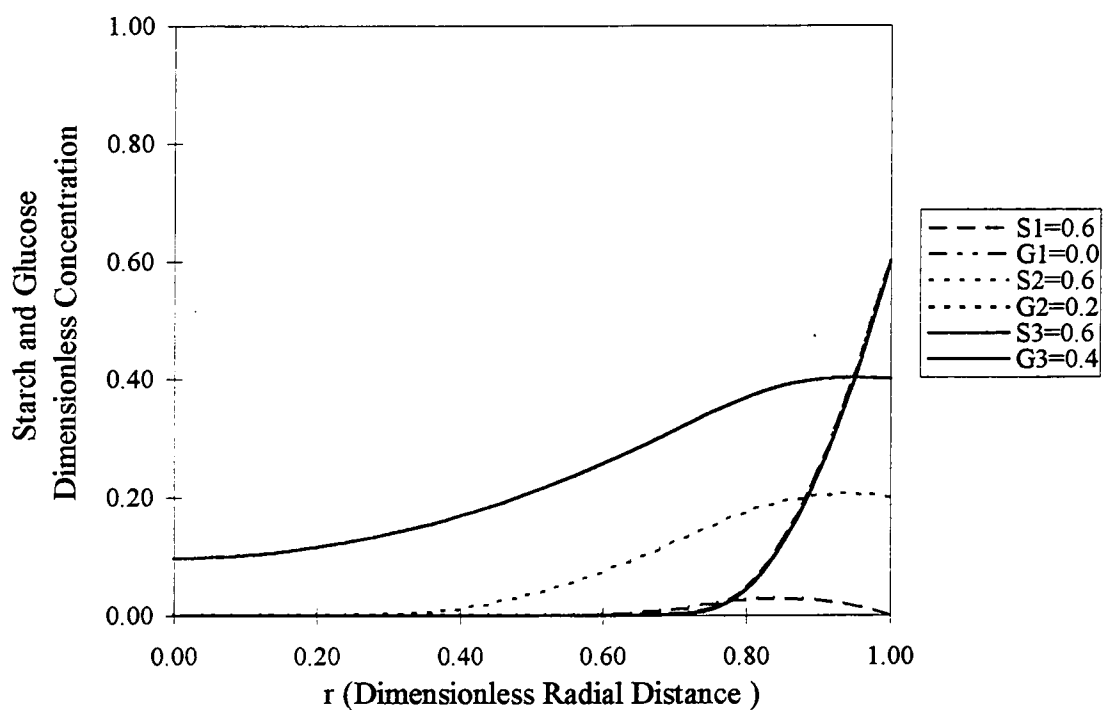


Figure 5.2.3. Starch and glucose concentration profiles in the bead at same starch and different glucose bulk concentrations. A. Starch bulk concentration

$S1 = 0.6$, Glucose bulk concentration $G1 = 0.0$; $S2 = 0.6$, $G2=0.2$;

$S3 = 0.6$, $G3 = 0.4$

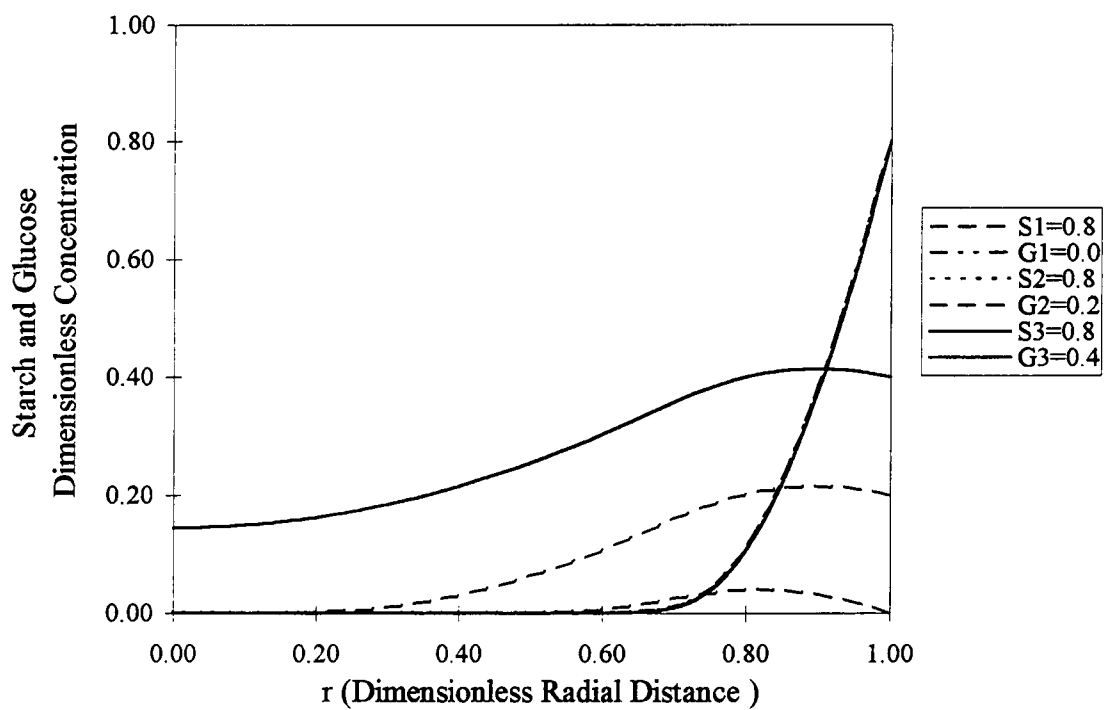


Figure 5.2.3 (continued)

B. Starch bulk concentration $S1 = 0.8$, Glucose bulk concentration $G1 = 0.0$;

$S2 = 0.8$, $G2 = 0.2$; $S3 = 0.8$, $G3 = 0.4$

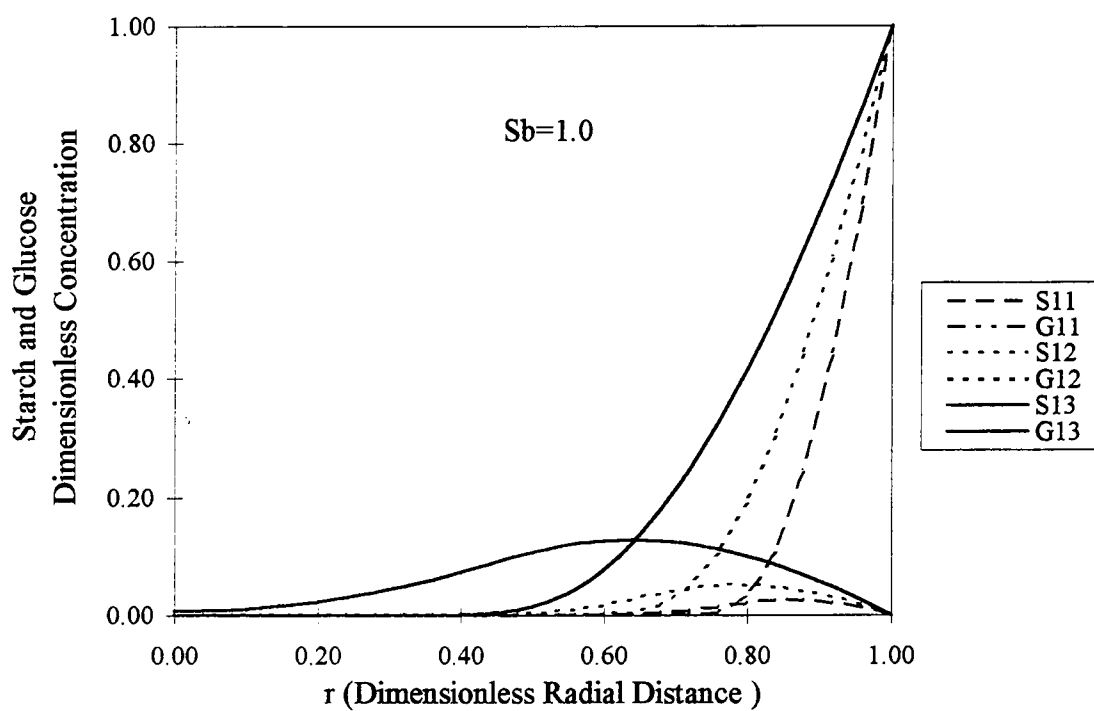


Figure 5.2.4 Starch and glucose concentration profiles at the different starch diffusivities. Bead Radius=2mm, Starch bulk concentration $S_b=1.0$, glucose bulk concentration = 0.0, starch S11, glucose G11 ($D_s = 0.002 \text{ cm}^2/\text{hr}$); S12, G12 ($D_s = 0.004 \text{ cm}^2/\text{hr}$); S13, G13 ($D_s=0.008 \text{ cm}^2/\text{hr}$)

immobilization carrier material and enzyme. Figure 5.2.5 shows different concentration profiles at different bead radii. This figure shows that for a small bead (radius $r = 1$ mm), starch can penetrate to the center of the bead. In this situation, the catalyst bead is used completely. However, for a large bead (radius $r = 2$ mm), starch can only reach 72% of the bead volume and glucose concentration becomes zero at a dimensionless radial distance of 0.65, therefore large beads are not used efficiently.

