To the Graduate Council:

I am submitting herewith a dissertation written by Hwai-Shen Liu entitled "Gas Stripping Ethanol Fermentation System." 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.

Hsien-Wen Hsu, Major Professor

We have read this dissertation
and recommend its acceptance:

Accepted for the Council:

Vice Provost
and Dean of The Graduate School

GAS STRIPPING ETHANOL FERMENTATION SYSTEM

A Dissertation

Presented for the

Doctor of Philosophy

Degree

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Hwai-Shen Liu

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DEDICATION

To my father (劉伉吾), mother (謝秀華),
wife (徐瑋玲) and Drew.

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ABSTRACT

The method of product removal from fermentation medium to prevent product inhibition and to improve the productivity by applying gas stripping in the ethanol fermentation (GSEF) is investigated experimentally and analytically.

In a GSEF system, the stripping effect was modeled by a quasi-steady-state assumption in the gas phase, using a newly defined "stripping factor". The effects of temperature and gas flow rate on the stripping factor were investigated in a batch ethanol-water system experimentally. The mass transfer effect was negligible in the experimental range investigated. The Henry's law constants are obtained from the stripping experiments for 35°C, 50°C and 60°C. They are to be used in the estimation of the ethanol removal rate associated with stripping factor in a GSEF system. Also, a simple stripper-condenser experiment has shown effectively not only in product removal but also in product concentration in the overhead condenser.

The advantages of the GSEF system are demonstrated by using a modification of Ghose and Tyagi growth kinetics (1979) together with Luedeking-Piret (1959) production model; namely, the cell concentration, productivity and substrate consumption increase with an increase in the stripping factor, and the ethanol concentration in the broth decreases with an increase in the stripping factor. The enhancement of productivity is mainly due to an increase in the cell concentration and to an increase in the substrate utilization in the system.

In the product inhibition case, there is always one (nontrivial) steady state provided that the dilution rate is less than the maximum growth rate. However, in the combined product-substrate inhibition case, it may have as many as three nontrivial steady states and the hysteresis effect is also observed. Local stability analysis and numerical simulation have shown that the one with the best substrate utilization (optimal) is always stable. The attraction region of the optimal steady state indicates that the higher cell concentration and the lower substrate and ethanol concentrations are in favor of the system to move toward the optimal steady state. In this model, the removal of inhibitory product has shown to enable the system to operate at a higher dilution rate and a higher feed substrate concentration.

Analysis of a batch GSEF system reveals that the removal of inhibitory product is not always favored in the total yield. A criterion is obtained to distinguish three different cases (increase, decrease or no effect of the total yield).

A thermophilic anaerobic bacterium, *Clostridium thermosaccharolyticum* (ATCC 31925, HG-6), was evaluated to incorporate with the GSEF system. However, the strain seems to be incapable of growing at high xylose concentration and, consequently, the ethanol concentration produced by this strain is not sufficient to demonstrate the alleviation of the product and of the product-substrate inhibitions by the gas stripping.

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1. INTRODUCTION

The use of ethanol as a fuel is not a new concept since it was first suggested about a century ago (Cheremisinoff, 1979). Presently, ethanol is used at 10% level in gasoline in many states of the United States. Ethanol has also been blended into gasoline in Brazil as a successful extender (Scheller, 1973). However, most of the ethanol produced in the United States is from the catalytic conversion of ethylene. It has been estimated that the U. S. generates 1 billion tons of various fermentable residues (agricultural, municipal and industrial) annually. Conversion of these residues could produce as much as 60 million gallons of ethanol per year or about half of the U. S. motor fuel requirements (Clausen and Gaddy, 1983).

For an ideal alcohol fermentation process the following characteristics are desirable:

(1) Continuous operation: This mode of operation provides steady production rates and ease of controlling the operation variables to achieve the optimum yields.

(2) High cell concentration: This provides a high reactor performance to optimize the maximum productivity; gram ethanol/L-h.

(3) Single sugar substrate: Sequential metabolism of sugars, often with a time lag between sugars, (diauxic effects) may be avoided and also the formation of toxic high molecular weight alcohols as by-products from multiple-sugar substrate may be reduced.

(4) High sugar concentration: This might provides an enhancement of substrate conversion to ethanol and also reduces the recovery, purification and concentration costs of the products.

(5) High ethanol concentration: This demonstrates high conversion of substrate to ethanol and thus also reduces the recovery cost.

The development of a system which meets the above characteristics hinges on two limitations that appear to be common to conventional ethanol-yielding fermentation: low cell concentration and ethanol toxicity (Weigel, 1980 and Zeikus, 1980). The toxicity affects the cell growth and the yield as well as the viability of microorganisms. However, the degree of effect strongly depends on the strain chosen.

In the case of ethanol production using *Saccharomyces cerevisiae*, ethanol noncompetitively inhibits the reaction kinetics. It was reported that ethanol producing capability of the cells was completely inhibited at approximately 110 [g/l] ethanol. Below a concentration of 30 [g/l], the inhibition was negligible (Luong, 1985; Holzberg et al., 1967). The inhibitory and poisonous effect of ethanol was thought to be caused by ethanol's effect on the lipid cell membrane (Leao and Van Uden, 1982). The addition of a protein-phospholipid complex as a protective agent that shields the cells against ethanol inhibition was proposed to reduce the inhibition effect (Jin et al., 1981). However, the mechanism of ethanol inhibition is not quite clear. Generally speaking, there are two schemes to approach this problem. Strain improvement by mutation, selection and adaptation procedures could result in higher ethanol tolerance characteristics. The other means of alleviating this problem is by continuously removing ethanol *in situ* (directly from

fermentation) such as solvent extraction, membrane separation, vacuum fermentation and supercritical fluid extraction systems.

Gas stripping is another alternative for ethanol *in situ* removal to alleviate the product inhibition with special application to thermophilic microorganisms. This alternative may offer a simple and economical design option and has been suggested by several authors (Hartley and Payton, 1983; Hartley et al., 1983; Ljunghdahl and Carrirea, 1983; Wang and Cooney, 1980). However, there has not been much systematic work done. The goal of this research, therefore, can be divided as the following aspects:

(1) To investigate experimentally and model the process of ethanol removal rate in the gas stripping ethanol fermentation (GSEF) system; thus, useful information can be provided for comparison with other processes and for design purpose.

(2) To analyze the effect of gas stripping on substrate utilization, productivity enhancement etc. in the GSEF system for both batch and continuous systems.

(3) To evaluate the possibility of incorporating the GSEF system with a ethanol-producing thermophilic bacterium, *Clostridium thermosaccharolyticum* (ATCC 31925, HG-6).

(4) To investigate the stability and possible multiplicity in the continuous fermentation systems.

2. LITERATURE REVIEW

Distillation is the current method of separating ethanol from fermentation broth (Busche, 1984; Abrams, 1975), although there are a number of alternatives under development such as reverse osmosis, adsorption (molecular sieve), dehydration using absorbent solids and solvent extraction. Distillation is an energy-intensive procedure, and it frequently uses more energy than is available from the ethanol recovered. It is evident that the energy needed varies not only with the technique adopted but also with the ethanol concentration of the broth. Several empirical correlations of energy requirement to produce 95%(wt) ethanol from various feed concentration for distillation (Federico, 1983; Leppanen et al., 1983; Wettstein and DeVos, 1980) show that the costs become more favorable when the fermentation broth contains more than 5% ethanol, especially in the range higher than 7 to 9% (Cysewki and Wilke, 1976). Hence, a great deal of attention has been directed toward *in situ* removal of ethanol from fermentation broth not only for lowering the inhibition and possibly higher productivity but also for reducing recovery cost in the downstream process. A review of published fermentation processes with *in situ* ethanol removal are presented and discussed in the following sections.

2.1. Solvent Extraction System

Solvent extraction system, or "extractive fermentation", offers the possibility of reducing energy cost and eliminating the product inhibition. Liquid-liquid extraction system is particularly attractive for large-scale operations because of the ease with which it can be scaled up and its tolerance for feed stream impurities, i.e. undissolved solids. In the extractive process,

ethanol is continuously removed from the beer by solvent extraction. A side stream of beer is tapped from the fermentor vessel, the cells are removed and recycled, and the clear beer is contacted with liquid extractant. The extractant, which is immiscible in the beer absorbs most of ethanol. The purified beer can now be returned to the reaction vessel, and the ethanol saturated extractant can be processed through a distillation train for ethanol recovery and solvent recycle. In term of process evaluation criteria, extractive fermentation, with a modest increase in complexity, yields a very high productivity, low energy system. However, a good extractant should have the following properties to be successful in ethanol removal (Daugulis, 1988; Phillips and Humphrey, 1983; Maiorella et al., 1981; Pye and Humphrey, 1980):

(1) high distribution coefficient for ethanol,

(2) non-toxic to microorganisms,

(3) chemical and thermal stability,

(4) relatively nonvolatile to ethanol to minimize solvent recovery cost,

(5) selective for ethanol over water and secondary products,

(6) immiscible with fermentation broth, and

(7) non-hazardous nature.

It becomes apparent that solvents with higher ethanol distribution coefficient (i.e. polar solvents) are also more soluble in water (Tedder, 1984; Finn and Fiechter, 1979). Further, most of the polar solvents are highly toxic to the microorganisms (Phillips and Humphrey, 1983; Minier and Goma, 1982; Finn and Fiechter, 1979). Nontoxic solvents, on the other hand (usually nonpolar and more than 12 carbon atoms), have a low distribution coefficient for ethanol. For ethanol *in situ* separation, no extractant having all the required properties listed above has been found yet (Slapack, 1987; Honda et al., 1986).

However, examples of potential extractants that have been examined include tributylphosphate (Roddy, 1981), decanol and activated carbon (Wang et al., 1981), dodecane (Finn, 1966), fluorocarbons (Levy, 1981), aqueous two-phases system, e.g. dextran, polyethylene (Mattiasson et al., 1981), a range of "high boiling" solvents (Matsumura and Markl, 1984) and oleyl alcohol (Kollerup and Daugulis, 1987, 1986, 1985a, 1985b).

Wiegel (1982) has suggested that the recovery of ethanol from the fermentation broth through solvent extraction does not seem feasible for thermophilic processes using wastes. Since interfering materials, possibly deleterious, might also be extracted from the wastes, Wiegel believes that methane production from wastes is a more practical alternative.

It may be possible to couple liquid-liquid extraction with extractive distillation (Tedder, 1984). Aqueous two-phase extractive bioconversion processes continuously partition ethanol from the bottom phase to the top phase (Larsson and Mattiasson, 1984; Hahn-Hageral and Mattiasson, 1981). This prevents ethanol build-up in the bottom phase, where the bioconversion takes place.

2.2. Membrane System

Membrane fermentation system was first developed as a batch technique, using a simple dialysis membrane flask fermentor (Schultz and Gerhardt, 1969; Gerhardt and Gallup, 1963). The process is readily adapted to continuous culture. In a simple continuous membrane system, a culture of active yeast is maintained in a confined zone of the vessel. Substrate enters the reaction zone by diffusing through a membrane from the medium zone. Reaction then takes place and product ethanol diffuses back through the

membrane into the medium zone where it is recovered in an overflow. Yeast can not escape the reaction zone and high cell densities can be achieved. Usually this process is diffusion-controlled rather than reaction-controlled because of limited surface area of membrane. This practical limitation prevents very rapid reaction and makes it difficult to use membrane for industrial application to ethanol production. Also, possible membrane fouling might block the pores and prevent further flow in a continuous process.

Although the fouling problem and slow diffusion rate could be lessened by the design of rotorfermentor (Margaritis, 1978). The rotorfermentor is a continuous pressure membrane reactor. The simple fixed membrane is replaced by a rapidly rotating membrane cylinder. Feed and gas are pumped into the annular reaction zone where yeast grow and are retained. This zone is maintained under pressure (2-20 psig), so that filtrate continuously flows out through the membrane. Because the membrane is rotating, a strong centrifugal force is developed at the membrane surface and large molecules impinging on the surface are thrown back into the annular zone. The rotorfermentor appears attractive in terms of its high productivity. It fails, however, when rated on mechanical and operating simplicity. The rotating membrane unit is mechanically complicated, and seals for this unit will be made especially complex as they must not leak against the vessel pressure.

Hollow fiber reactors, providing extremely large membrane area, are another approach to achieve high reaction rate. The hollow fiber reactor is arranged like a shell and tube heat exchanger. The tubes are fine hollow fiber (in the order 0.05 cm I.D.) of membrane material. A bundle of hundreds or thousands of these fiber tubes can be packed into a single shell with a few centimeter in diameter. The shell side is then inoculated with growing cells.

Medium is fed into the fiber tubes at one end of the reactor and substrate diffuses into shell side where bioconversion takes place. The product, ethanol, diffuses back to tube side and is carried out at the other end of the reactor. The hollow fiber reactor has potential to achieve yeast cell densities approaching the maximum cell packing density (200 [g/l]) and the corresponding high ethanol productivity would be possible. However, they are quite complex and costly with the problems of membrane fouling and product gas venting.