Glucose concentration inside the bead relates to starch availability and the enzymatic reaction which produces glucose. Figure 5.2.2 shows bead glucose concentration when no glucose is present in the bulk. The profile shows increasing glucose concentration followed by a decrease due to low starch concentrations. However, the glucose decrease varies for different conditions. For example, in Figure 5.2.4 glucose concentration (G13) decreases slowly to zero at the center of the bead at high starch diffusivity and G12 decreases rapidly at low starch diffusivity. In this case starch diffusion is the rate limiting step. Both Figure 5.2.2 and Figure 5.2.4 show the concentration profiles for a 2 mm catalyst bead. Figure 5.2.5 shows the starch and glucose concentration for different bead sizes. For a 1 mm bead, starch and glucose are found through the bead. This indicates that for smaller beads, starch diffusion is not the rate limiting step and suggests that the enzyme concentration is high. Accumulating glucose in the bead is an undesirable situation and the enzyme loading should be adjusted. Figure 5.2.6 shows the starch and glucose concentration profiles at different enzyme loading values for a 1 mm catalyst bead. The dimensionless bulk starch concentration is 1.0 (225 g/L). When enzyme loading is 150 mL, the glucose

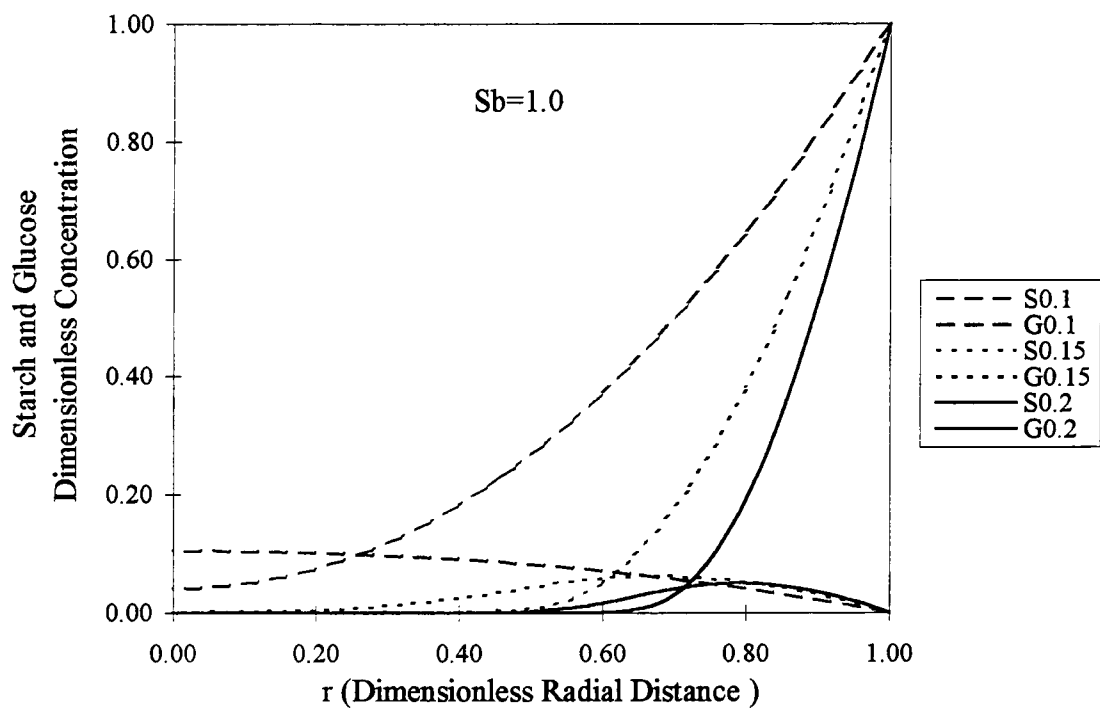


Figure 5.2.5 Starch and glucose concentration profiles for different sizes of catalyst bead. S0.1 is starch concentration, G0.1 is glucose concentration at $r = 0.1$ cm; S0.15, G0.15 ($r = 0.15$ cm); S0.2, G0.2 ($r = 0.2$ cm)

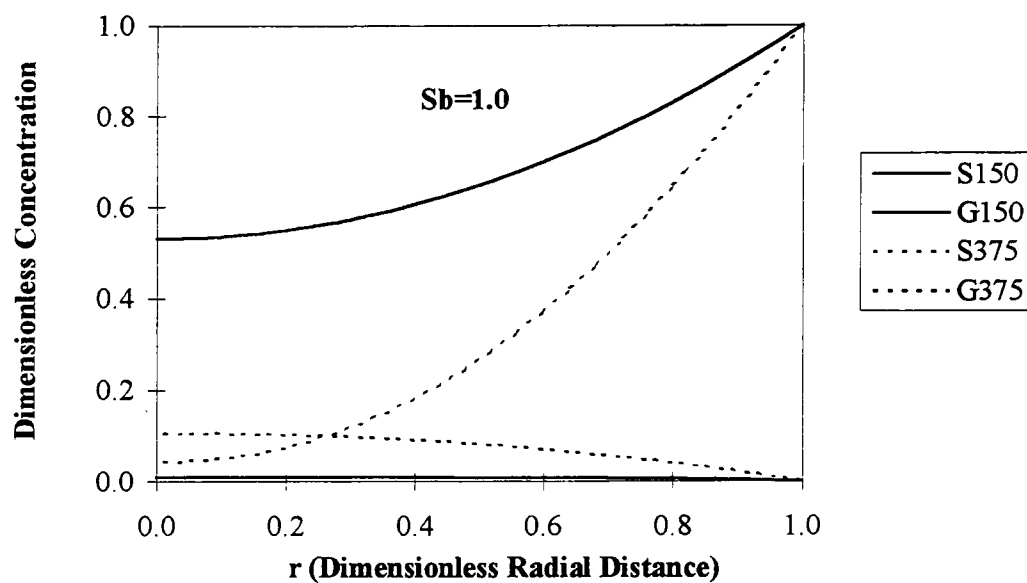


Figure 5.2.6. Concentration profiles at different biocatalyst enzyme loadings.

Starch bulk concentration $S_b = 1.0$ (225 g/L)

S150 - starch concentration, G150 - glucose concentration (enzyme loading = 150 mL)

S375 - starch concentration, G375 - glucose concentration (enzyme loading = 375 mL)

concentration is close to zero. However, when the enzyme loading increases to 375 mL, the glucose concentration increases and glucose accumulates inside the catalyst bead. Figure 5.2.7 shows the concentration profile at low bulk starch concentration (45 g/L) for 1 mm bead and Figure 5.2.8 is for a 2 mm catalyst bead. When the enzyme loading increases, the glucose concentration increases faster, and glucose distributes to more portions of the bead. Both Figure 5.2.7 and Figure 5.2.9 suggest that under the conditions of very low starch bulk concentrations, adjustment of enzyme will not improve the performance of the catalyst due to low starch concentrations in the bead.

5.2.2 The influence of variation of catalyst bead on effectiveness factor

The effectiveness factor is important for the mathematical model calculations. It is important to know the catalyst's influence on the effectiveness factor under different enzyme loading and bead sizes. This information helps to further understand the effectiveness factor and its application.

The influence of enzyme loading on the effectiveness factor

Enzyme loading is a key variable for the experimental system. Enzyme loading directly effects catalyst activity. Figures 5.2.10 A and B show the relationship of the effectiveness factor to different enzyme loading. Figure 5.2.10 A uses a logarithmic scale for the Y axis and helps to visualize the effectiveness factor at high bulk concentration. Figure 5.2.10 B uses a logarithmic scale for both the X and Y axis and helps to visualize the effectiveness factor at low bulk starch concentration. These two figures indicate that higher enzyme loading causes the effectiveness factor to go far away

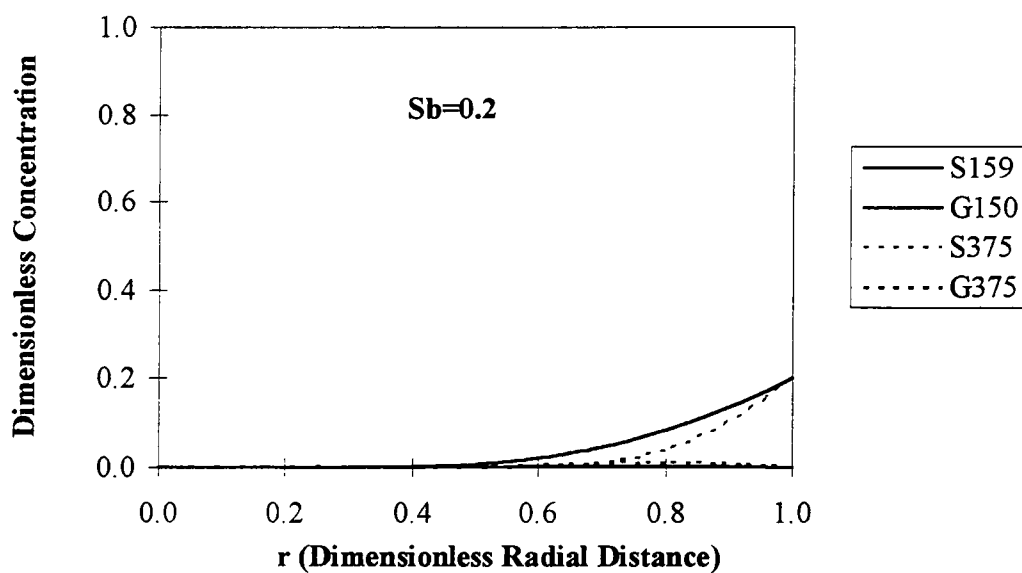


Figure 5.2.7 Concentration profiles at different biocatalyst enzyme loadings.

Starch bulk concentration $S_b = 0.2$ (45 g/L)

S150 - starch concentration, G150 - glucose concentration (enzyme loading = 150 mL)

S375 - starch concentration, G375 - glucose concentration (enzyme loading = 375 mL)

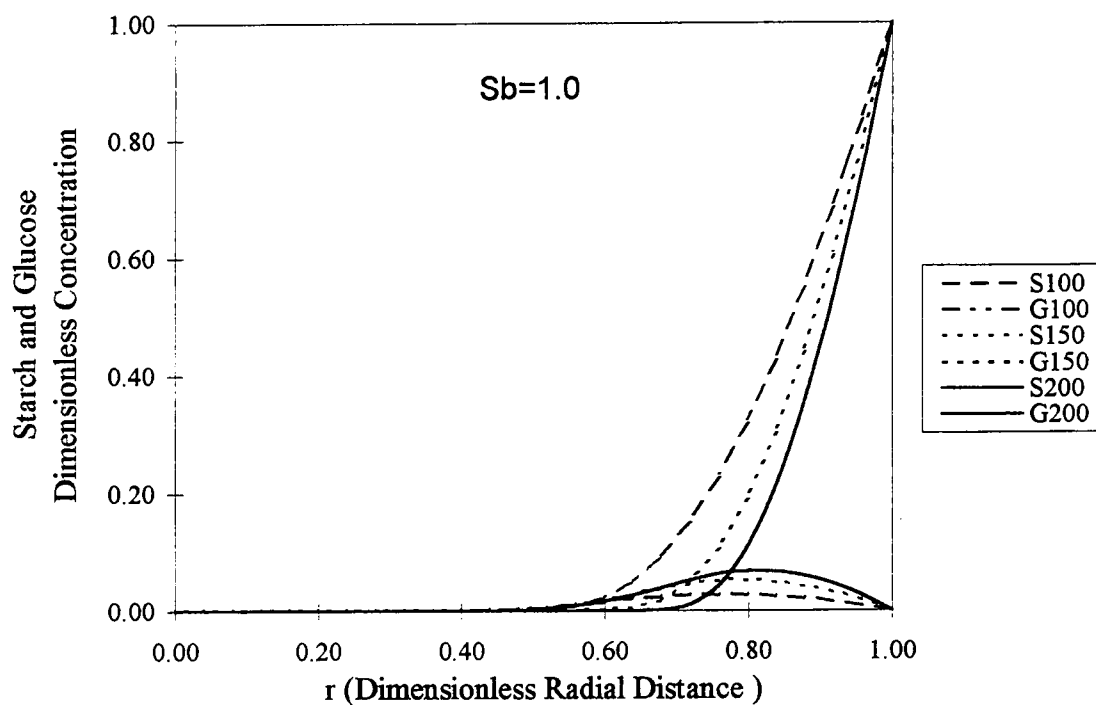


Figure 5.2.8 Concentration profiles at different biocatalyst enzyme loadings.

Starch bulk concentration $S_b = 1.0$ (225 g/L)

S100 - starch concentration, G100 - glucose concentration (enzyme loading = 100 mL)

S150 - starch concentration, G150 - glucose concentration (enzyme loading = 150 mL)

S200 - starch concentration, G200 - glucose concentration (enzyme loading = 200 mL)

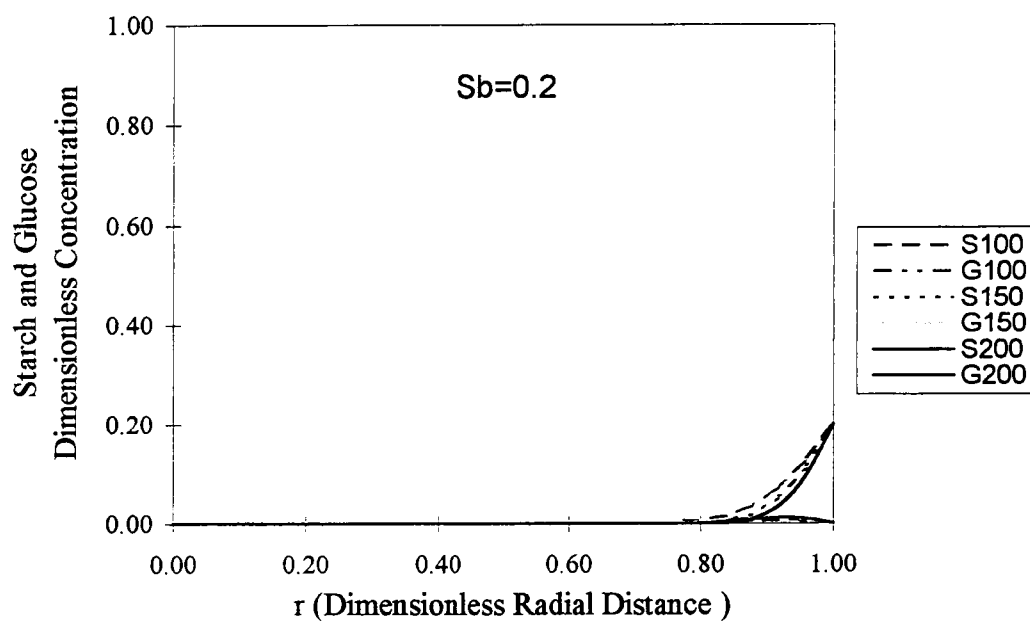


Figure 5.2.9 Concentration profiles at the different biocatalyst enzyme loadings.

Starch bulk concentration $S_b = 0.2$ (45g/L)

S100 - starch concentration, G100 - glucose concentration (enzyme loading = 100 mL)

S150 - starch concentration, G150 - glucose concentration (enzyme loading = 150 mL)

S200 - starch concentration, G200 - glucose concentration (enzyme loading = 200 mL)

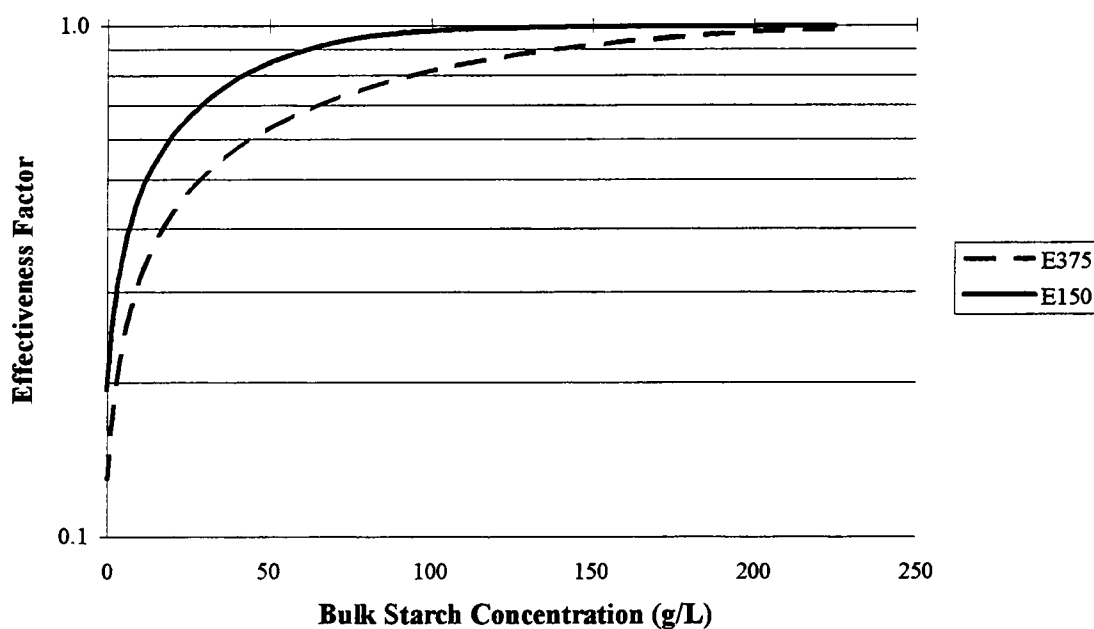


Figure 5.2.10 The effectiveness factor profile at different bulk starch concentrations with two different enzyme loadings (1) enzyme loading is 375 mL (E375); (2) enzyme loading is 150 mL (E150). A. This figure helps to visualize the effectiveness factor at high bulk glucose concentration.

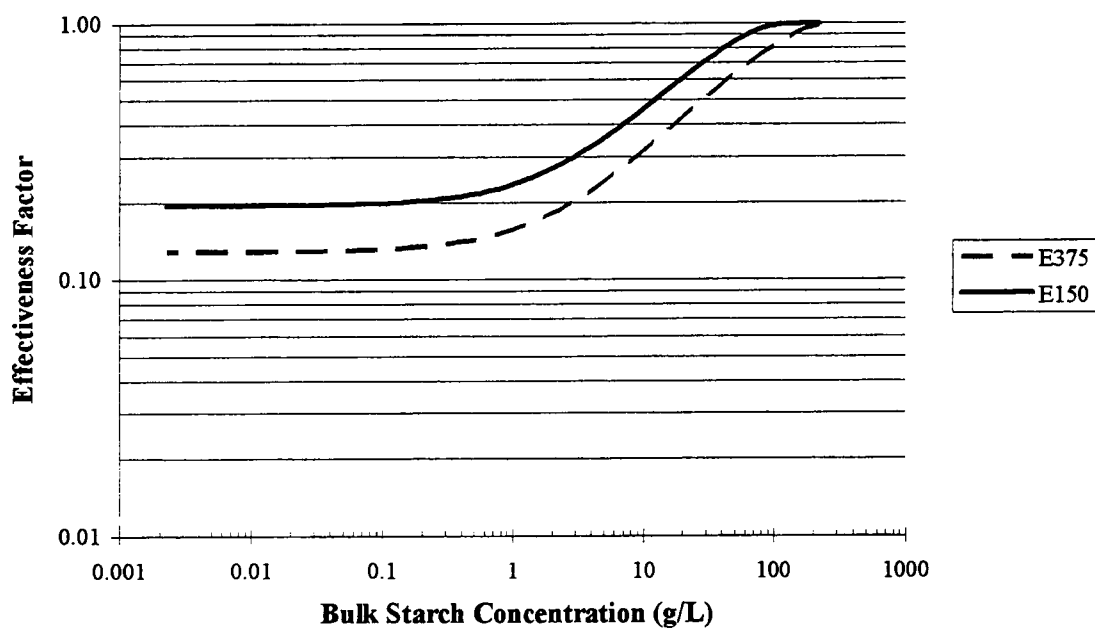


Figure 5.2.10 continued

B. This figure helps to visualize the effectiveness factor at low bulk glucose concentration.

from unity. The effectiveness factor as discussed in Section 4.3 is defined as the ratio of the actual starch consumption rate by the enzyme within the biocatalyst particle to the consumption rate of starch if all the enzyme were exposed to the exterior bulk concentration. The starch consumption rate calculated by using bulk concentrations is larger at higher enzyme loading than the one at lower enzyme loading. The observed reaction rate is a function of the effectiveness factor and the reaction rate calculated with the bulk concentration. Therefore, the effectiveness factor with higher enzyme loading is smaller than the value of the effectiveness factor with lower enzyme loading.

The influence of catalyst size on the effectiveness factor

The starch concentration profile inside the catalyst with different size beads has already been discussed in Section 5.2.1. Figures 5.2.11 A and B present the differing performance of the effectiveness factor with different catalyst radii. These two figures indicate that the larger catalyst causes the effectiveness factor to deviate more from unity. From Section 5.2.1 (Figure 5.2.5), it was shown that with the same bulk starch concentration, the larger the catalyst is, the smaller the portion of the catalyst is used. The calculation of the reaction rate was made using the assumption that all catalyst volume is used and exposed to the bulk concentration. This assumption will cause more error with a larger catalyst. Therefore, the smaller effectiveness factor is used to modify the calculated reaction rate for the larger catalyst bead.