2.3. Vacuum Fermentation System

The vacuum fermentation processes, first developed by Cyseweski and Wilke (1979) and Ramalingham and Finn (1979), provide another alternative with *in situ* ethanol removal. In those systems, a substrate stream is continuously fed into the reactor to maintain constant liquid level while ethanol-water solution is boiled away under vacuum conditions. Ramalingam and Finn (1979) demonstrated that it was possible to ferment a threefold higher molasses concentration in only one third the amount of time needed in a conventional process. Independently, Cysewski and Wilke (1979) developed a similar vacuum system, but in more detail. Using a 33.4% continuous glucose feed and a total pressure of 50 mmHg, ethanol productivities achieved were as high as 82 and 40 [g/l/hr] with and without cell recycle, respectively. Since ethanol is more volatile than water, this flash operation acts as single stage distillation. With a process temperature of 35^{o}C and pressure of 51 mmHg, the ethanol concentration is maintained at 35 [g/l] while the ethanol concentration in the vapor product is increased to more than 200 [g/l], thus simplifying later distillation steps. Besides the lowering distillation cost and elimination of ethanol inhibition, no cooling is required for this process. In fact, the heat of fermentation may be insufficient to sustain isothermal

evaporation in a reduced pressure system (Bu'Lock, 1979). In this respect, the use of thermophilic microbes would improve fermentation under reduced pressure or would permit higher pressure in the fermentor. Both of these would further reduce the energy costs. The major concerns of this vacuum fermentation process are energy requirements for the vacuum system, capital cost, control difficulty of the unusual low-pressure compressor, and high risk of contamination because of reduced pressure. In view of the highly anaerobic conditions and elevated growth temperatures, special nutrients are required such as sparging pure oxygen as well as other physiological problems (Finn and Fiechter, 1979).

The flash fermentation process modifies the simple vacuum fermentation process to overcome some difficulties (Maiorella, 1979). Reaction is carried out in an atmospheric pressure fermentor so that the yeast oxygen maintenance requirement can be cheaply met with sparged air. Carbon dioxide produced can be vented directly from the vessel and thus needs not be compressed with vapor product as in the vacuum fermentation process. To remove ethanol, beer is rapidly cycled through a small auxiliary flash vessel where it boils. Ethanol is recovered as the flash vessel overheated vapor product and ethanol depleted beer is returned to the vessel. The problem of contamination is greatly reduced as only the small flash vessel is under vacuum. Energy requirements for this process are also slightly lower than for the vacuum fermentation process. The flash fermentation process is somewhat more complicated than the vacuum fermentation process, the former requiring an added vessel and the associated liquid cycling pumps, but this is probably more than compensated by the advantages mentioned above. Like the vacuum process, it is not clear whether the high productivities

possible with vacuum operation offset the operation and control difficulties and the likely high compressor cost.

2.4. Supercritical Fluid Extraction System

Since the economics of ethanol production centers around the energy-intensive step of ethanol separation from the fermentation step, the process used to remove ethanol from the fermentation broth should be more efficient and economical than is traditional distillation. Supercritical carbon dioxide extraction seems to be the promising method because of (1) very high selectivity, (2) easy separation of the solvent (supercritical carbon dioxide) from extractant and (3) low heat of vaporization of the solvent (Kuk and Montagua, 1983; DeFilippi, 1982). Experimental results indicate that dehydration of ethanol by the supercritical carbon dioxide is technically feasible and carbon dioxide becomes supercritical under relatively mild conditions: about 304K and 7.5MPa (1100 psi). The solubility of supercritical carbon dioxide in water is small whereas it is completely miscible with low-molecular weight oxygenated hydrocarbons, and as a result, it can be used efficiently to extract ethanol from an aqueous solution. While viscosity remains low, diffusivity and solvent power of supercritical carbon dioxide for nonpolar molecules become very high. Such a process has been used successfully for some years for decaffeinating coffee. However, a recent study showed that using supercritical carbon dioxide does not lead to significant energy saving or to completely dehydrate alcohols (Brignole, 1987). Moreover, pressure was reported to have a significant inhibiting effect on the initial rate of ethanol production as well as the final ethanol concentration. The effect is more significant as the pressure increases (only 17% of ethanol concentration at normal conditions obtained under 7 MPa CO_2),

especially when carbon dioxide is used to pressurize the fermentor (Thibault, 1987). These disadvantages as well as capital and operating costs of high pressure system might discourage the application of supercritical fluid extraction fermentation as an *in situ* ethanol removal method.

2.5. Gas Stripping System

Recently, thermophilic microbes have attracted more attention because of several practical advantages (Weigel, 1980; Zeikus, 1980) such as (1) fast growth rate, (2) low celluar yield leading to high product/substrate ratios, (3) high metabolic activity leading to fast fermentation and an increase in production rates, (4) fermentation with wide range of substrate, pH as well as temperature, (5) potential advantages of thermophilic enzymes, (6) low risk of contaminations, and (7) high solubility of substrate. Fermentation by thermophilic microbes with *in situ* gas stripping seems promising because higher stripping efficiency is expected for higher temperature. It might alleviate ethanol toxicity to the microorganism, resulting in higher productivity and drastically reduced distillation cost. It also may provide a gentle way of mixing. Best of all, it may offer a simple and economical design option without complicated sealing problems, requires less energy than the vacuum and supercritical fluid systems and, furthermore, there is no need for cleaning equipment like the membrane system. Also, cleaner product (only volatile components) can be obtained more easily compared with a solvent extraction system which might contain solids. For aerobic fermentation, a stream of air can serve for both aerating and stripping purposes while an oxygen free gas can help the system to be anaerobic for strictly anaerobes. Since the pressure in the system is higher than in the surroundings, less chance of contamination is anticipated. Also, recovery of other volatile

fermentation products such as acetone and butanol might be good candidates for this system.

Although several authors mentioned the possible application of this process, only a few studies were found that have direct application of this system in ethanol production (Mulholland, 1984; Dale et al., 1985) and butanol production (Groot et al., 1989; Ennis et al., 1986). Mulholland tested two strains of *S. cerevisiae* (ATCC 4126 and 4132) for fermentation at 40^{o}C in an 11 liter glass cylinder with an inner draft tube to help circulation. The ethanol removal rate reportedly increased approximately twofold for every 10^{o}C rise in operation temperature. However, operating difficulties such as substrate build-up and occasional decrease in cell viability were observed in the continuous fermentation. This was attributed mainly to the lack of good automatic control (sugar feed, liquid level, flow of gas and specially pH) by the author. Dale used *Kluyveromyces fragilis* (NRLL 2415) at 31^{o}C in an immobilized cell reactor in which the majority of microbial conversion was carried out followed by a stripper for separation. The continuous fermentation was modelled by equilibrium stage approach resulting in height equivalent of a theoretical plate (HTEP). A suspicious point was observed: the vapor liquid equilibrium data of ethanol water system at boiling points (78-100^{o}C) was extended for 31^{o}C for the purpose of modelling. In batch butanol fermentations, Groot et al. (1989) and Ennis (1986) all observed enhanced substrate utilization rate with butanol removal by gas stripping, but lower yields were also noticed. Nevertheless, the gas stripping fermentation system appears promising in practical application and offers potential advantages over most conventional systems as described above. This warrants further study and; is the objective of this research.

3. THEORY

The equations pertaining to the GSEF system are presented below to facilitate analysis.

3.1 Formulation

In a GSEF system, a CSTR fermentor of liquid volume (V_L) is fed with a stream of volumetric flow rate (F) containing the limiting substrate concentration of (S_F) and ethanol concentration of (P_F) while an inert gas of constant volumetric flow rate (G) is stripping through the fermentor to remove the product (ethanol) in the broth. The mass balance equations of cell (X), of product in liquid (P_L) and in gas phases (P_G) and of substrate (S) are:

(1) Cell balance:

$$\frac{dX}{dt} = (\mu - D)X \qquad (3.1.1)$$

where D ($= \frac{F}{V_L}$) is the dilution rate and μ is the specific growth rate. Since substrate and product inhibitions are not unusual in ethanol fermentation, various modifications of Monod equation as well as other form of models that include these inhibition effects have been proposed (Lenbury and Chiaranai, 1987; Lee, Poulard and Coulman, 1983; Jin, Chang and Wang, 1981; Sevely et al., 1981; Ghose and Thyagi, 1979; Bazua and Wilke, 1977; Holzberg, Finn and Steinkraus, 1967; Hinshelwood, 1964; Monod, 1942).

(2) Product balances:

gas phase: $$V_G \frac{dP_G}{dt} = - GP_G + V_L K_G a(P_G^* - P_G) \quad (3.1.2)$$

liquid phase: $$\frac{dP_L}{dt} = \sigma X + D(P_F - P_L) - K_L a(P_L - P_L^*)$$

$$= \sigma X + D(P_F - P_L) - K_G a(P_G^* - P_G) \quad (3.1.3)$$

where V_G is the volume of gas phase ($V_G + V_L$ = reactor volume); σ is the specific production rate which is categorized mainly as growth-associated, nongrowth-associated and combination of both (Luedeking and Piret, 1959); i.e.

growth-associated: $\sigma = \alpha\mu$ (3.1.4)

nongrowth-associated: $\sigma = \omega$ (3.1.5)

Luedeking-Piret model: $\sigma = \alpha\mu + \omega$ (3.1.6)

Where α and ω are constants determined by experiment; K_L and K_G are the overall mass transfer coefficients based on liquid phase and gas phase, respectively; a is the specific area. $K_L a(P_L - P_L^*)$ and $K_G a(P_G^* - P_G)$ are used interchangeably with the assumption of no accumulation at interphase; P_L^* and P_G^* are the hypothetical equilibrium concentrations with P_G (product concentration in gas phase) and P_L (product concentration in liquid phase) through the use of K_L and K_G. The equilibrium concentration is given by

$$H = \frac{P_G^*}{P_L} \quad (3.1.7)$$

where H is the distribution coefficient based on the liquid bulk concentration and is a function of temperature and composition. For a dilute solution it is known as Henry's law constant.

(3) Substrate balance:

$$\frac{dS}{dt} = \upsilon X + D(S_F - S) \qquad (3.1.8)$$

where υ is the specific substrate consumption rate and is related to the specific growth rate μ or the specific production rate σ by the yield factors as

$$\upsilon = -\mu / Y_{x/s} \qquad (3.1.9)$$

$$\upsilon = -\sigma / Y_{p/s} \qquad (3.1.10)$$

where $Y_{x/s}$: yield of cell-growth based on limiting substrate ($-\frac{\Delta X}{\Delta S}$)

$Y_{p/s}$: yield of product based on limiting substrate ($-\frac{\Delta P}{\Delta S}$)

These yield factors in equations (3.1.9) and (3.1.10) are usually taken as constants. However, a possibility of these yield factors as a function of substrate concentration has been also mentioned (Koga and Humphrey, 1967).

3.2 Stripping Factor

With a quasi-steady-state approximation in the gas phase, equation (3.1.2) may be simplified to

$$P_G = \frac{V_L K_G a}{G + V_L K_G a} P_G^* \qquad (3.2.1)$$

Then, substituting equation (3.2.1) into equation (3.1.3), one has

$$\frac{dP_L}{dt} = \sigma X + D(P_F - P_L) - \frac{GK_Ga}{G + V_L K_G a} P_G^* \qquad (3.2.2)$$

With Henry's law given in equation (3.1.7), equation (3.2.2) reduces to

$$\frac{dP_L}{dt} = \sigma X + D(P_F - P_L) - \beta P_L \qquad (3.2.3)$$

$$\beta = \frac{GHK_Ga}{G + V_L K_G a} \qquad (3.2.4)$$

where β is defined as a "stripping factor". As can be seen from the definition of β, it is a function of the gas volumetric flow rate (G), the product distribution coefficient (H), liquid volume of fermentor (V_L), and the mass transfer coefficient ($K_G a$). Also, the product concentrations in gas and liquid phases are related through equations (3.1.7), (3.2.1) and (3.2.4) by stripping factor as:

$$P_G = \frac{V_L}{G} \beta P_L \qquad (3.2.5)$$

Thus, the GSEF system is described by equations (3.1.1), (3.1.8) and (3.2.3) and the stability behavior of the system can be explored by investigating these simultaneously coupled equations. The magnitude of β should reflect the stripping effect in the GSEF system and two limiting cases are reduced from equation (3.2.4);

(i) For low gas rate ($G << V_L K_G a$), $\beta \approx \frac{G}{V_L} H$. (3.2.6)

(ii) For high gas rate ($G >> V_L K_G a$), $\beta \approx HK_G a = K_L a$. (3.2.7)

In case (i), the stripping factor is proportional to gas rate and inversely to liquid volume while in case (ii), it is a function of the mass transfer

coefficient. In viewing of these limiting cases, it is clear that there are mainly two mechanisms involved in the stripping process. First, ethanol diffuses from the liquid phase into the gas phase which is expressed by the mass transfer. There is an ultimate (equilibrium) ethanol concentration in the gas phase that can be reached for the ethanol concentration in the liquid phase. Second, the ethanol in the gas phase is swept away by the gas stream out of system. When the gas rate is relatively low, the ethanol concentration in the gas phase is allowed to reach equilibrium before swept out of system. Thus, the ethanol removal rate is controlled by gas rate as case (i) indicates. In case (ii), the ethanol removal rate is controlled by the mass transfer, whereas the gas rate is too fast to allow the system to reach equilibrium.

3.3 Determination of Stripping Factor

The order of magnitude of β may be estimated in ethanol-water system either by stripping in a continuous system at steady state or by stripping in a batch system. For the continuous case at steady state ($\frac{dP_L}{dt} = 0$) with negligible cell effect (X=0), equation (3.2.3) reduces to

$$\beta = D\left(\frac{P_F}{P_{L.ss}} - 1\right) \qquad (3.3.1)$$

where the subscript "ss" denotes the values at steady state.

Similarly, for a batch process (i.e. D=0) without microorganism (X=0), equation (3.2.3) becomes

$$\frac{dP_L}{dt} = -\beta P_L \qquad (3.3.2)$$

which can be used for the estimation of ß value. If the stripping factor is a constant, equation (3.3.2) can be integrated with the initial condition t=0, $P_L=P_{L.i}$ to give

$$\ln(P_L) = -\beta t+\ln(P_{L.i}) \qquad (3.3.3)$$

where ß may be determined from the slope of $\ln(P_L)$ vs t. However, the effects of necessary nutrients as well as cells in the fermentor are unknown and can only be found by experiments.

The Henry's law constant may also be estimated from the stripping experiment. By rearranging equation (3.2.4) in a reciprocal form, one has

$$\frac{1}{\beta} = \frac{1}{HK_Ga} + \frac{1}{H}\frac{V_L}{G} \qquad (3.3.4)$$

Thus, the Henry law constant, H, may be determined from the slope of $\frac{1}{\beta}$ vs $\frac{V_L}{G}$ when $\frac{1}{HK_Ga}$ is a constant. For example, the plot of $\frac{1}{\beta}$ vs $\frac{V_L}{G}$ would be a straight line with slope of $\frac{1}{H}$ through origin point in the case of low gas rate, i.e. $\frac{1}{HK_Ga}$ is much smaller than $\frac{1}{H}\frac{V_L}{G}$.

If the Antonine vapor pressure equation is used for temperature effect, the relation between Henry's law constant and temperature can be obtained as

$$H = \frac{P_G^*}{P_L} = \gamma\frac{\exp(A - \frac{B}{T})}{P_{tot}} \qquad (3.3.5)$$

$$\text{i.e.} \qquad \ln(H) = \{\ln(\frac{\gamma}{P_{tot}}) + A\} - \frac{B}{T} \qquad (3.3.6)$$

Where γ, P_{tot} and T are the activity coefficient, total pressure, and absolute temperature while A, and B are the Antonine constants. If γ is a constant, the plot of ln(H) vs 1/T should result in a straight line with a negative slope.

3.4 Steady State(s) in CSTR

In equation (3.2.3), P_F (ethanol feed concentration) is introduced to the system for completeness. In a practical application, one would prefer P_F being zero to avoid product inhibition. Therefore, in a steady state operation with P_F=0 the governing equations, (3.1.1), (3.1.8) and (3.2.3), reduce to

$$(\mu_{ss} - D)X_{ss} = 0 \tag{3.4.1}$$

$$\upsilon_{ss}X_{ss} + D\,(S_F - S_{ss}) = 0 \tag{3.4.2}$$

$$\sigma_{ss}X_{ss} - (D + \beta)P_{L.ss} = 0 \tag{3.4.3}$$

The solution to the above three coupled equations, $X_{ss} = 0$, $S_{ss} = S_F$, and $P_{L.ss}$=0 is a trivial one which is recognized as a washout point. For nontrivial solution ($X_{ss}\neq 0$), the above three equations can be further simplified. They are

$$\mu_{ss}\,(P_L\,,\,S) = D \tag{3.4.4}$$

which is the solution of equation (3.4.1); and by eliminating X_{ss} from equations (3.4.2) and (3.4.3) , one obtains

$$\frac{S_{ss} - S_F}{P_{L.ss}} = \frac{\upsilon_{ss}}{\sigma_{ss}}\left(1 + \frac{\beta}{D}\right) \tag{3.4.5}$$

Evidently, equation (3.4.5) represents an "operating line" always passing through feed point $W(0,S_F)$ and all the possible steady state points in a P_L-S phase plane. Thus, the nontrivial steady state(s) for the system is(are) the

intersection point(s) between the operating line given by equation (3.4.5) and the contour curves given by equation (3.4.4). The operating line is generally a straight line, since $\frac{\upsilon_{ss}}{\sigma_{ss}}(1+\frac{\beta}{D})$ is a constant, e.g., υ as well as σ (as seen in equations (3.1.4), (3.1.5), (3.1.6), (3.1.9) and (3.1.10)) depend only on μ which is equal to D from equation (3.4.4) and β may be assumed to be a constant for the concentration range of interest. The introduction (or increase) of stripping effect will increase the slope of the operating line, therefore, change the corresponding steady state. Mainly, there are two types of steady state change, depending on the growth kinetics and explained in the following.

For the kinetics without product inhibition such as Monod model, the specific growth contour curve in CSTR (equation 3.4.4) is a horizontal straight line as shown in Figure 3.4.1 because the growth rate is independent of product concentration. The increase of stripping effect shifts the steady state to lower product concentration remained in the reactor, but leaves the substrate concentration unchanged. That is, the stripping effect provides product removal, but does not enhance substrate utilization. In the case of product inhibition model, the specific growth curve is bent upward more or less as shown in Figure 3.4.2, depending on the significance of product inhibition. The increase in the slope of operating line moves the steady states not only to a lower product concentration but also to a lower substrate concentration. The extent of steady state shift, due to stripping, depends on the magnitude of stripping factor as well as the characteristics of specific growth curve in a P_L-S plane.

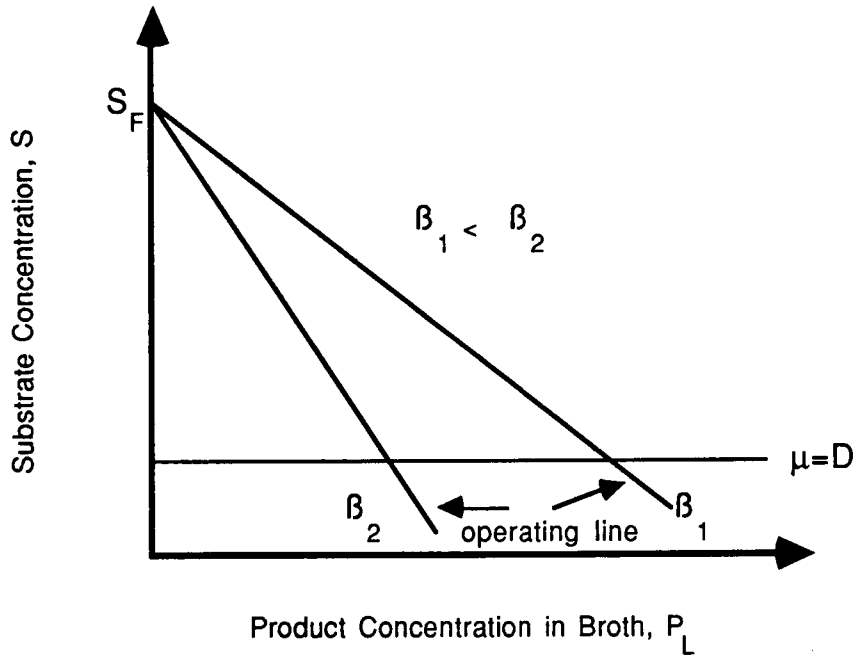


Figure 3.4.1 Graphical Illustration of Stripping Effect on Steady State for Monod Kinetics

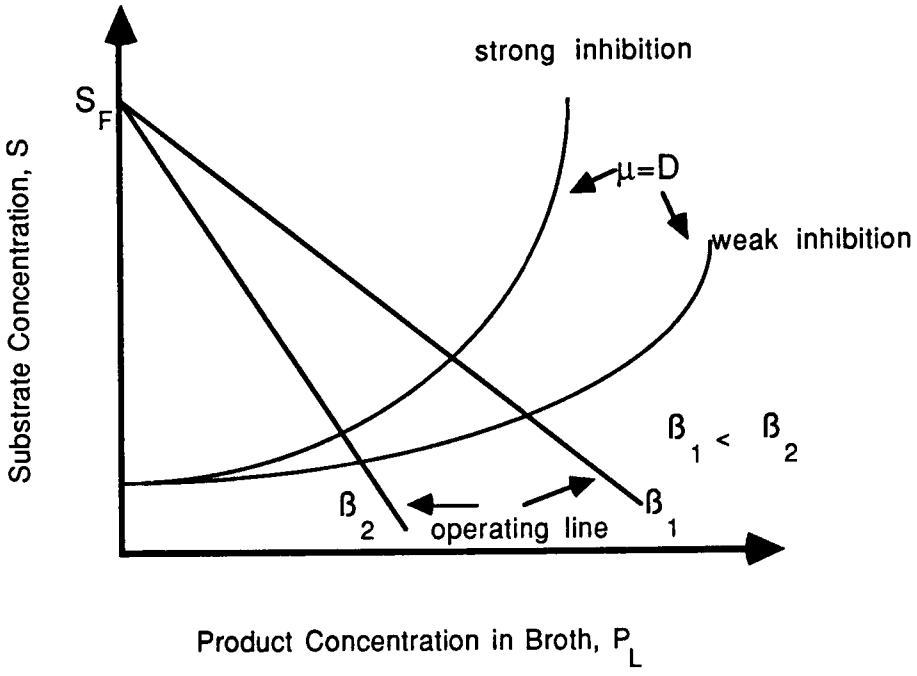


Figure 3.4.2 Graphical Illustration of Stripping Effect on Steady State for Product Inhibition Kinetics

3.5. Productivity Enhancement

The productivity (ψ) is defined as the total product produced per unit time per unit liquid volume from the system. For the GSEF system, it becomes

$$\psi^{GSEF}=(FP_L+GP_G)/V_L \tag{3.5.1}$$

By replacing P_G with P_L by equations (3.2.5) and substituting P_L into equation (3.4.3), one obtains the productivity of GSEF system at steady state as

$$\psi^{GSEF}=\sigma_{ss}X_{ss} \tag{3.5.2}$$

For the CSTR fermentation without stripping ($\beta = 0$), the productivity at steady state is defined as

$$\psi^{CSTR}=\sigma'_{ss}X'_{ss} \tag{3.5.3}$$

where the prime quantity " ' " is used to distinguish CSTR with $\beta = 0$ from the GSEF system. With equations (3.5.2), (3.5.3) and an expression of X_{ss} and X'_{ss} from equations (3.4.2) and (3.4.3), one may define a product enhancement factor by stripping, η, as

$$\eta=\frac{\psi^{GSEF}}{\psi^{CSTR}}=\frac{\sigma_{ss}X_{ss}}{\sigma'_{ss}X'_{ss}}=\frac{\sigma_{ss}(S_F - S_{ss})/\upsilon_{ss}}{\sigma'_{ss}(S_F - S'_{ss})/\upsilon'_{ss}} \tag{3.5.4}$$

For a linear operating line, both the specific production rate and the specific substrate consumption rate depend only on the specific growth rate μ which is equal to the dilution rate D at steady state as indicated by equation (3.4.4), i.e. υ_{ss} and σ_{ss} will be the same value as υ'_{ss} and σ'_{ss}. Thus, equation (3.5.4) reduces to

$$\eta = \frac{\psi^{GSEF}}{\psi^{CSTR}} = \frac{X_{ss}}{X'_{ss}} = \frac{S_F - S_{ss}}{S_F - S'_{ss}} \qquad (3.5.5)$$

Equation (3.5.5) demonstrates that the productivity enhancement factor (η) is proportional to the biomass or the extent of substrate utilization. Therefore, the shift of steady state to lower substrate coordinate does not imply only a better substrate utilization but also a higher productivity.

3.6 Apparent Yield Factor

Since the cost of raw materials makes up about 70% of ethanol sale price in a typical fermentation process (Mairolla, 1984), the yield factor (ethanol produced/ substrate consumed) is as important as the productivity. For a process to be economical viable, one needs to produce ethanol with a good yield ratio in reasonable time.

Apparent yield factor is often referred to the variation of yield in fermentation process, especially for the dilution rate effect on yield in a CSTR. Usually it is attributed to a result of endogeneous metabolism or maintenance energy requirement (Bailey and Ollis; 1986). For example, the parallel consumption of substrate for growth and for other energetic, or maintenance requirement in a continuous fermentor results in the following kinetic.

$$\upsilon X = -\left(\frac{1}{Y_{x/s}}\mu + m\right)X = -\left(\frac{1}{Y_{a.x/s}}\mu\right)X \qquad (3.6.1)$$

where $Y_{x/s}$ is the "true" biomass yield factor, m is the specific maintenance rate and $Y_{a.x/s}$ is the apparent biomass yield factor, which is the yield actually observed. At steady state operation (μ=D), the relation between true biomass yield factor and apparent biomass yield factor becomes

$$Y_{a.x/s} = \frac{DY_{x/s}}{D + mY_{x/s}} \qquad (3.6.2)$$

The variation of (apparent) biomass yield factor due to dilution rate has been noticed and identified as a result of maintenance energy requirement by Lacis, Lawford (1985) and Pirt (1965). However, there is a concern raised by Lacis and Lawford (1989) that there seems no consistent trend about dilution rate effect on apparent product yield factor in ethanol fermentation. This point will be discussed in section 5.2 again.