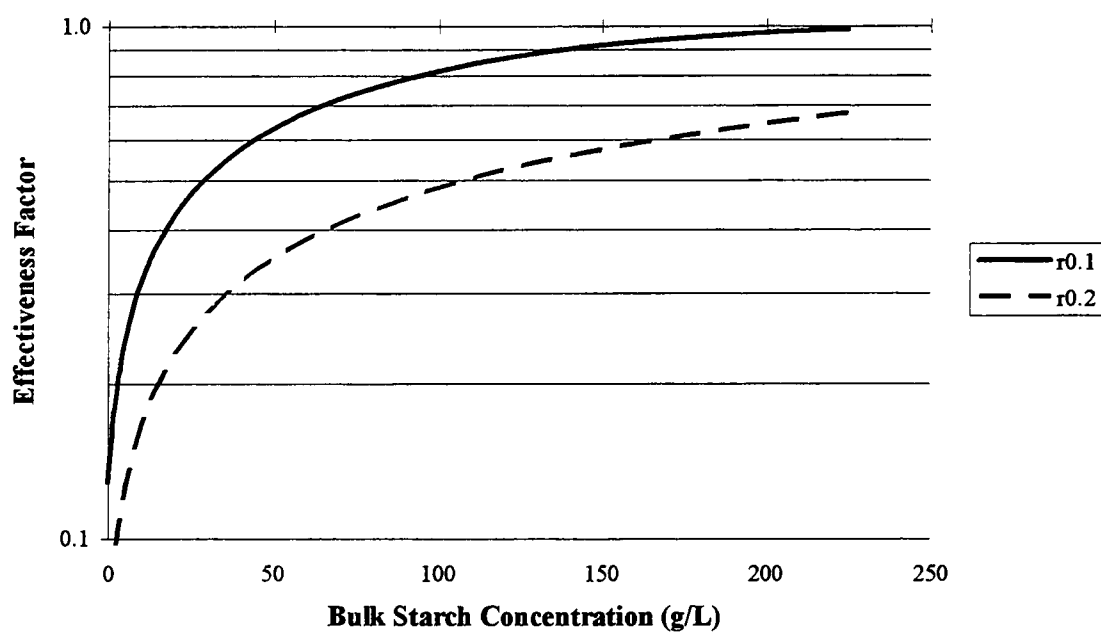


Figure 5.2.11 The effectiveness factor profile at different bulk starch concentrations with two different sizes of catalyst (1) the radius is 1 mm ($r_{0.1}$); (2) the radius is 2 mm ($r_{0.2}$). A. This figure helps to visualize the effectiveness factor at high bulk glucose concentration.

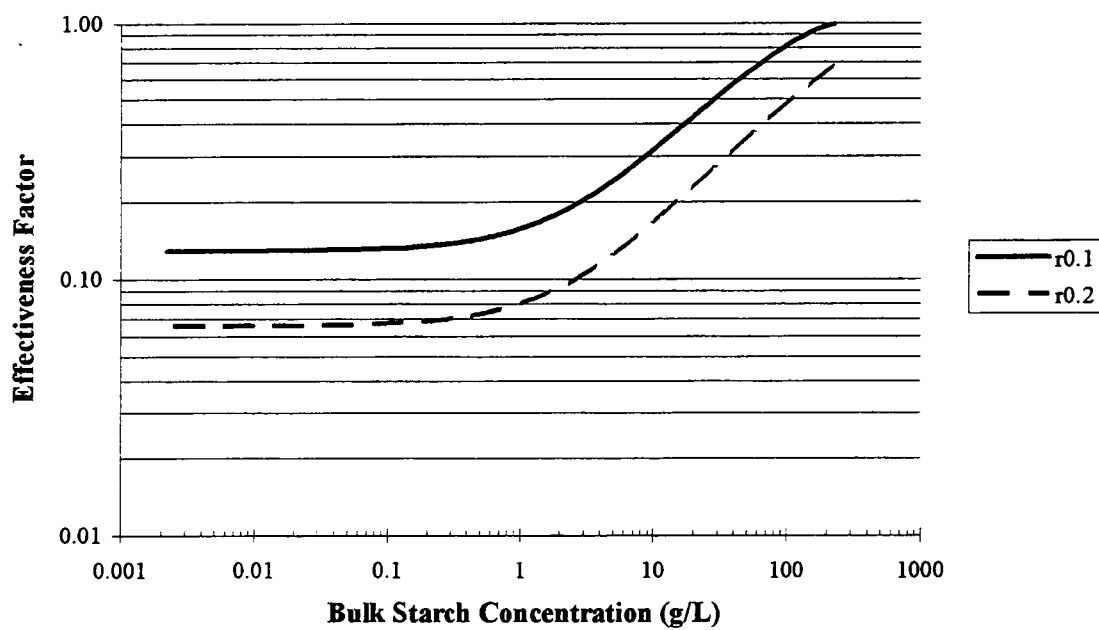


Figure 5.2.11 continued

B. This figure helps to visualize the effectiveness factor at low bulk glucose concentration.

5.2.3 The Performance of Fluidized Bed Reactor at Different Conditions

Enzyme loading influences the activity of the catalyst which effects the overall performance of the reactor. Figure 5.2.12 shows that the starch concentration in the effluent of the reactor with higher enzyme loading is lower than the one with lower enzyme loading. Obviously, there is more starch consumed for the high enzyme loading reactor. Consequently, there is more ethanol produced with the high enzyme loading reactor. In the actual experimental process, the enzyme loading is limited by the technique of the bead making. There is a difficulty for making beads when there is too large a volume of enzyme in the bead gel solution. The gel solution can block the outlet nozzle (see Section 3.2) because of very high viscosity of the gel solution.

As discussed in section 5.2.1, the size of the catalyst affects the efficiency of the catalyst. Figure 5.2.13 presents the concentration profile inside the reactor with different size of the bead. For the same total volume of the catalyst which is loaded in the reactor, the smaller catalyst performs better than the larger catalyst. For the smaller catalyst bead, a larger portion of each bead is used (see Figure 5.2.5). This indicated that a larger portion of the total volume of catalyst is used. Therefore, more starch is consumed with a smaller size catalyst and a higher concentration of ethanol is obtained at the outlet of the reactor. However, this does not mean that the beads should be made as small as possible. The size of the catalyst bead should be decided with the consideration of the other experimental conditions. For example, the industrial feed

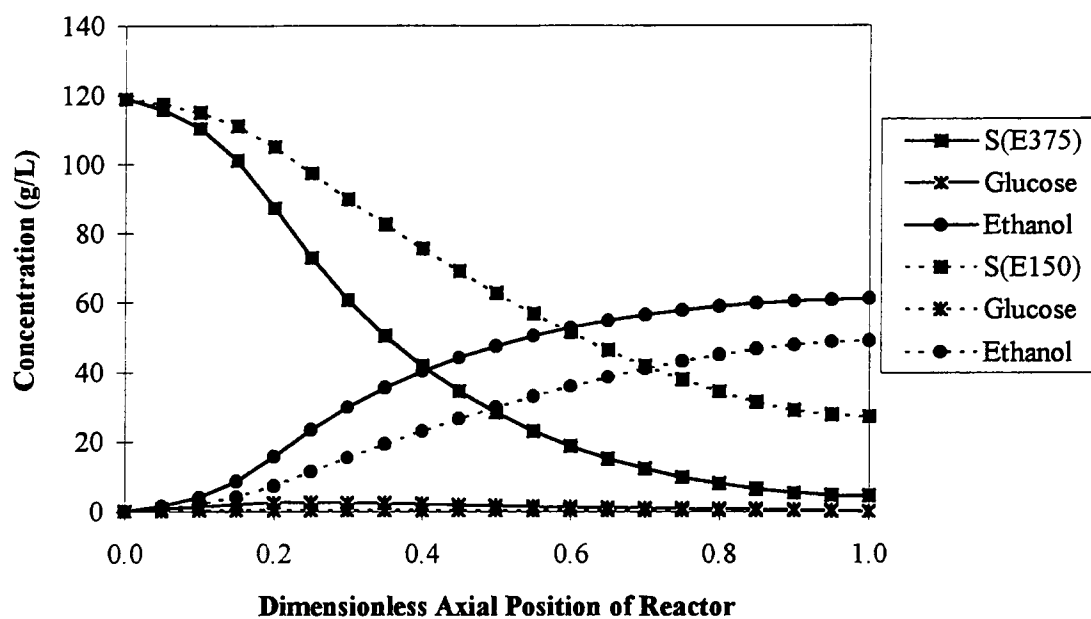


Figure 5.2.12 The comparison of concentration profiles
in the reactor with different catalyst enzyme loading

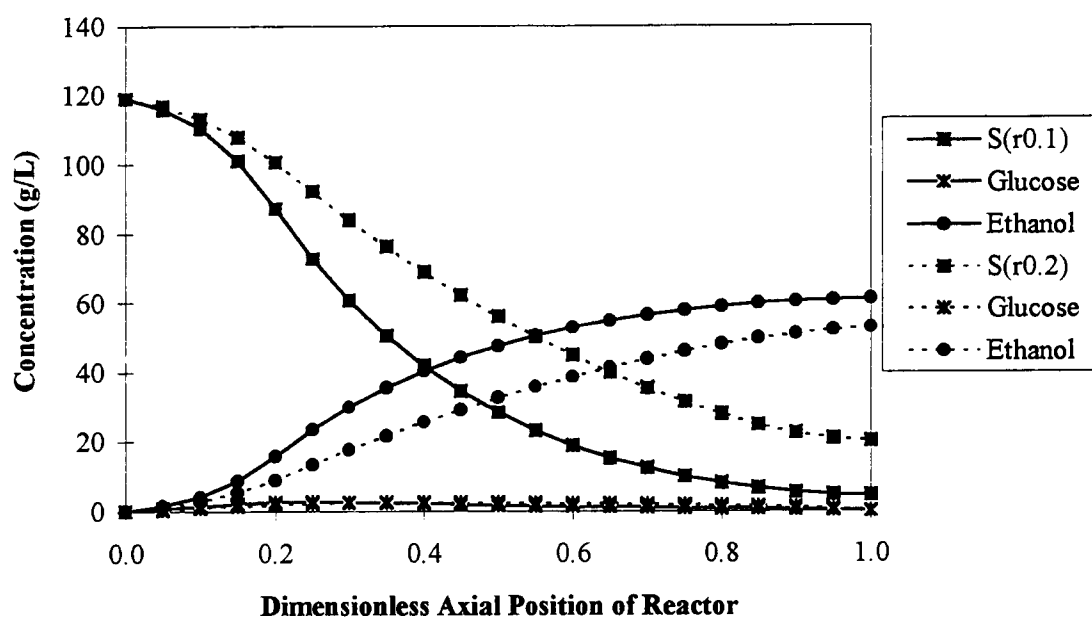


Figure 5.2.13 The comparison of concentration profiles
in the reactor with different catalyst enzyme loading.

solutions contain the particles. In this situation, beads that are too small could be washed out with the solution and it would be hard to separate the catalyst from the effluent solution by using simple screen sieving method.