In a continuous GSEF system, the apparent product yield factor ($Y_{a.p/s}$) becomes the ratio of ethanol production rate ($\sigma_{ss}X_{ss}$) to substrate consumption rate ($-\upsilon_{ss}X_{ss}$), i.e.

$$Y_{a.p/s} = \frac{\sigma_{ss}X_{ss}}{-\upsilon_{ss}X_{ss}} = \frac{\sigma_{ss}}{-\upsilon_{ss}} \qquad (3.6.3)$$

Generally, σ_{ss} and υ_{ss} depend on specific growth rate (μ) which is equal to dilution rate (D) at steady state. Accordingly, an introduction of stripping will not change yield factor. From this point of view, the productivity enhancement by stripping is mainly due to an increase in cell numbers as indicated in equation (3.5.5), resulting from the inhibition removal.

3.7 Stability

For a GSEF system which is an autonomous system, it is expressed in a compact vector form.

$$\frac{dC}{dt} = f(C, q) \qquad (3.7.1)$$

where C is a vector of concentrations of dimension 3 (C^T= [X, S, P_L]), vector f is equal to the right hand side of equations (3.1.1), (3.1.8), (3.2.3) and q denotes a vector of model parameters such as feed concentrations, dilution rate, stripping factor and kinetic parameters. The steady states, or equilibrium points, of a dynamical system occur when there are no change in time, so time derivatives vanish. Then the steady states, C_s, is the solution of the following algebraic equations, which is a vector form of equations (3.4.1), (3.4.2) and (3.4.3).

$$f(C_s, q) = 0 \qquad (3.7.2)$$

A steady state is called regionally asymptotically stable if the system will return to the steady state for any disturbance within the region. If the region covers the whole feasible domain, the steady state is said to be globally asymptoically stable. If not, the region is often called "domain of attraction" which is important if more than one steady state exists. The steady state is locally asymptotically stable if the convergence is maintained for a sufficiently small disturbance. For linear systems, the local stability can be analyzed easily by investigating the eigenvalues of the Jacobian matrix (Denn, 1975). Although no similar technique is available for nonlinear systems, the stability of a nonlinear system can generally be inferred from the stability of a linear approximation. Use of the linearized equations to study the stability behavior of a nonlinear system is sometimes called Liapunov's first method.

By restricting the attention to the region close to steady state, it is convenient to change the variables so that the steady state becomes a reference point, i.e. if the perturbation variables (ξ) is defined as

$$\xi(t) = C(t) - C_s \tag{3.7.3}$$

which represents the vector of deviation from steady states. Since C_s is the time invariant, $\frac{d\xi}{dt}$ is equal to $\frac{dC}{dt}$. The perturbation equation similar to equation (3.7.1) is given as:

$$\frac{d\xi}{dt} = g(\xi, q) \tag{3.7.4}$$

Expanding the right hand side of equation (3.7.4) in a Taylor's series about C_s and neglecting all terms of higher than the first order, the following linearized equation is obtained.

$$\frac{d\xi}{dt} = J\,\xi(t) \tag{3.7.5}$$

The matrix J is the so-called Jacobian matrix, or community matrix, whose element k_{ij} is defined as :

$$k_{ij} = \frac{\partial f_i(C_s, q)}{\partial C_j} \qquad i, j=1, 2, 3 \tag{3.7.6}$$

With equations (3.1.1), (3.1.8,) and (3.2.3), the Jacobian matrix becomes

$$J = \begin{bmatrix} \mu\text{-}D & \frac{\delta\mu}{\delta S}X & \frac{\delta\mu}{\delta P_L}X \\ \upsilon & (\frac{\delta\upsilon}{\delta S}X)\text{-}D & \frac{\delta\upsilon}{\delta P_L}X \\ \sigma & \frac{\delta\sigma}{\delta S}X & (\frac{\delta\sigma}{\delta P_L}X)\text{-}(D+\beta) \end{bmatrix} C_s \tag{3.7.7}$$

For a system with more than one steady state, there will be a different matrix J corresponding to each steady state. The solutions of the linearized system of equation (3.7.5) usually take the form

$$\xi(t) = \Sigma \kappa_i e^{\lambda_i t} \tag{3.7.8}$$

The quantities λ_i and κ_i are the corresponding pairs of eigenvalues and eigenvectors of **J**. If the real parts of all λ_i are negative, then $\xi(t)$ goes to zero as t approaches infinity, and the system is asymptotically stable. The eigenvalue vector $\lambda = [\lambda_i]$ can be shown to have the following property, which is often called characteristic equation.

$$\det (\mathbf{J} - \lambda \mathbf{I}) = 0 \tag{3.7.9}$$

where **I** is the identity matrix and κ_i satisfies the relation, $(\mathbf{J} - \lambda_i \mathbf{I})\kappa_i = 0$. Local stability is determined in most cases by the eigenvalues λ_i of the matrix **J** defined in equation (3.7.9), i.e. if all eigenvalues of **J** have negative real parts, the steady state is locally asymptotically stable. The characteristic equation for a GSEF system is a third order equation, which is obtained by expanding equation (3.7.9), which is given by

$$\lambda^3 + a_1\lambda^2 + a_2\lambda + a_3 = 0 \tag{3.7.10}$$

The a_i's in term of the components of **J** in equation (3.7.7) are presented as (D66), (D67) and (D68) in Appendix B which is an output of a symbolic language, MACSYMA. The direct calculation of the roots, eigenvalues, is not straightforward for this third order equation and sometimes impossible. The Routh-Hurwitz criteria provides a better way to ensure the stability of the system and May (1973) summarizes these conditions as:

the necessary condition: $a_1 > 0, \quad a_3 > 0,$ (3.7.11a)

the sufficient condition: $a_1 a_2 > a_3$ (3.7.11b)

If these inequalities are satisfied, then all the eigenvalues have negative real parts; that is, the corresponding steady sate is locally stable.

4. EXPERIMENTAL INVESTIGATION

4.1 Stripping of Ethanol from Aqueous Solution

The stripping experiments were performed in a 6.3cm ID x 22.7cm height cylindrical glass vessel, which was connected to a 0.83cm ID x 115cm in length condenser placed in a 0°C refrigerated box. The schematic of the experimental set-up is presented in Figure 4.1.1. Air was used as a stripping gas through a porous sparger. The flowrate was measured with a rotameter, model 1110-01F1B1Z17, Brooks Instrument Division, Hatfield, PA. Ethanol concentrations in the solution and the condensate were determined with a gas chromatography (Varian Vista 6000) equipped with a 2m x 3.175mm Chromosorb 101 (80/100) column, a FID detector and a Hewlett-Packard 3390A integrator.

The stripping experiments were carried out in a batch mode to verify equation (3.3.3). Typical stripping data of three different flowrates, V_L/G equals 0.0015, 0.0030, 0.0045 [hr] at 60°C are shown in Figure 4.1.2. The linear relationship between $\ln(P_L)$ and stripping time (t) were observed and, therefore, the stripping factors for each flowrate were obtained form the slopes. The experiments were also performed at two other temperatures, 35°C and 50°C, to evaluate the temperature effect. These results are plotted in a reciprocal form, as given in equation (3.3.4) for estimation of the Henry's law constant, which is shown in Figure 4.1.3. The existence of a linear relationship between $\frac{1}{\beta}$ and $\frac{V_L}{G}$ and the line through the origin verify equation (3.3.4) for the case of low gas rate which implies that the mass transfer effect is negligible in these experiments. Therefore, Henry's law constants for ethanol-water at 35°C, 50°C, and 60°C were obtained from the

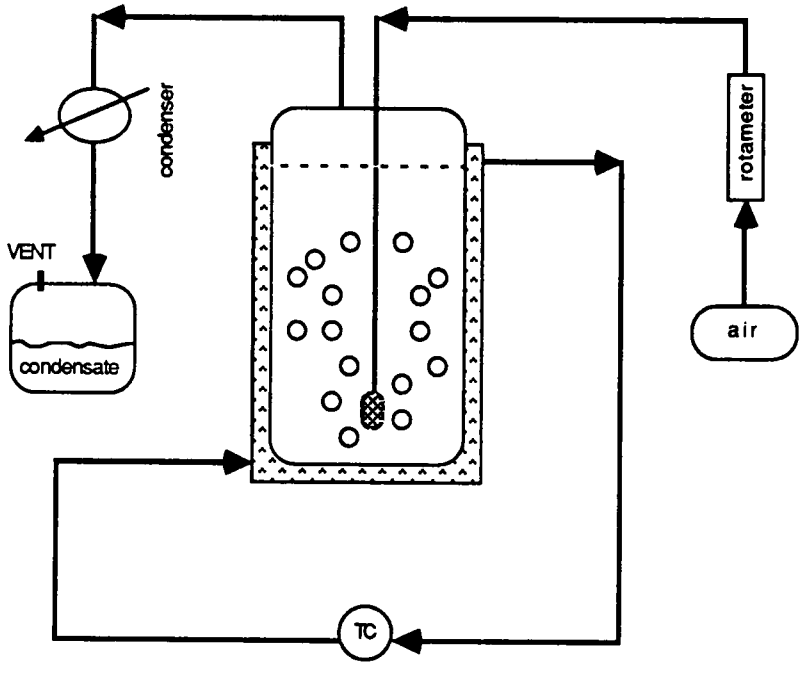


Figure 4.1.1 Schematic Diagram of Stripping Experiment Set-up

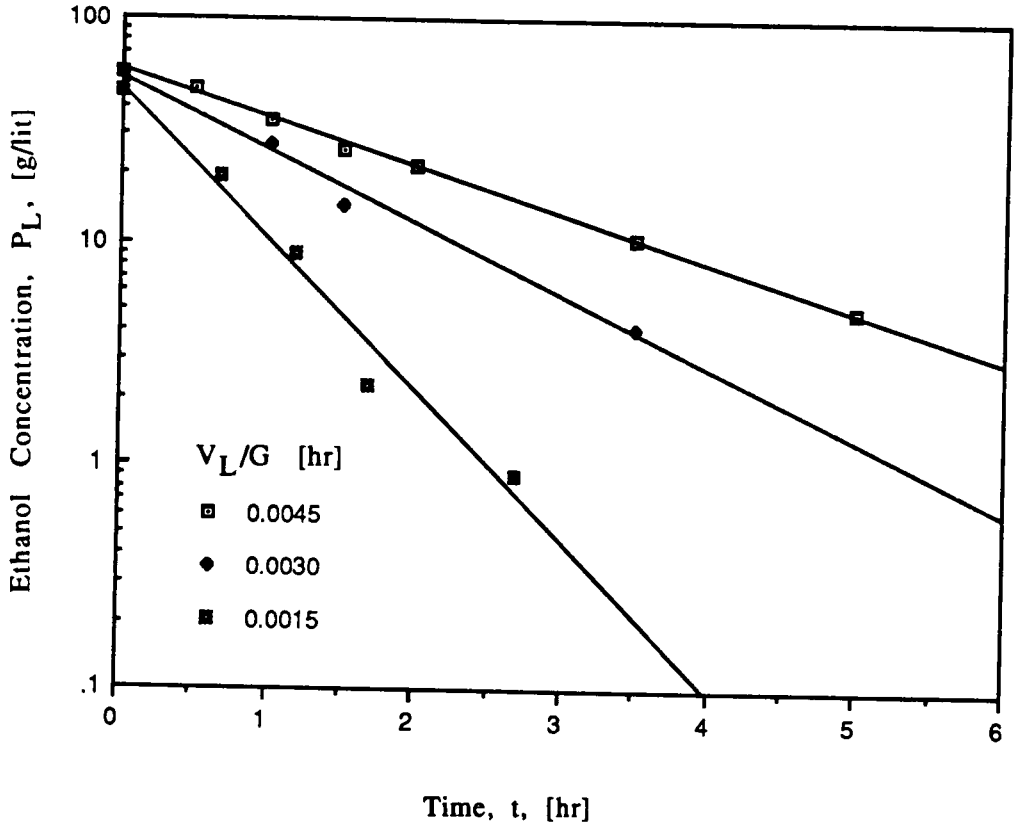


Figure 4.1.2 Ethanol Concentration in the Vessel for Various Stripping Time at $60^{o}C$

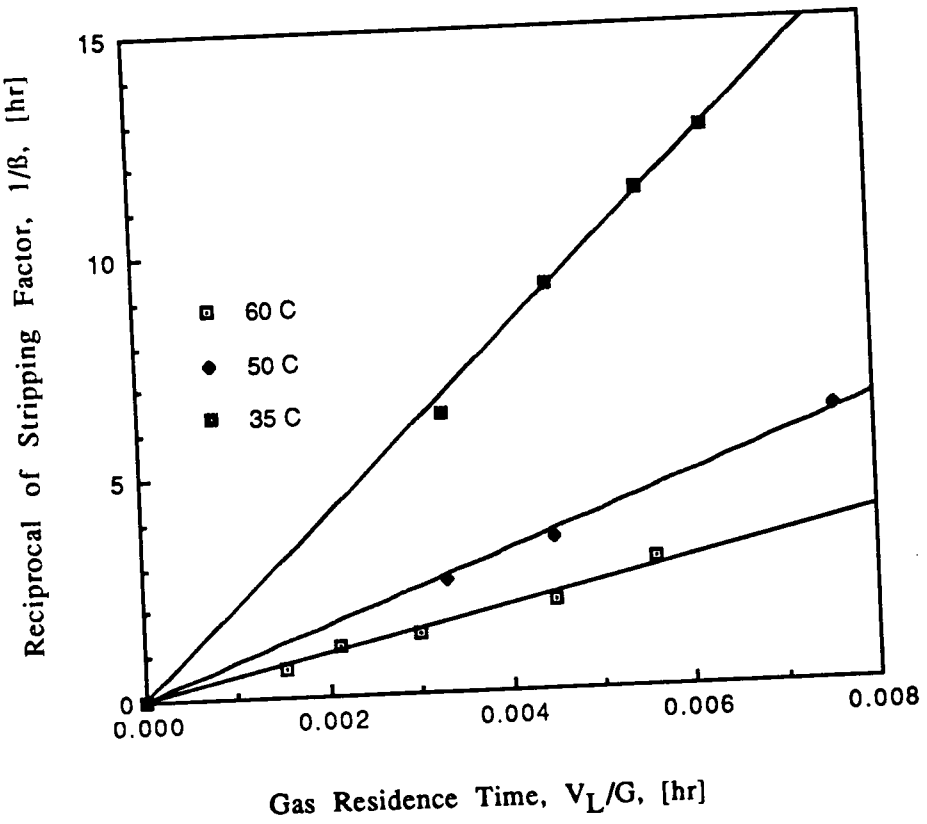


Figure 4.1.3 The Reciprocal Plot of Stripping Factor (ß) against the Stripping Gas Residence Time (V_L/G)

slopes of the plots in Figure 4.1.3. The estimated Henry's law constants from the stripping data and a value calculated from experimental data of Mulholland (1984) at 40°C with corresponding operating condition are presented in Table 4.1.1. A plot of ln(H) vs 1/T for verification of consistence and a constant activity coefficient in equation (3.3.6) is also presented in Figure 4.1.4.

To the best of authors' knowledge, this is the only information available for Henry's law constant in ethanol-water system below their bubbling point since most research interest in an ethanol-water system was for distillation. Air was used as a stripping gas for convenience because nitrogen, carbon dioxide and air have shown similar characteristics as noted by Mulholland (1984). Otherwise the choice of gas depends on the requirement of microorganism. Lee et al. (1981) have shown little cell effect in comparison between their vacuum ethanol fermentation system using *Z. mobilis* ZM4 and a simulated pure ethanol-water system. As to sugar and nutrients effects in ethanol-water system, Tan at al. (1988) and Roychoudhury et al. (1986) have all reported a positive effect in ethanol vapor pressure. Therefore, the results presented in Figure 4.1.4 may be used in the design conservatively for the ethanol removal rate.

Ethanol concentrations recovered in the condenser from stripping and the corresponding ethanol concentration in the vessel at 60°C are shown in Figure 4.1.5. The similar result for vacuum fermentation at 50mmHg, 35°C by Lee at al. (1981) is reproduced and presented in the same Figure for comparison. It is interesting to note that the concentration factor in this particular stripper-condenser can reach approximately five to eight times. This provides a distillation effect for the product concentration and

Table 4.1.1

Henry's Law Constants from Stripping and Their Experimental Conditions

Temperature [°C]	H [-] x 10^4	min. V_L/G [hr] x 10^4	max. $P_{L.i}$ [g/lit]	Corr. Coeff. [-]
35	4.95	33	60	0.99
40	7.20	84	78	*
50	12.50	33	60	0.99
60	20.50	15	60	0.99

* calculated from Mulholland (1984)

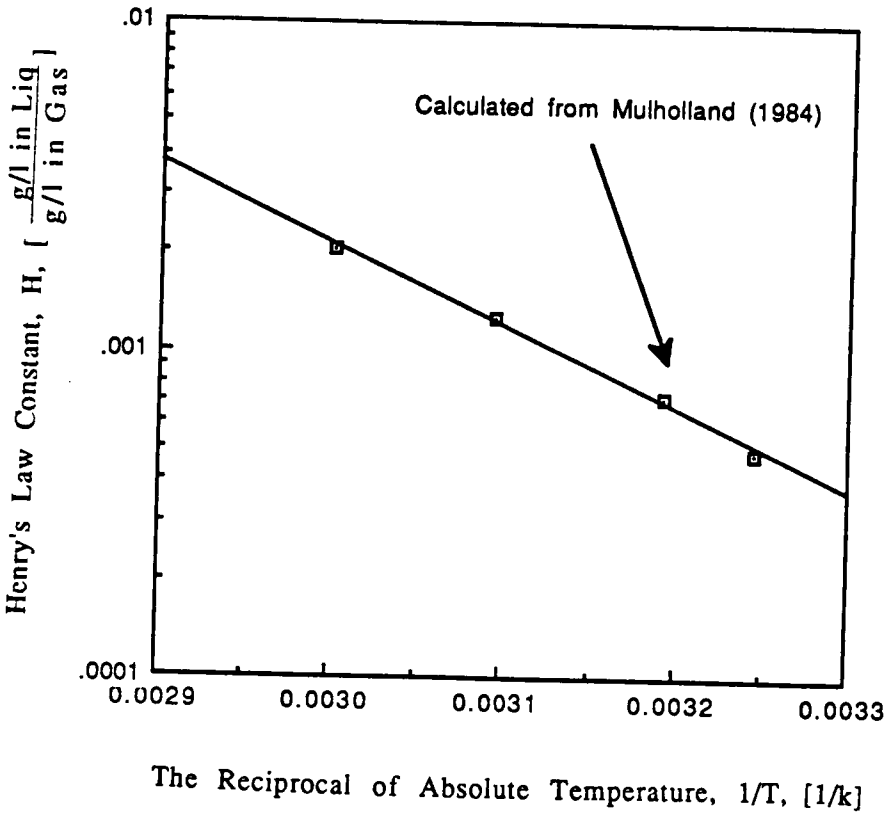


Figure 4.1.4 A Plot of Henry's Law Constants against the Reciprocal of Absolute Temperature

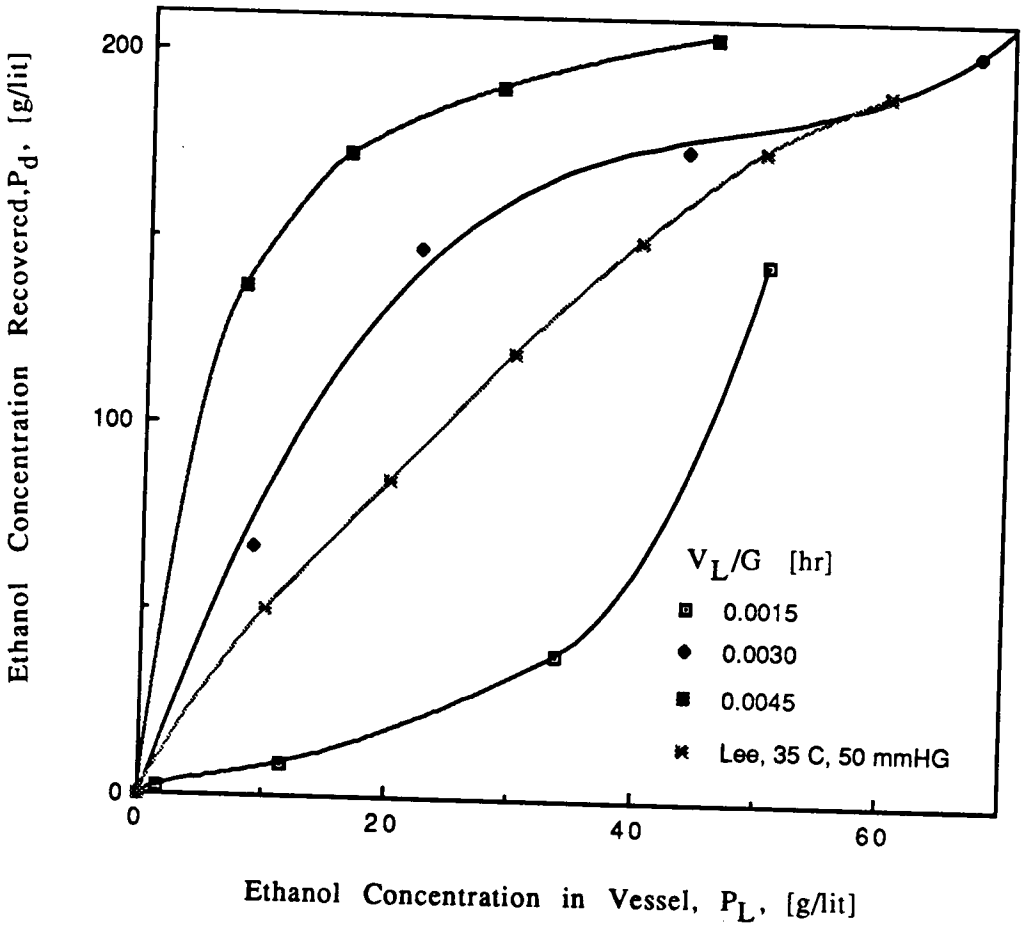


Figure 4.1.5 The Relationship between Ethanol Concentration Recovered in the Condenser and in the Vessel at $60^{o}C$
(*data of vacuum system from Lee et al., 1981)

purification and reduces the downstream separation cost significantly. However, the efficiency of ethanol recovery in the condenser, based on a material balance of ethanol, ranges from 35 to 65%, depending on the stripping gas rate. This implies that the recycle of the unrecovered ethanol might be necessary for a GSEF system to minimize the ethanol loss.

There are two major factors concerned in an *in-situ* removal system; namely, ethanol removal rate factor such as stripping factor in this study and concentration factor after the ethanol is recovered. For the comparison of ethanol removal rate factor, data of vacuum fermentation system by Lee at al. (1981) which was operated at 50 mmHg, 35°C and D=0.24 [1/hr] with various feed concentrations are listed in Table 4.1.2. The ethanol removal rate factor by Lee et al. is equivalent to stripping factor in this study. The stripping factor for Lee's data were calculated by rearranging equation (3.4.3) to give

$$\beta = \left(\frac{\sigma_{ss} X_{ss}}{P_{L.ss}} - D\right) \qquad (4.1.1)$$

where $\sigma_{ss} X_{ss}$ is the productivity at steady state. It is emphasized that a vacuum fermentation system and a GSEF system share a strong resemblance in taking the advantage of ethanol volatility, but with the difference in operating pressure. In the vacuum fermentation of Lee et al. at 35°C, the average corresponding ß is 0.565 [1/hr]. If a system is operated at 60°C with identical kinetics at atmospheric pressure (i.e. GSEF system) instead of vacuum by the same stripping value of 0.565 [1/hr], it is interesting to note from Figure 4.1.3 that the V_L/G for the GSEF system for the case is 0.0036 [hr]. Then, from Figure 4.1.5, it can be seen that the ethanol concentration in the condenser for the

Table 4.1.2

Estimation of Ethanol Removal Rate in Vacuum System by Lee et al. (1981)*

Glucose feed [g/l]	Productivity [g/l-hr]	Ethanol in broth [g/l]	Corresponding ß** [1/hr]
200	20.89	25.45	0.580
250	25.67	32.73	0.544
300	30.00	38.00	0.539
350	36.11	43.09	0.598
			av. 0.565

* dilution rate=0.24 [1/hr], without cell recycle at 35°C, 50 mmHg

** calculated by equation (4.1.1)

GSEF system at $60^{o}C$ is always higher than the vacuum fermentation at $35^{o}C$. The advantage of using thermophilic microorganisms in the GSEF system is obvious and equation (4.1.1) provides a way to compare the ethanol removal rate factor in other *in-situ* removal systems.

4.2 Fermentation with *Clostridium thermosaccharolyticum* (ATCC 31925)

An anaerobic thermophilic bacteria, *Clostridium thermosaccharolyticum* (ATCC 31925, HG-6), obtained from American Type Culture Collection (Rockville, MD) was investigated for the possibility of the incorporation with the GSEF system. D-xylose was used throughout this study as a carbon source and analyzed by HPLC (Varian 5500) equipped with a μBondapack NH_2 column (Waters Associated, Milford, MA) and a RI detector using water/acetonitrile (v/v: 15/85) as a solvent. The cultural medium, preparation procedures, and other analytical methods are described in Liu, Hsu and Salyer (1988).