The performance of the reactor is also affected by the operating conditions. Figure 5.2.14 shows the concentration profile of the reactor under different feed flow rates. For the same feed concentration, a higher flow rate causes less starch consumption, mainly because the high flow rate corresponds to the short residence time. The feed starch does not have enough time to be converted before it flows through the reactor. However, a higher flow rate introduces better fluidization, better external mass transfer environment, and also high productivity. In the future, one way to overcome this weakness is to build a close loop feed system. This means that the effluent from the reactor is pumped back into the reactor forming a closed loop. This operation could continue till the starch conversion rate reaches a certain level. A longer column could be another way to reduce the starch concentration of the effluent. However, these two reactor configurations have the same kind of disadvantage, that is CO_2 build up in the reactor. For overcoming CO_2 build up in the reactor, staged columns could be an alternative. A gas disengaging system could be built between each columns. The inlet CO_2 of the next column will be reduced.

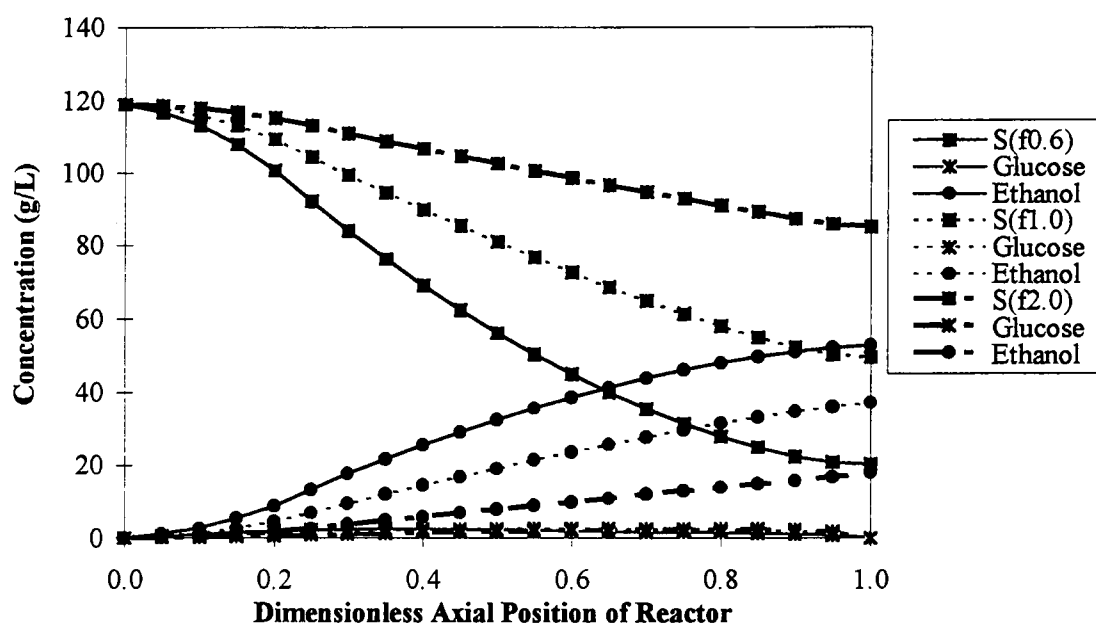


Figure 5.2.14 The influence of the feed flow rate
on the concentration profile in the reactor

5.3 Conclusions

A fully predictive mathematical model of a three-phase fluidized-bed reactor with a co-immobilized catalyst has been developed. There are two key parts in the model, the catalyst model to describe the catalyst performance, and the model for the reactor system. The catalyst model includes the effects of diffusion caused by the immobilization on consecutive reactions. In the reactor model, correlations from literature are used to describe the dispersion coefficient and the holdup of the various phases as a function of the axial position in the reactor. This mathematical model is numerically solved and the predictions compare favorably with the experimental data.

Model results show that the effectiveness factor has a significant influence on the reaction rate inside of the immobilized catalyst, therefore the calculated reaction rate must be modified by this factor. This model not only predicts the concentration inside the catalyst bead under different conditions but also shows that enzyme loading and catalyst size affect both the concentration profile inside the catalyst and the effectiveness. The model can be used to gain a better appreciation of the conditions that limit reaction rates in such systems and to design a high-efficiency catalyst bead. This model can also be used to predict an axial concentration profile under different conditions and to understand observed phenomena in the reactor. The model can then be used in designing the optimal conditions for which maximal conversion of the ethanol is achieved.

Because no adjustments were made in the model parameters, this model can be considered fully predictive. This model is generally applicable, rather than situation

specific. The structure of this mathematical approach may be broadly applicable. With different kinetic expressions, the catalyst model could be used to describe other co-immobilization system or the reactor model could be used to describe fluidized bed with co-immobilized catalyst. Such simulations can assist the scale-up and economic analyses of these systems.

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APPENDIX

APPENDIX

Computer Program for Fluidized Bed Reactor Mathematical Model

```
implicit double precision (a-h,o-z)
character*10 dfn
dimension zeta(5),tol(5),fixpnt(2),z(5),retf(2)
integer m(3),ipar(11),ltol(5)
external fsub3,dfsub3,gsub3,dgsub3,solutn
include 'spaces.for'
include 'commons.for'

c
c  variables
c
c  c0-temporary storage of inlet concentration
c  c0c-inlet concentration
c  difs-Diffusivity of starch
c  difp-Diffusivity of product
c  lt-length of tapered section
c  mul-Viscosity of liquid, g/cm/s
c  p is the bulk product concentration
c  p0d-Product concentration
c  qpm-
c  rg-reaction rate g/L/hr
c  rb-bead radius
c  rhof-specific gravity of fluid
c  rhos-Density of bead, g/cm^3
c  s is the bulk substrate concentration
c  temp-Temperature of reactor, C
c  x-Cell concentration in bead
c  xd-dimensionless position in bead
c  ypg-ethanol yield 0.48 g/g
c  End Declaration Statements*****
c
  open(unit=57,file='jacobbcc.dat',status='unknown')
  call init
  call effcal
  nred=2
  pstep=1.d-4
2  vf=vfc
  icntr=1
  icntr2=1
  itoggle=0
  epslmin=1.0d0-esmax
```

```

c0=c0c
co2max=c0c*ygs*vf*0.49d0
ks=ksd/c0
pi=pid/c0
pm=pmd/c0
ki=kid/c0
ag=agd/c0
p0=p0d/c0
c parameter for colsys
m(1)=1
m(2)=2
m(3)=2
ncomp=3
aleft=0.d0
aright=1.d0
zeta(1)=0.d0
zeta(2)=0.d0
zeta(3)=0.d0
zeta(4)=1.d0
zeta(5)=1.d0
do 5 i=1,11
5 ipar(i)=0
ipar(1)=1
ipar(3)=1500
ipar(4)=5
ipar(5)=1000000
ipar(6)=200000
do 6 ispf=1,ipar(5)
6 fspace(ispf)=0.d0
do 7 ispf=1,ipar(6)
7 ispace(ispf)=0
ipar(7)=1
ltol(1)=1
tol(1)=1.d-5*10.d0**nred
ltol(2)=2
tol(2)=1.d-5*10.d0**nred
ltol(3)=3
tol(3)=1.d-5*10.d0**nred
ltol(4)=4
tol(4)=1.d-5*10.d0**nred
ltol(5)=5
tol(5)=1.d-5*10.d0**nred
ipar(9)=1 !use initial guess of the solution

8 call colsys(ncomp,m,aleft,aright,zeta,ipar,ltol,tol,
* fixpnt,ispace,fspace,iflag,fsub3,dfsub3,gsub3,dgsub3,

```

```

* solutn)
print*, 'flag'
if (iflag.ne.1) then
  print *, 'Unable to reach a solution, IFLAG = ', iflag
  if (iflag.eq.-1) then
    nred=nred+1
    if (nred.ge.4) stop
    print *, 'reducing the tolerance by', nred, 'orders of magnitude'
    goto 2
  endif
  if (iflag.eq.0) then
    nred=nred+1
    ipar(10)=1
    ipar(3)=ipar(3)+1000
    print *, 'reducing the tolerance by', nred, 'orders of magnitude'
    print *, 'also setting ipar(10)=1'
    goto 8
  endif
  stop
endif
print*, 'ipar(3)', ipar(3)
nred=0
ics=c0c
write(dfn, 4999) ics
4999 format('o', i4.4, '.dat')
5555 format(11e12.5)
5556 format(f9.4)
  open(unit=14, file=dfn, status='unknown', recl=1024)
  open(unit=55, file='output.dat', status='unknown')
  xd=0.d0
  jnp2=20
  do 31 jnp1=1, 21
c
  call appsln(xd, z, fspace, ispace)
c
  call retres(xd, z, p, disp, epsg, epsl, es, rg, rs, f)
c
  zd1=z(1)
  zd2=z(2)*c0
  zd3=z(4)*c0
  p=p*c0
  print *, xd, zd1, zd2, zd3
  write(55, *) xd, zd2, zd1
  write(14, 5555) xd, zd2, zd3, p, zd1, disp, epsg, epsl, es, rg, rs
  xd=xd+0.05
31 continue

```

```

c  z(1) (bulk phase) production rate of co2 g/hr
c  z(2) (bulk phase) substrate concentration
c  z(3) (bulk phase) derivative of substrate concentration with respect to position
sconsum=(1.d0-z(2))*vfc*c0c
theo2=((1.d0-z(2))*ygs-z(4))*0.49*vfc*c0c
write(14,5556) c0c
write(14,5556) sconsum
write(14,5556) theo2
write(14,5556) db
write(14,5556) dt
write(14,5556) lt
write(14,5556) dc
write(14,5556) vfc
write(14,5556) l
write(14,5556) esmax
write(14,5556) epslmin
write(14,5556) conskim
write(14,5556) co2frac
write(14,5556) qpm
write(14,5556) ygs
write(14,5556) ypg
write(14,5556) ksd
write(14,5556) pid
write(14,5556) pmd
write(14,5556) kid
write(14,5556) agd
write(14,5556) vm
write(14,5556) p0d
write(14,5556) x
write(14,5556) rb
write(14,5556) difs
write(14,5556) difg
write(14,5556) difp
write(14,5556) mul
write(14,5556) rhos
write(14,5556) temp-273.15
write(14,5556) rhof
write(14,5556) bctype
write(14,5556) xe
write(55,5556) sconsum
write(55,5556) theo2
write(55,5556) db
write(55,5556) dt
write(55,5556) lt
write(55,5556) dc
write(55,5556) vfc

```

```

write(55,5556) l
write(55,5556) esmax
write(55,5556) epslmin
write(55,5556) conskim
write(55,5556) co2frac
write(55,5556) qpm
write(55,5556) ypg
write(55,5556) ypg
write(55,5556) ksd
write(55,5556) pid
write(55,5556) pmd
write(55,5556) kid
write(55,5556) agd
write(55,5556) vm
write(55,5556) p0d
write(55,5556) x
write(55,5556) rb
write(55,5556) difs
write(55,5556) difg
write(55,5556) difp
write(55,5556) mul
write(55,5556) rhos
write(55,5556) temp-273.15
write(55,5556) rhof
write(55,5556) bctype
write(55,5556) xe

```

```

close(14)
close(55)
!print 9998,x,difs,difp,g(1)
stop
end

```

c

c

```

subroutine init
implicit double precision (a-h,o-z)
include 'commons.for'
character*80 dummy !
open(unit=10,file='parm.dat',status='unknown')
open(unit=100,file='conc.dat',status='unknown')
!   for each case that is run.

```

```

read(10,*) db,dummy
write(*,*) db,dummy
read(10,*) dt,dummy
write(*,*) dt,dummy

```

```

read(10,*) lt,dummy
write(*,*) lt,dummy
read(10,*) dc,dummy
write(*,*) dc,dummy
read(10,*) vfc,dummy
write(*,*) vfc,dummy
read(10,*) l, dummy
write(*,*) l,dummy
read(10,*) esmax, dummy
write(*,*) esmax, dummy
read(10,*) conskim, dummy
write(*,*) conskim, dummy
read(10,*) co2frac, dummy
write(*,*) co2frac, dummy

```

c

- c if the column is straight, ensure that the bottom
- c has the same diameter as the rest of the column

c

```

if (lt.le.0.d0) db=dc !
print *, 'Input the feed dextrose concentration'
read(100,*) c0c      !read in the current value of the inlet concentration
read(10,*) qpm,dummy
write(*,*) qpm,dummy
read(10,*) ygs,dummy
write(*,*) ygs,dummy
read(10,*) ypg,dummy
write(*,*) ypg,dummy
read(10,*) ksd,dummy
write(*,*) ksd,dummy
read(10,*) pid,dummy
write(*,*) pid,dummy
read(10,*) pmd,dummy
write(*,*) pmd,dummy
read(10,*) kid,dummy
write(*,*) kid,dummy
read(10,*) agd,dummy
write(*,*) agd,dummy
read(10,*) vm,dummy
write(*,*) vm,dummy
read(10,*) p0d,dummy
write(*,*) p0d,dummy
read(10,*) x,dummy
write(*,*) x,dummy
read(10,*) rb,dummy
write(*,*) rb,dummy
read(10,*) difs,dummy

```

```

write(*,*) difs,dummy
read(10,*) difg,dummy
write(*,*) difg,dummy
read(10,*) difp,dummy
write(*,*) difp,dummy
read(10,*) mul,dummy
write(*,*) mul,dummy
read(10,*) rhos,dummy
write(*,*) rhos,dummy
read(10,*) temp,dummy
write(*,*) temp,dummy
temp=temp+273.15d0
read(10,*) rhof,dummy
write(*,*) rhof,dummy
read(10,*) bctype,dummy
write(*,*) bctype,dummy
read(10,*) xe,dummy
write(*,*) xe,dummy
return
end

```

c

c

```

double precision function eff1()
implicit real*8(a-h,o-z)
include 'commons.for'
sc1=s1*c0/225.d0
if(sc1.gt.1.d0) then !check limits
    eff1=effic1(1)
    return
endif
if(sc1.lt.1.d-5) then
    eff1=effic1(101)
    return
endif
a=-dlog10(sc1)*100.d0/5.d0
n1=int(a)
a=a-n1
eff1=effic1(n1+1)+a*(effic1(n1+2)-effic1(n1+1))
return
end

```

c

```

double precision function eff2()
implicit real*8(a-h,o-z)
double precision tem1,tem2
include 'commons.for'
sc1=s1*c0/225.d0

```

```

sc2=s2*c0/225.d0
if(sc1.gt.1.d0) then
  if (sc2.gt.1.d0)then
    eff2=effic2(1,1)
  else if (sc2.gt.1.0d-5.and.sc2.lt.1.d0) then
    a=-dlog10(sc2)*100.d0/5.d0
    n1=int(a)
    a=a-n1
    if (effic2(1,n1+2).gt.effic2(1,n1+1)) then
      eff2=effic2(1,n1+1)+a*(effic2(1,n1+2)-effic2(1,n1+1))
    else if (effic2(1,n1+2).lt.effic2(1,n1+1)) then
      eff2=effic2(1,n1+1)-a*(effic2(1,n1+1)-effic2(1,n1+2))
    end if
  else if (sc2.lt.1.d-5) then
    eff2=effic2(1,101)
  endif
  return
endif

if(sc1.lt.1.d0.and.sc1.gt.1.d-5) then
  if (sc2.gt.1.d0) then
    a=-dlog10(sc1)*100.d0/5.d0
    n1=int(a)
    a=a-n1
    if (effic2(n1+2,1).gt.effic2(n1+1,1)) then
      eff2=effic2(n1+1,1)+a*(effic2(n1+2,1)-effic2(n1+1,1))
    else if (effic2(n1+2,1).lt.effic2(n1+1,1)) then
      eff2=effic2(n1+1,1)-a*(effic2(n1+1,1)-effic2(n1+2,1))
    end if
  else if (sc2.lt.1.d-5) then
    a=-dlog10(sc1)*100.d0/5.d0
    n1=int(a)
    a=a-n1
    if (effic2(n1+2,101).gt.effic2(n1+1,101)) then
      eff2=effic2(n1+1,101)+a*(effic2(n1+2,101)-effic2(n1+1,101))
    else if (effic2(n1+2,101).lt.effic2(n1+1,101)) then
      eff2=effic2(n1+1,101)-a*(effic2(n1+1,101)-effic2(n1+2,101))
    end if
  else if (sc2.gt.1.0d-5.and.sc2.lt.1.d0) then
    a=-dlog10(sc1)*100.d0/5.d0
    b=-dlog10(sc2)*100.d0/5.d0
    n1=int(a)
    n2=int(b)
    a=a-n1
    b=b-n2
    if (effic2(n1+2,n2+1).gt.effic2(n1+1,n2+1)) then

```

```

tem1=effic2(n1+1,n2+1)+a*(effic2(n1+2,n2+1)-effic2(n1+1,n2+1))
  else if (effic2(n1+2,n2+1).lt.effic2(n1+1,n2+1)) then
tem1=effic2(n1+1,n2+1)-a*(effic2(n1+1,n2+1)-effic2(n1+2,n2+1))
  endif
  if (effic2(n1+2,n2+2).gt.effic2(n1+1,n2+2)) then
tem2=effic2(n1+1,n2+2)+a*(effic2(n1+2,n2+2)-effic2(n1+1,n2+2))
  else if (effic2(n1+2,n2+2).lt.effic2(n1+1,n2+2)) then
tem2=effic2(n1+1,n2+2)-a*(effic2(n1+1,n2+2)-effic2(n1+2,n2+2))
  endif
  if (tem2.gt.tem1) then
    eff2=tem1+b*(tem2-tem1)
  else if (tem2.lt.tem1) then
    eff2=tem1-b*(tem1-tem2)
  end if
end if
return
endif

```

```

if(sc1.lt.1.d-5) then
  if (sc2.gt.1.d0) then
    eff2=effic2(101,1)
  else if (sc2.gt.1.0d-5.and.sc2.lt.1.d0) then
    a=-dlog10(sc2)*100.d0/5.d0
    n1=int(a)
    a=a-n1
    if (effic2(101,n1+2).gt.effic2(101,n1+1)) then
      eff2=effic2(101,n1+1)+a*(effic2(101,n1+2)-effic2(101,n1+1))
    else if (effic2(101,n1+2).lt.effic2(101,n1+1)) then
      eff2=effic2(101,n1+1)-a*(effic2(101,n1+1)-effic2(101,n1+2))
    end if
  else if (sc2.lt.1.d-5) then
    eff2=effic2(101,101)
  endif
return
endif
end

```

c

c

```

subroutine effcal
implicit real*8(a-h,o-z)
include 'commons.for'
double precision z(2)
external effc1,effic2
irdit=0
write (*,*) 'enter 0 if you want new effectiveness factor table,'
write (*,*) ' 1 if use current eff.dat'

```

```

read (*,*) irdit
open (unit=12,file='eff1.dat',status='unknown')
open (unit=13,file='eff2.dat',status='unknown')

if (irdit.eq.1) then
  do 1 i=1,101
    read(12,*)s1,effic1(i)
    do 2 j=1,101
      read(13,*)s1,s2,effic2(i,j)
2      continue
1      continue
    return
  endif

c0=225.d0
ks=ksd/c0
pi=pid/c0
pm=pmd/c0
ki=kid/c0
ag=agd/c0
p0=p0d/c0
s1=1.d0
fac=10.d0**0.05d0
ncl=0
do 10 i=1,101
  effic1(i)=effic1(ncl)
  write(12,*)s1,effic1(i)
  write(*,*)s1,effic1(i)
  s1=s1/fac
10 continue
  s1=1.0
  do 20 i=1,101
    s2=1.0
    do 15 j=1,101
      if(((1.d0-s1)*ygs).le.s2) then
        p=0
      else
        p=((1.d0-s1)*ygs-s2)*ypg
      endif
      effic2(i,j)=effic2(ncl)
      write(13,*)s1,s2,effic2(i,j)
      write(*,*)s1,s2,effic2(i,j)
      s2=s2/fac
15      continue
    s1=s1/fac
20      continue