Batch fermentations with various initial D-xylose concentrations in the Hungate tube (Hungate, 1969) containing 10 ml medium were carried out to evaluate the upper-limit of substrate concentration of this strain. Ethanol concentrations were measured at 48 hour fermentation time. The results are presented in Figure 4.2.1. It was found that the highest ethanol concentration obtained was 4.01 [g/lit] and cell growth ceased for xylose concentration higher than 40 [g/lit].

Continuous fermentations without stripping were performed in the same vessel as shown in Figure 4.1.1 with a magnetic stirrer. All the continuous fermentations were started-up by introducing an appropriate substrate feed into the reactor after a 24 hours batch fermentation with initial xylose of 6 [g/lit]. The steady states were reached as effluent (ethanol, xylose, cell) concentrations reaches constant values. Each fermentation steady state was ran more than 96 hours. Those results are shown in Table 4.2.1. The yields of ethanol concentration were very low in these experiments. When a feed xylose concentration was increased to 40 [g/lit] to see whether the cell could

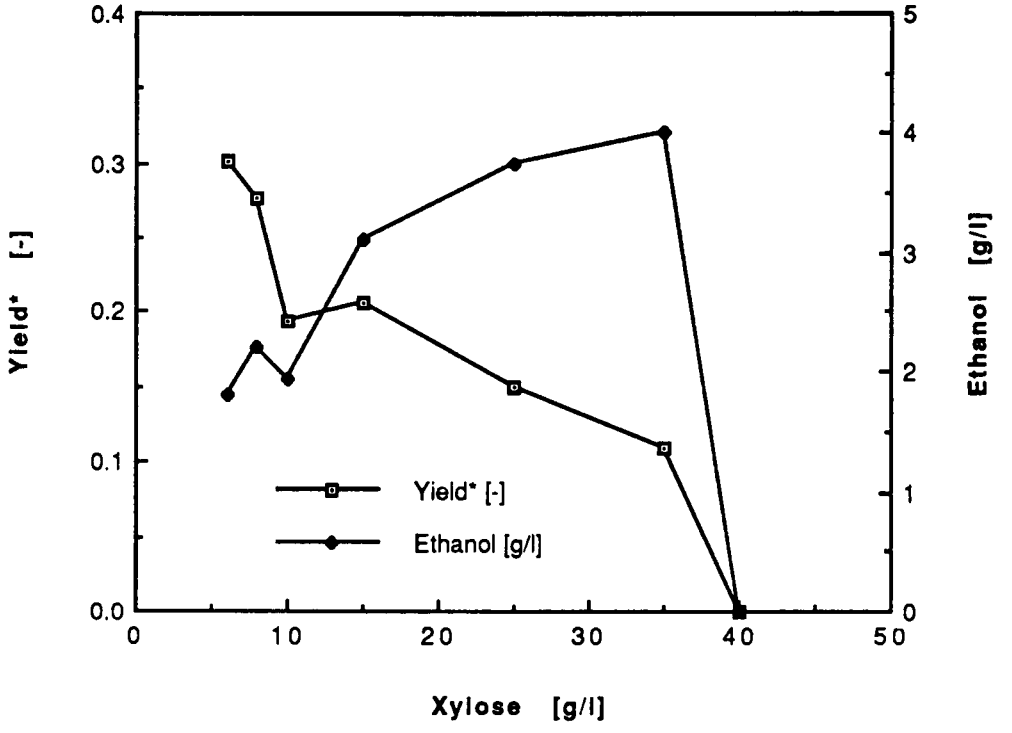


Figure 4.2.1 Ethanol Yield of Batch Fermentation for Various Initial D-xylose Concentrations Using *C. thermosaccharolyticum* (*Yield is defined as gram ethanol produced per gram of xylose.)

Table 4.2.1

Steady State Data of Continuous Fermentation in a CSTR

Dilution rate [1/hr]	Xylose in [g/l]	Xylose out [g/l]	EtoH out [g/l]	Cell out [g/l]
0.05	25	1.4	3.4	1.1
0.12	25	11	1.9	1.2
0.22	25	12	1.8	0.9
0.22	10	0.5	2.2	0.8
0.05	40	-	-	-

-Unable to reach steady state (washout)

be pushed to grow at higher sugar concentration, a decreasing cell density was observed with stronger smell of lactic acid and the system finally failed to reach a steady state (washout). The whole operation was operated more than a week and repeated twice. However, the results were the same.

The recent publications of Mistry and Cooney (1989) reported that 7.7 [g/l] ethanol can be produced by an improved strain *C. thermosaccharolyticum* HG-8 using 25 [g/l] xylose feed and 100% cell recycle while Kersey and Lynd (1989) reached 11.2 [g/l] ethanol at 35 [g/l] xylose feed, but fail to reach a steady state at 42 [g/l] xylose feed. A vacuum fermentation system was also investigated by Kersey and Lynd (1989) at same condition. However, the difference compared with the one without ethanol removal was not observed. Probably, the ethanol concentrations in these thermophilic bacteria were not sufficient to impose inhibition while the authors attribute the effect of hydrogen removal in the headspace (generated during fermentation) which is thought to be important in ethanol production (Sundquist et al., 1986; Doremus, et al., 1982; Su, et al., 1981).

The upper-limit of substrate concentration of *Clostridium thermosaccharolyticum* (ATCC 31925, HG-6) is too low to yield a reasonable ethanol concentration. Further improvements of this strain by mutation, selection or genetic technique are required before a practical application can be made. Thus, the investigation of a GSEF system moved to a general consideration instead of this strain. The emphasis in next chapter was placed on the analytical studies.

5 ANALYTICAL STUDIES

5.1 Continuous GSEF System

The kinetic models used are modification of Ghose and Tyagi (1979) with finite K_{si} and $K_{si} = \infty$ together with Luedeking-Piret (1959) production model, which are :

modification of Ghose and Tyagi (1979) model:

$$\mu = \frac{\mu_m S}{K_s + S + \frac{S^2}{K_{si}}} \left[\frac{K_p}{K_p + P_L}\right]\left[1 - \left(\frac{P_L}{P_{max}}\right)\right] \qquad (5.1.1)$$

$$\upsilon = -\sigma / Y_{p/s} - \mu / Y_{x/s} \qquad (5.1.2)$$

Luedeking-Piret (1959) production model:

$$\sigma = \alpha \mu + \omega \qquad (5.1.3)$$

where the quantities μ_m, K_s, K_{si}, K_p, and P_{max} are the model parameters which have to be determined experimentally. The model is a combination of Monod type growth with product and substrate inhibitions. If the substrate inhibition is negligible, $K_{si} = \infty$.

A. Effect of Stripping Factor in CSTR with Product Inhibition Kinetics

A nontrivial steady-state analytical solution for X_{ss}, S_{ss}, and $P_{L.ss}$ as a function of dilution rate D was obtained from equations (3.4.1), (3.4.2), and (3.4.3) using the kinetic model given in equations (5.1.1), (5.1.2), and (5.1.3) without substrate inhibition ($K_{si} = \infty$). The derivation of the solution is

presented in equation (A4), Appendix A. Equation (A4) is a second order algebraic equation in P_L for which an exact analytical expression can be obtained easily.

Profiles of steady-state solutions, X_{ss}, S_{ss}, and $P_{L.ss}$ as a function of dilution rate (D) with various values of stripping factor, β = 0.00 (0.02) 0.08 [1/hr] (the number in parentheses between two numbers is an increment), are presented in Figures 5.1.1, 5.1.2, and 5.1.3 to permit evaluation of the characteristics of the stripping effect. The parameters used for the kinetic model in equations (5.1.1)-(5.1.3) with S_F=80 [g/l] are presented in Table 5.1.1.

Figure 5.1.1 illustrates the effect of variation of the stripping factor on the cell concentration in the broth. It shows that the higher the stripping factor the higher the cell concentration in the reactor which demonstrates the product inhibition is reduced with an increase in the stripping factor. Figure 5.1.2 shows the substrate concentration profile as a function of dilution rate with various values of ß. As the stripping factor increases, the steady state substrate concentration decreases. That is, the stripping factor enhances the substrate utilization for all dilution rate. It is interesting to note that with β=0 (no stripping) and D close to 0.0 the residual substrate concentration approaches zero; i.e. the continuous operation approaches to a batch reactor operation. The best improvement was obtained when ß was changed from 0 to 0.02. Not much improvement was observed when ß was increased from 0.02 to 0.08. There are two reason for this phenomena, all resulting from lower ethanol concentration in the broth. First, the ethanol removal rate depends on not only stripping factor but also ethanol concentration in the broth as equation (3.3.2) shows. Second, the major part of inhibition is drastically

Table 5.1.1

The Numerical Parameters Used in Equations (5.1.1)-(5.1.3) for Simulation*

μ_m	=	0.3	[1/hr]	K_s	=	5.0	[g/lit]
K_p	=	4.5	[g/lit]	P_{max}	=	85.0	[g/lit]
$Y_{p/s}$	=	0.5	[-]	$Y_{x/s}$	=	0.83	[-]
α	=	13.0	[-]	ω	=	0.05	[1/hr]
K_{si}	=	∞ and 8	[g/lit]**				

*These values are taken from Sevely et al. (1980) except K_{si} = 8 [g/lit].

** $K_{si} = \infty$ [g/lit] is used for product inhibition kinetics.

K_{si} = 8 [g/lit] is used for combined product-substrate inhibition kinetics.

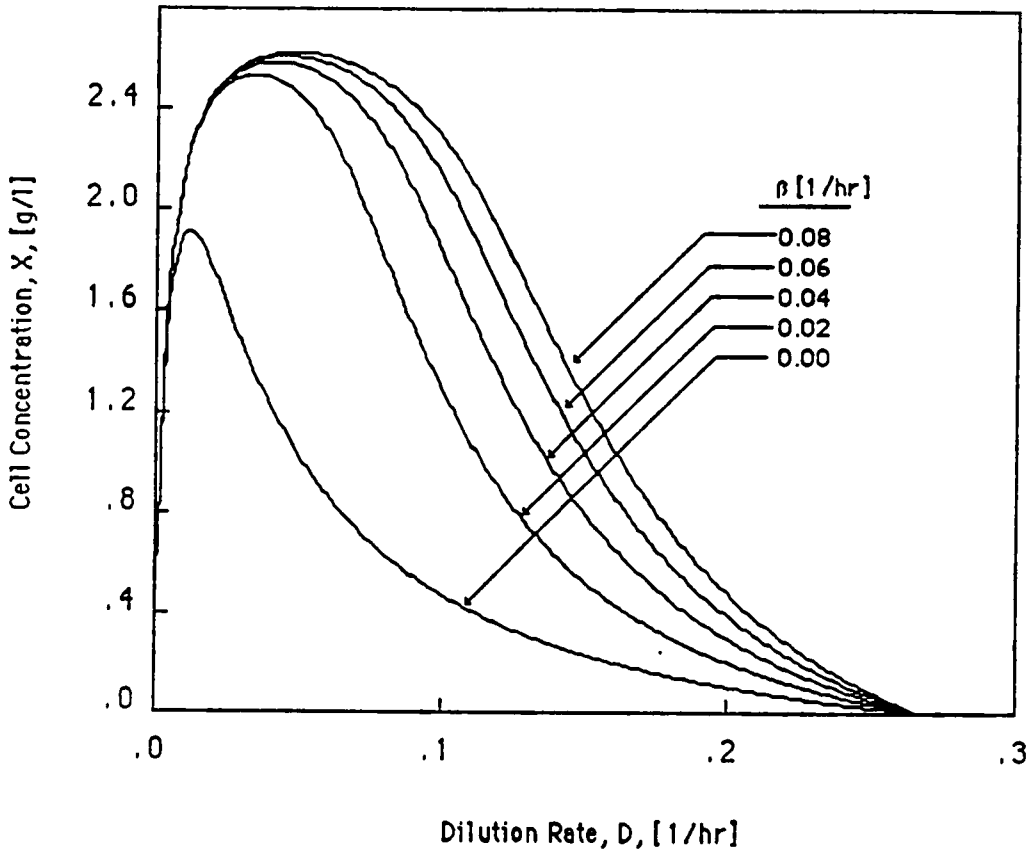


Figure 5.1.1 Effect of Stripping Factor on Steady State Cell Concentration as a Function of Dilution Rate for Product Inhibition Kinetics (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with $K_{si}=\infty$ [g/l] and $S_F=80$ [g/l].)