```

```

close(12)
close(13)
end
c
c
double precision function effc1(ncl)
implicit real*8(a-h,o-z)
double precision zeta(2),tol(2),z(2),rs,p
integer m(1),ipar(11),ltol(2)
include 'spaces.for'
include 'commons.for'
external fsub1,dfsub1,gsub1,dgsub1,dummy
nredp=0.0
4  m(1)=2
   ncomp=1
   aleft=0.d0
   aright=1.d0
   zeta(1)=0.d0
   zeta(2)=1.d0
   if(ncl.eq.0) then
   do 5 i=1,11
5    ipar(i)=0
   endif
   ipar(1)=1
   ipar(2)=3
   if(ncl.eq.0) ipar(3)=30
   ipar(4)=2
   ipar(5)=1000000
   ipar(6)=200000
   ipar(7)=1
   ltol(1)=1
   tol(1)=1.d-3
   ltol(2)=2
   tol(2)=1.d-3

20  call colsys(ncomp,m,aleft,aright,zeta,ipar,ltol,tol,
*   fixpnt,inspace,fspace,iflag,fsub1,dfsub1,gsub1,dgsub1,
*   dummy)
   if(iflag.ne.1)then
   print *,'Error in COLSYD, IFLAG =', iflag
   if(iflag.eq.-1 .and. ncl.ne.0) then
   ncl=0
   goto 4
   endif
   endif
   xd=1.d0

```

```

c
call appsln(xd,z,ospace,ospace)
c
rs=vm*s1*xs/((ag+s1)*ygs)
if(s1.lt.0.d0)rs=0.d0
effc1=3.d0*difs*z(2)*c0/(rb*rb*rs)
ncl=1
return
end
c
c
double precision function effc2(ncl)
implicit real*8(a-h,o-z)
double precision zeta(4),tol(4),z(4),p
integer m(2),ipar(11),ltol(4)
include 'spaces.for'
include 'commons.for'
external fsub2,dfsub2,gsub2,dgsub2,dummy
nredp=0.0
24  m(1)=2
    m(2)=2
    ncomp=2
    aleft=0.d0
    aright=1.d0
    zeta(1)=0.d0
    zeta(2)=0.d0
    zeta(3)=1.d0
    zeta(4)=1.d0
    if(ncl.eq.0) then
      do 25 i=1,11
25    ipar(i)=0
      endif
      ipar(1)=1
      ipar(2)=3
      if(ncl.eq.0) ipar(3)=40
      ipar(4)=4
      ipar(5)=1000000
      ipar(6)=200000
      ipar(7)=1
      ltol(1)=1
      tol(1)=1.d-3
      ltol(2)=3
      tol(2)=1.d-3
      ltol(3)=3
      tol(3)=1.d-3
      ltol(4)=4

```

```

tol(4)=1.d-3

40  call colsys(ncomp,m,aleft,aright,zeta,ipar,ltol,tol,
*   fixpnt,ispac,fspac,iflag,fsub2,dfsub2,gsub2,dgsub2,
*   dummy)

if(iflag.ne.1)then
print *,'Error in COLSYS, IFLAG =', iflag
if(iflag.eq.-1 .and. ncl.ne.0) then
ncl=0
goto 24
else
stop
endif
endif
xd=1.d0
call appsln(xd,z,fspac,ispac)
fp=max(min(1.d0-(p-pi)/(pm-pi),1.d0),0.d0)
fs=ki/(ki+s2)
rg=(qpm/ypg)*(s2/(ks+s2))*fp*fs*x
if(s2.lt.0.0d0)rg=0.d0
effc2=3.d0*c0*(ygs*difs*z(2)+difg*z(4))/(rb*rb*rg)
ncl=1
return
end

c
c
subroutine fsub1(xd,z,f)
implicit real*8(a-h,o-z)
dimension f(1),z(2)
include 'commons.for'
sr1=z(1)
rs=vm*sr1*xs/((ag+sr1)*ygs)
if(sr1.lt.0.0d0)rs=0.d0
if(xd.eq.0.d0) then
f(1)=rb*rb/(3.d0*c0*difs)*rs
else
f(1)=rb*rb/(c0*difs)*rs-2.d0*z(2)/xd
endif
return
end

c
c
subroutine dfsub1(xd,z,df)
implicit real*8(a-h,o-z)
dimension df(1,2),z(2)

```

```

include 'commons.for'
drsdz1=((ag+z(1))*vm-vm*z(1))/((ag+z(1))*(ag+z(1)))*xe/ygs
df(1,1)=rb*rb*drsdz1/(c0*difs)
if(xd.ne.0.d0) then
  df(1,2)=2.d0/xd
else
  df(1,2)=1.d75
end if
return
end

c
c
subroutine gsub1(i,z,g)
implicit real*8(a-h,o-z)
dimension z(2)
include 'commons.for'
goto (10,20),i

10  g=z(2)-0.d0
    return
20  g=z(1)-s1
    return
end

c
subroutine dgsub1(i,z,dg)
implicit real*8(a-h,o-z)
dimension z(2),dg(2)
include 'commons.for'
goto (10,20),i
10  dg(1)=0.d0
    dg(2)=1.d0
    return
20  dg(1)=1.d0
    dg(2)=0.d0
    return
end

c
c
subroutine fsub2(xd,z,f)
implicit real*8(a-h,o-z)
dimension f(2),z(4)
include 'commons.for'
sr1=z(1)
sr2=z(3) !Use Lee and Rodgers kinetics
pp=p+ygs*ypg*(s1-sr1)*difs/difp+ypg*difg/difp*(s2-sr2)
fp=max(min(1.d0-(pp-pi)/(pm-pi),1.d0),0.d0)

```

```

fs=ki/(ki+sr2)
rs=vm*sr1*xe/((ag+sr1)*ygs)
rg=(qpm/ypg)*(sr2/(ks+sr2))*fp*fs*x
if(sr2.lt.0.0d0)rg=0.d0
if(sr1.lt.0.0d0)rs=0.d0
if(xd.eq.0.d0) then
f(1)=rb*rb/(3.d0*c0*difs)*rs
f(2)=rb*rb/(3.d0*c0*difg)*(rg-ygs*rs)
else
f(1)=rb*rb/(c0*difs)*rs-2.d0*z(2)/xd
f(2)=rb*rb/(c0*difg)*(rg-ygs*rs)-2.d0*z(4)/xd
endif
return
end

```

c

c

```

subroutine dfsub2(xd,z,df)
implicit real*8(a-h,o-z)
dimension df(2,4),z(4)
include 'commons.for'
sr1=z(1)
sr2=z(3)
drsdz1=((ag+z(1))*vm-vm*z(1))/((ag+z(1))*(ag+z(1)))*xe/ygs
pp=p+ygs*ypg*(s1-sr1)*difs/difp+ypg*difg/difp*(s2-sr2)
fp=max(min(1.d0-(pp-pi)/(pm-pi),1.d0),0.d0)
if (pp.le.pi.or.pp.ge.pm) then
  dfpdz3=0
  dfpdz1=0
else
  dfpdz3=ypg*difg/difp/(pm-pi)
  dfpdz1=ygs*ypg*difs/difp/(pm-pi)
end if
drgdz1=(qpm/ypg)*x*(sr2/(ks+sr2))*(ki/(ki+sr2))*dfpdz1
drgdz3=(qpm/ypg)*x*(ks*ki*fp/((ks+sr2)*(ks+sr2)*(ki+sr2))-
1      ki*sr2*fp/((ki+sr2)*(ki+sr2)*(ks+sr2))+
2      ki*sr2*dfpdz3/((ks+sr2)*(ki+sr2)))
df(1,1)=rb*rb*drsdz1/(c0*difs)
if(xd.ne.0.d0) then
df(1,2)=-2.d0/xd
else
df(1,2)=-1.d75
end if
df(1,3)=0.d0
df(1,4)=0.d0
df(2,1)=rb*rb*(drgdz1-ygs*drsdz1)/(c0*difg)
df(2,2)=0.d0

```

```

df(2,3)=rb*rb*drgdz3/(c0*difg)
  if(xd.ne.0.d0) then
df(2,4)=2.d0/xd
  else
df(2,4)=1.d75
  end if
return
end

```

c

c

```

subroutine gsub2(i,z,g)
implicit real*8(a-h,o-z)
dimension z(4)
include 'commons.for'
goto (10,20,30,40),i

```

```

10  g=z(2)-0.d0
    return

```

```

20  g=z(4)-0.d0
    return

```

```

30  g=z(1)-s1
    return

```

```

40  g=z(3)-s2
    return
end

```

c

c

```

subroutine dgsub2(i,z,dg)
implicit real*8(a-h,o-z)
dimension z(4),dg(4)
include 'commons.for'
do 5 j=1,4

```

```

5   dg(j)=0.d0
    goto (10,20,30,40),i

```

```

10  dg(2)=1.d0
    return

```

```

20  dg(4)=1.d0
    return

```

```

30  dg(1)=1.d0
    return

```

```

40  dg(3)=1.d0
    return
end

```

c

c

```

subroutine dummy

```

```

return
end
c
c
subroutine fsub3(xd,z,f)
implicit double precision (a-h,o-x)
double precision f(3),z(5)
call retres(xd,z,p,disp,epsg,epsl,es,rg,rs,f)
return
end
subroutine retres(xd,z,p,disp,epsg,epsl,es,rg,rs,f)
implicit double precision (a-h,o-z)
double precision m,mw,k1,k2
dimension f(3),z(5)
include 'commons.for'
double precision bw(11) !Begovich and Watson constants
data bw/0.058d0,0.692d0,0.107d0,-0.037d0,0.388d0,0.26d0,
1 0.036d0,-0.327d0,-0.278d0,0.047d0,-0.02216d0/
data mw/44.0d0/ !molecular weight of CO2
data piv/3.14159265359d0/
! print 9995,xd,z(1),z(2),z(3),z(4),z(5)
9995 format(4g12.5)
k1=rhof/70.306958
k2=14.7d0
press=k1*I*(1.d0-xd)+k2
s1=max(1.d-5*(1.d0-xd),min(1.d0,z(2)))
s2=max(0.0,min(0.25d0,z(4)))
co2=min(c0c*.49d0*vf*ygs,max(0.d0,z(1)))
atemp=c0c*.49d0*vf*ygs
97 format('f.p. z(1)=',e12.3,',',e12.3)
98 format('f.p. z(2)=',e12.3,',',e12.3,',',e12.3,',',e12.3)
99 format('f.p. f(1)=',e12.3,',',e12.3,',',e12.3,',',e12.3)
p=max(0.d0,p0+((1.d0-s1)*ygs-s2)*ypg)
fp=max(min(1.d0-(p-pi)/(pm-pi),1.d0),0.d0)
fs=ki/(ki+s2)
fs1=s2/(ks+s2)
zt=lt/l
zb=xd
rbot=db/2.0d0
if(zb.lt.zt) then
rt=dt/2.d0
rbot=db/2.d0
r=(rt-rbot)/zt*zb+rbot
m=(rt-rbot)/lt
else
r=dc/2.d0

```

```

    m=0.d0
endif
rideal=1.206d0
ug=co2/mw/press*rideal*temp*1000.d0
1 /3.1415926535d0/r/r*co2frac
if (ug.lt.0.d0) ug=0.d0
ul=1000.d0*vf/piv/r/r
peclet=conskim*(ul/(ul+ug))**1.03d0*(rb/r)**1.66d0
disp=rb*2.d0*ul/peclet
ug2=c0*.01/mw/press*rideal*temp*1000.d0
1 /3.1415926535d0/r/r !
ug=ug/3600.d0
ug2=ug2/3600.d0
ul=ul/3600.d0
epsg=bw(1)*ug**bw(2)*(rb*2.d0)**bw(3)*(r*2.d0)**bw(4)
eps1=bw(5)*ul**bw(6)*ug**bw(7)*(rhos-rhof)**bw(8)
1 *(rb*2.d0)**bw(9)*mul**bw(10)*(r*2.d0)**bw(11)-epsg
eps12=bw(5)*ul**bw(6)*ug2**bw(7)*(rhos-rhof)**bw(8)
1 *(rb*2.d0)**bw(9)*mul**bw(10)*(r*2.d0)**bw(11)-epsg
eps1=max(eps1,eps12,1.d0-esmax-epsg)
es=max(0.d0, 1.d0-epsg-eps1)
ug=ug*3600.d0
ul=ul*3600.d0
rg=(qpm/ypg)*fs1*fp*fs*x*eff2()
if(s2.lt.0.0d0)rg=0.d0
f(1)=rg*es*0.49d0*1/ul*vf
q=vf+co2*rideal*temp/(press*mw)!l/hr
term1=1.03d0*rideal*temp/(mw*q)
term2=k1*co2/press/press
term3=f(1)/press/l
term4=0.34*m/r
ddispdx=disp*1*
1 (term1*(term2+term3)-term4)

93 format('ddispdx=',f7.4,1x,f12.4,1x,f12.4)
rs=vm*s1/ygs*xe/(ag+s1)*eff1()
f(2)=(1/disp)*(ul-ddispdx/1-2.d0*disp*m/r)*z(3)+1*l/disp/c0c*rs*
* es
f(3)=(1/disp)*(ul-ddispdx/1-2.d0*disp*m/r)*z(5)+1*l/disp/c0c*es*
* (rg-ygs*rs)
z(1)=min(co2max,max(0.d0,z(1)))
return
end

c
c
subroutine dfsub3(xd,z,df)

```

```

implicit real*8(a-h,o-z)
dimension df(3,5),z(5)
dimension ff(5),f(5)
include 'commons.for'
dzmin=0.1d0*pstep
do 200 i=1,5
  dz=dabs(z(i))*pstep
  if (dz.lt.dzmin) dz=dzmin
  if(i.eq.2.and.z(i).ge.1.0d0) dz=-dz
  if(i.eq.4.and.z(i).ge.1.0d0) dz=-dz
  ts=z(i)
  z(i)=z(i)+dz
  call fsub3(xd,z,ff)
  z(i)=ts
  call fsub3(xd,z,f)
  do 150 lc=1,3
    df(lc,i)=(ff(lc)-f(lc))/dz
150  continue
200  continue
  return
end
c
c
  subroutine gsub3(i,z,g)
  implicit real*8(a-h,o-z)
  dimension z(5)
  include 'commons.for'
  goto (10,20,30,40,50),i
10  g=z(1)-0.d0
  return
20  if(bctype .eq. 0.0)then
    call smplbc(z,g)
  else
    call cmplxbc(z,g)
  endif
  return
30  g=z(4)-0.d0
  return
40  g=z(3)-0.d0
  return
50  g=z(5)-0.d0
  return
end
c
  subroutine smplbc(z,g)
  implicit real*8(a-h,o-z)

```

```

dimension z(5)
include 'commons.for'
g=z(2)-1.0
return
end

c
c
subroutine cmplxbc(z,g)
implicit real*8(a-h,o-z)
double precision m,mw,k1,k2
dimension z(5)
double precision bw(11) !Begovich and Watson constants
include 'commons.for'
data bw/0.058d0,0.692d0,0.107d0,-0.037d0,0.388d0,0.26d0,
1 0.036d0,-0.327d0,-0.278d0,0.047d0,-0.02216d0/
data mw/44.0d0/ !molecular weight of CO2
data piv/3.14159265359d0/
k1=rhof/70.306958
k2=14.7d0
press=k1*1+k2
xd=0.d0
s1=z(2)
co2=min(c0c*ygs*.49d0*vf,max(0.d0,z(1))*co2max)
atemp=c0c*.49d0*vf
if(z(1).lt.0.d0)write(*,*)'b.c. error co2=',z(1)
if(z(1).gt.atemp)write(*,*)'b.c. error co2=',z(1)
if(z(2).gt.1.0d0)write(*,*)'b.c. error z(2)='s
if(z(2).lt.0.0d0)write(*,*)'b.c. error z(2)='s
p=p0+((1.d0-s1)*ygs-s2)*ypg
fp=max(min(1.d0-(p-pi)/(pm-pi),1.d0),0.d0)
fs=ki/(ki+s2)
fs1=s2/(ks+s2)
zt=lt/l
zb=0.d0
if(zb.lt.zt) then
  rt=dt/2.d0
  rbot=db/2.d0
  r=(rt-rbot)/zt*zb+rbot ! radius at our length
  m=(rt-rbot)/lt
else
  r=dc/2.d0
  m=0.d0
endif
rideal=1.206d0
ug=co2/mw/press*rideal*temp*1000.d0
1 /3.1415926535d0/r/r*co2frac !

```

```

ul=1000.d0*vf/piv/r/r
peclet=conskim*(ul/(ul+ug))**1.03d0*(rb/r)**1.66d0
disp=rb*2.d0*ul/peclet
ug2=c0*.01/mw/press*rideal*temp*1000.d0
1 /3.1415926535d0/r/r !
ug=ug/3600.d0
ug2=ug2/3600.d0
ul=ul/3600.d0
epsg=bw(1)*ug**bw(2)*(rb*2.d0)**bw(3)*(r*2.d0)**bw(4)
eps1=bw(5)*ul**bw(6)*ug**bw(7)*(rhos-rhof)**bw(8)
1 *(rb*2.d0)**bw(9)*mul**bw(10)*(r*2.d0)**bw(11)-epsg
eps12=bw(5)*ul**bw(6)*ug2**bw(7)*(rhos-rhof)**bw(8)
1 *(rb*2.d0)**bw(9)*mul**bw(10)*(r*2.d0)**bw(11)-epsg
eps1=max(eps1,eps12,1.d0-esmax-epsg)
es=1.d0-epsg-eps1
ug=ug*3600.d0
ul=ul*3600.d0
rg=(qpm/ypg)*fs1*fp*fs*x*eff2()
if(s2.lt.0.0d0)rg=0.d0
rs=vm*s1/ygs*xe/(ag+s1)*eff1()
if(s1.lt.0.0d0)rs=0.d0
f1=rg*es*0.49d0*1/ul*vf
alpha=2.d0/(conskim*piv*vf**0.03d0*rb**0.66d0)
q=vf+co2*rideal*temp/(press*mw)
term1=1.03d0*rideal*temp/(mw*q)
term2=k1*co2/press/press
term3=f1*co2max/press/l
term4=0.34*m/r
ddispdx=disp*1*
1 (term1*(term2+term3)-term4)
g=z(2)-1-(disp/l)*(1/(ul-ddispdx/l-2.d0*disp*m/r))*z(3)
return
end
c
c
subroutine dgsub3(i,z,dg)
implicit double precision (a-h,o-z)
dimension z(5),dg(5)
include 'commons.for'
do 5 j=1,5
dg(j)=0.d0
5 continue

goto (10,20,30,40,50),i
10 dg(1)=1.d0
return

```

```

20  if(bctype .eq. 0.0)then
      call smpljbc(dg)
    else
      call cmplxjbc(i,z,dg)
    endif
    return

30  dg(4)=1.d0
    return

40  dg(3)=1.d0
    return

50  dg(5)=1.d0
    return
    end

c
c
      subroutine smpljbc(dg)
      implicit double precision (a-h,o-z)
      dimension dg(5)
      include 'commons.for'
      dg(2)=1.d0
      dg(3)=0.d0
      dg(4)=0.d0
      dg(5)=0.d0
      return
      end

c
      subroutine cmplxjbc(i,z,dg)
      implicit double precision (a-h,o-z)
      dimension z(5),dg(5)
      include 'commons.for'
      write(*,*)'flag-gee1'
      pstep=1.d-6
      dzmin=0.1d0*pstep
      do 200 ic=1,5
        dz=dabs(z(ic))*pstep
        if (dz.lt.dzmin) dz=dzmin
        ts=z(ic)
        if(i.eq.2.and.z(ic).ge.1.0d0) dz=-dz
        z(ic)=z(ic)+dz
        bctype=1.0
        call gsub3(i,z,gg)

```

```

      z(ic)=ts
      bctype=1.0
      call gsub3(i,z,g2)
      dg(ic)=(g2-gg)/dz
      write(*,*)z(ic),z(ic),'ic',ic,'gg',gg,'g2',g2
200  continue
      write(*,*)dg(1)',dg(1)',dg(2)',dg(2),dg(3),dg(4),dg(5)
      return
      end
c
      subroutine solutn(x,z,dmval)
      implicit real*8 (a-h,o-z)
      dimension z(5), dmval(1)

      z(1)=-0.2d0*x*x+0.4d0*x
      z(2)=1.d0-(-1.d0*x*x+2.d0*x)
      z(3)=x-2.d0
      z(4)=-1.d0*x*x+0.5d0*x
      z(5)=x*x-2.17d0*x+1.d0
      return
      end

```

VITA

May Yongmei Sun entered East China University of Chemical Technology in July 1985 and received the Bachelor of Engineering in Biochemical Engineering with honor in July 1989. She then worked in a pharmaceutical company in China. After working for a year, she went to Biochemistry Institute of the University of Zurich, Switzerland, as research assistant. In 1992, she entered the Master's program in Chemical Engineering at the University of Tennessee, Knoxville, and received the Master's degree in 1994. She pursued the Doctor of Philosophy degree in Chemical Engineering at a joint program between Oak Ridge National Laboratory and University of Tennessee. The doctoral degree was received in December, 1997.