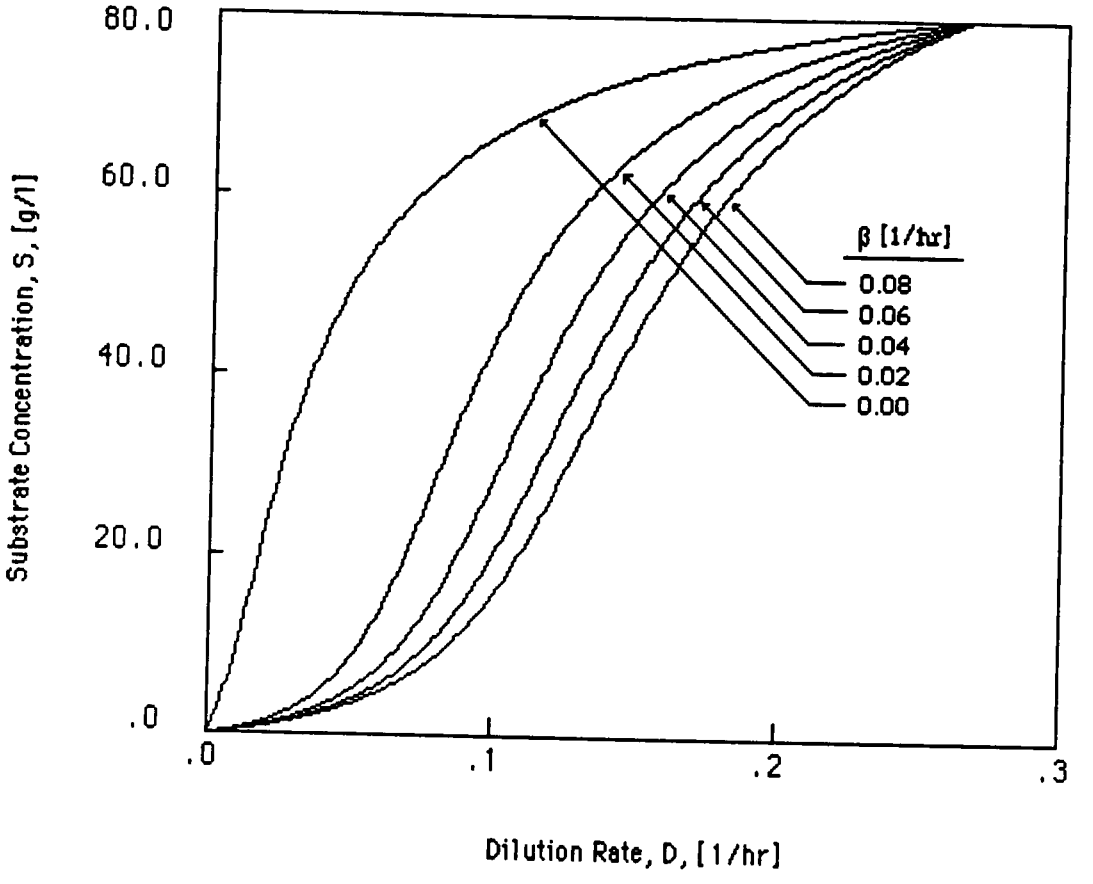


Figure 5.1.2 Effect of Stripping Factor on Steady State Substrate Concentration as a Function of Dilution Rate for Product Inhibition Kinetics (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with $K_{si}=\infty$ [g/l] and $S_F=80$ [g/l].)

removed when ß increases from 0.0 to 0.2 as shown in Figure 5.1.3. This figure demonstrates the product concentration reduction in fermentation broth resulting from the stripping. It can be seen that with S_F= 80 [g/l] the maximum ethanol concentration in the broth is reduced from 7.5 [g/l] to 2.5 [g/l] with increasing in the stripping factor from 0.02 to 0.08. Also, the highest improvement was obtained when β was increased from 0.0 to 0.02.

The productivity as a function of dilution rate with various values of β is presented in Figure 5.1.4. From the figure, the productivity has its maximum value which increases with increasing the stripping factor. This maximum productivity together with the stripping factor as a function of the optimum dilution rate is inferred from Figure 5.1.4 and presented in Figure 5.1.5 . As can be seen from the figure, the optimum productivity increases linearly with the optimum dilution rate, but the corresponding stripping factor increases exponentially with the dilution rate or the productivity. This result generalizes the effect of the stripping factor on various parameters in Figures 5.1.1-5.1.4. Also, can be seen from the figure, the optimum value of stripping factor is in the range of $0.01 < \beta < 0.05$.

It is interesting to note that by using the derived equation (3.5.5), the problem of cautionary discussion by Slapack et al. (1987) and Lencki et al. (1983) can be clarified. In analyzing the results of Cysewski and Wilke (1977), Lencki et al. reported that their claims of higher ethanol production rates in vacuum fermentation process were due to increased cell numbers in comparison to those in controls. When Lencki et al. standardized the results of Cysewski and Wilke on the basis of cell number (Figure 5.1.6), there was

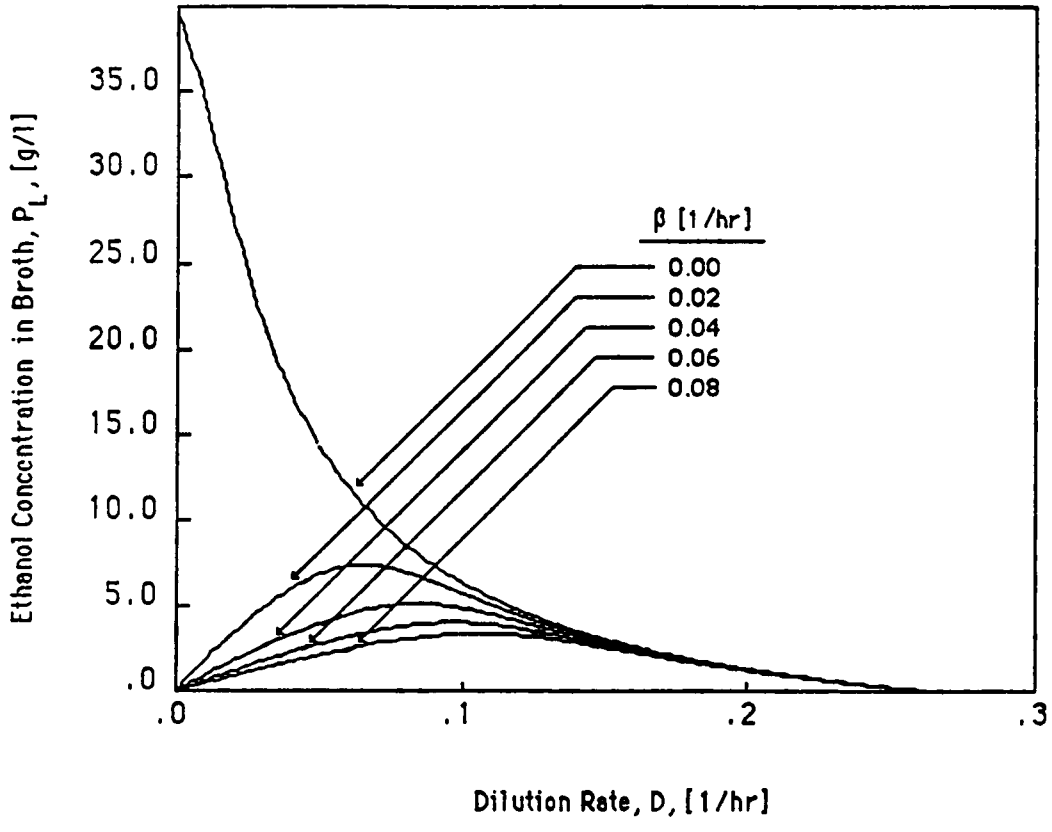


Figure 5.1.3 Effect of Stripping Factor on Steady State Ethanol Concentration in the Broth as a Function of Dilution Rate for Product Inhibition Kinetics (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with $K_{si}=\infty$ [g/l] and $S_F=80$ [g/l].)

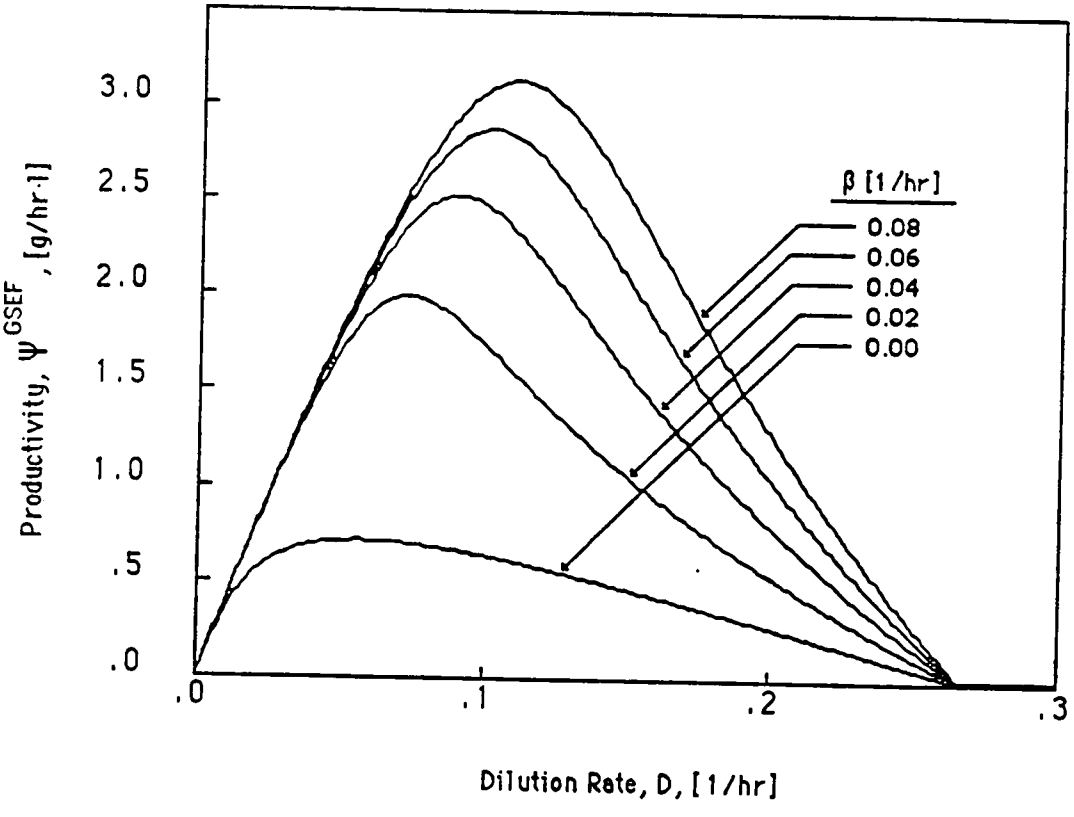


Figure 5.1.4 Effect of Stripping Factor on Steady State Productivity as a Function of Dilution Rate for Product Inhibition Kinetics (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with $K_{si}=\infty$ [g/l] and $S_F=80$ [g/l].)

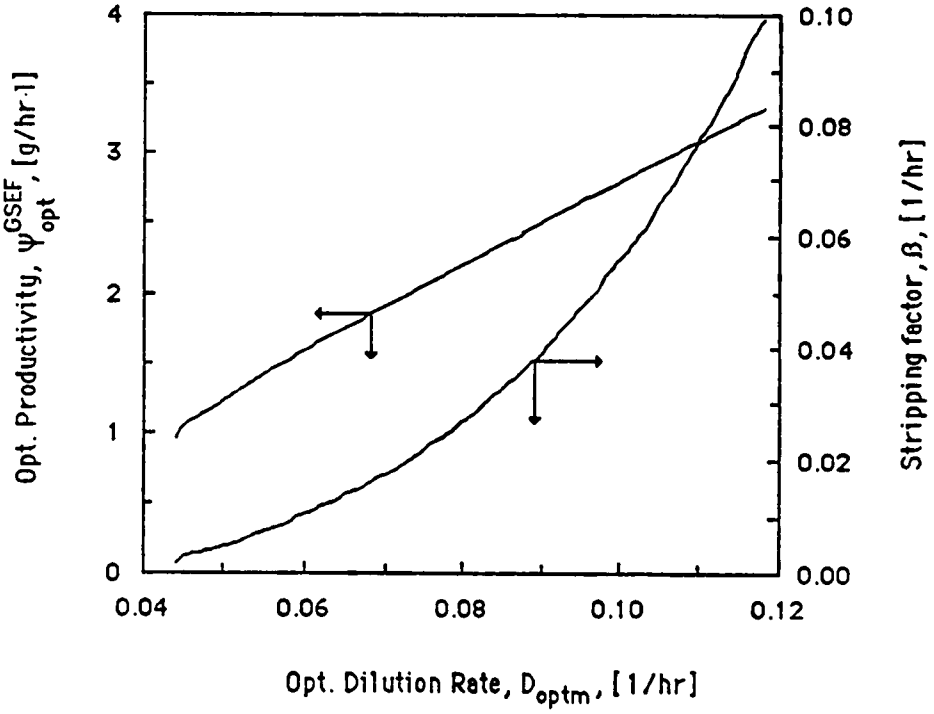


Figure 5.1.5 The Optimal Productivity and Dilution Rate as a Function of Stripping Factor (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with $K_{si}=\infty$ [g/l] and $S_F=80$ [g/l].)

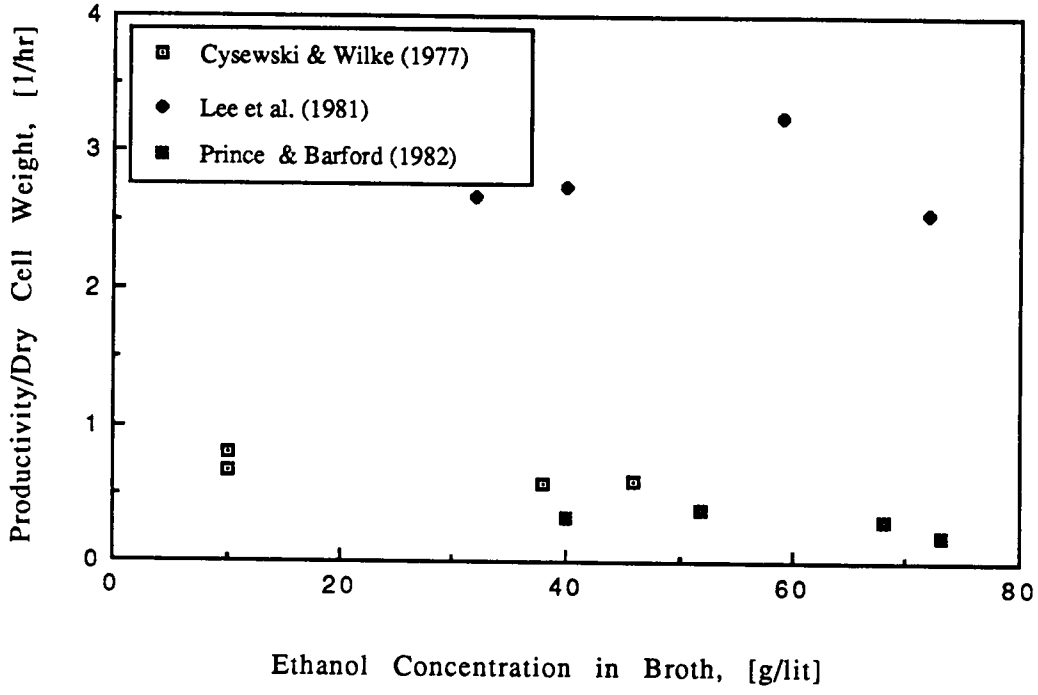


Figure 5.1.6 Standardization of Productivity by Cell Weight*
(*Reproduced from Lencki et al., 1983)

essentially no difference in ethanol production rates. By rearranging equation (3.5.5), it becomes

$$\frac{\psi^{GSEF}}{X_{ss}} = \frac{\psi^{CSTR}}{X'_{ss}} \qquad (5.1.4)$$

Equation (5.1.4) is essentially the basis of Lencki et al. standardization and yields a constant value as expected. This demonstrates the point which has been stated in sections 3.5 and 3.6. That is, the productivity enhancement is primarily a result of the biomass increase, due to the less inhibition effect in the system. Also, Groot et al. (1984) reported a productivity increase from 1.0 to 1.7 [Kg/m^3hr] accompanied with an increase of glucose conversion 65-70% by continuous product removal with pervaporation method using *Clostridium beyerinckii* LMD 27.6. The proportionality between productivity and substrate utilization was observed also demonstrated in equation (3.5.5).

B. Effect of Stripping Factor in CSTR with Combined Product-Substrate Inhibition and Multiplicity

Profiles of specific growth rate, for the product-inhibition only case (where $K_{si} = \infty$) and the corresponding operating lines, for various values of the stripping factor, β = 0.00, 0.02, 0.04 [1/hr] are presented in Figure 5.1.7 and for the case including the product and substrate inhibitions (K_{si} =8 [g/l]) in Figure 5.1.8.

Figure 5.1.7 shows that the profiles of the specific growth rate in the P_L-S phase plane are monotonically increasing contour curves. There is only one intersection point between an operating line and a contour curve of the specific growth rate. For this case there is only one nontrivial steady-state. As values of the stripping factor increase the slope of the operating line becomes steeper, so that the steady state (intersection point) moves to the lower substrate coordinate which means a better substrate utilization resulting in a higher productivity enhancement factor as shown in equation (3.5.5).

When a substrate inhibition is present together with the product inhibition, the contour curve of the specific growth rate is no longer monotonically increasing, as shown in Figure 5.1.8. The equivalence of equation (A4) for the substrate-product inhibition case is a third order algebraic equation in S, which is also presented as equation (A9) in Appendix A. The operating variables for this case are S_F, D, and β. There is a possibility of triple steady states. For some values of β, one can see from the figure that it is only possible to have one intersection point (a steady-state) between a contour curve and an operating line. The slope of the operating line increases with increasing in ß and with decreasing in dilution rate (for steady-state,

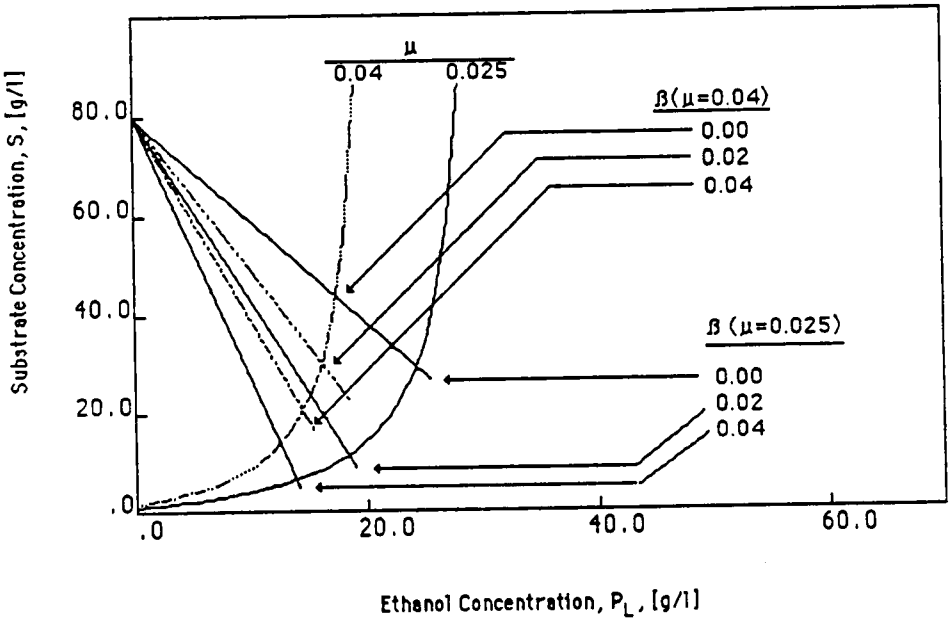


Figure 5.1.7 Stripping Effect on Steady State for Product Inhibition Kinetics at D=0.04 [1/hr] and 0.025 [1/hr] (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with $K_{si}=\infty$ [g/l] and $S_F=80$ [g/l].)

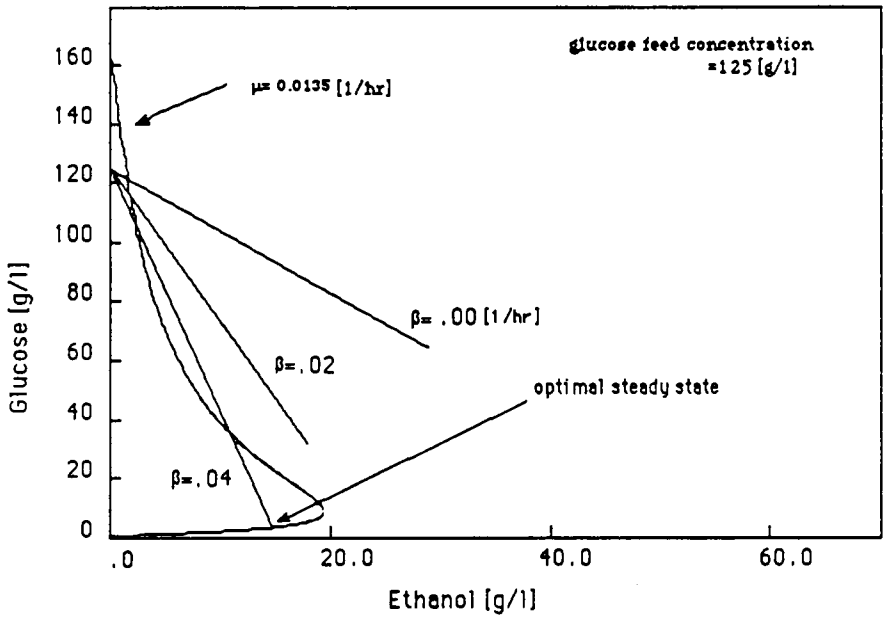


Figure 5.1.8 Stripping Effect on Steady State for Combined Product-Substrate Inhibition Kinetics at D=0.0135 [1/hr]: Multiplicity (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

$D=\mu$). In the substrate coordinate, the concentration difference between the substrate feed concentration and the substrate concentration at steady-state is the amount of the substrate utilization. For a shallow operating line, this concentration difference is small. While the slope of the operating line increases with increasing β, the steady state moves downward toward a lower substrate concentration which signifies a better substrate utilization. Thus, the substrate utilization increases with the stripping. For further increases in the stripping additional steady-states may be observed, such as the case for β = 0.04 [1/hr] and μ = 0.0135 [1/hr]. It is possible to have as many as three intersection points between an operating line and a specific growth contour curve in the phase plane depending on the values of ß and S_F. The optimal steady-state is the state with the highest substrate utilization, which also means the highest productivity. For the product-substrate inhibition case, there is an additional constraint in the dilution rate. The limiting dilution rate is further reduced from μ_m which is the model parameter used in the specific growth rate of biomass in equation (5.1.1). If the system exceeds this limiting dilution rate, it is impossible to have a nontrivial steady-state in the system. The limiting dilution rate, $D_{lim}(=\mu_{max})$, is obtained by finding the maximum dilution rate of which the steady state substrate concentration (equation A9) satisfying the constraint $0 < S < S_F$. The quantity μ_{max} is different from μ_m used in equation (5.1.1). The calculated limiting dilution rate as a function of S_F with various values of ß is presented in Figure 5.1.9. It can be seen from this Figure that if the substrate feed concentration, S_F, is less than 55 [g/l] and β is less than 0.1 [hr], the stripping factor does not affect the limiting dilution rate. The limiting dilution rate increases exponentially with reduction in the

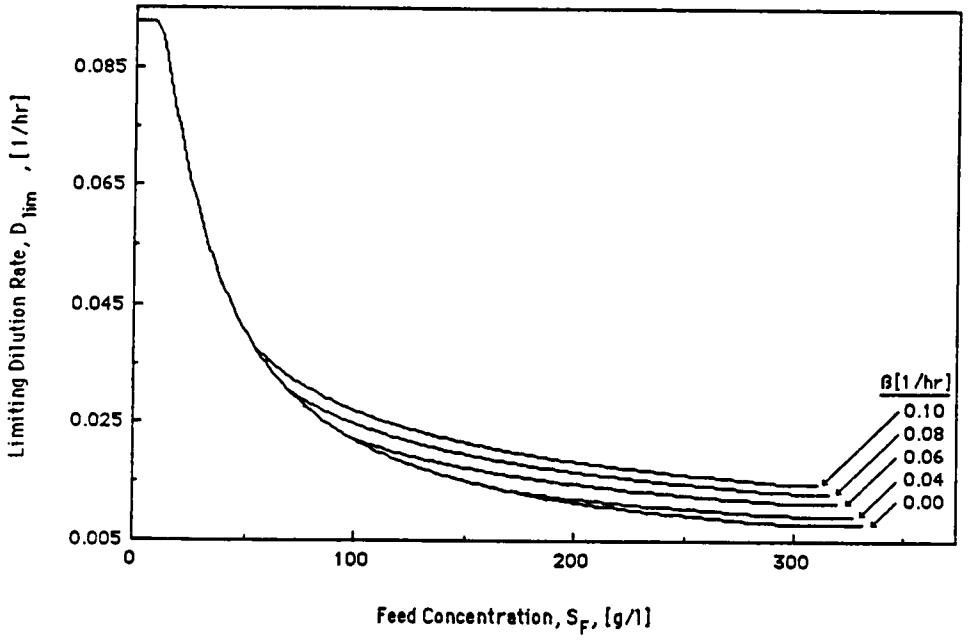


Figure 5.1.9 The Effect of Stripping Factor on Limiting Dilution Rate as a function of Feed Concentration for Combined Product-Substrate Inhibition Kinetics (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{Si}=8 [g/l].)

substrate feed concentration until it reaches the concentration around 10 [g/l]. If the concentration is less than 10 [g/l], the limiting dilution rate is constant at 0.093 [1/hr]. In general, the limiting dilution rate increases with increasing ß for high substrate feed concentrations. However, the general tendency is that the higher the substrate feed concentration the lower the limiting dilution rate. The advantages of the GSEF system are that the system can be operated with higher substrate concentrations and at higher dilution rates. The graphical illustration of this point is also shown as Figure 5.1.10 in a P_L-S phase plane. For the case of ß=0 and ß<0.04, there is no intersection between the operating line and the growth rate contour curve. However, with an increase in the stripping factor, the slope of operating line increases which results in the intersection(s) between the contour curve and operating line. Otherwise, there is no intersection possible.

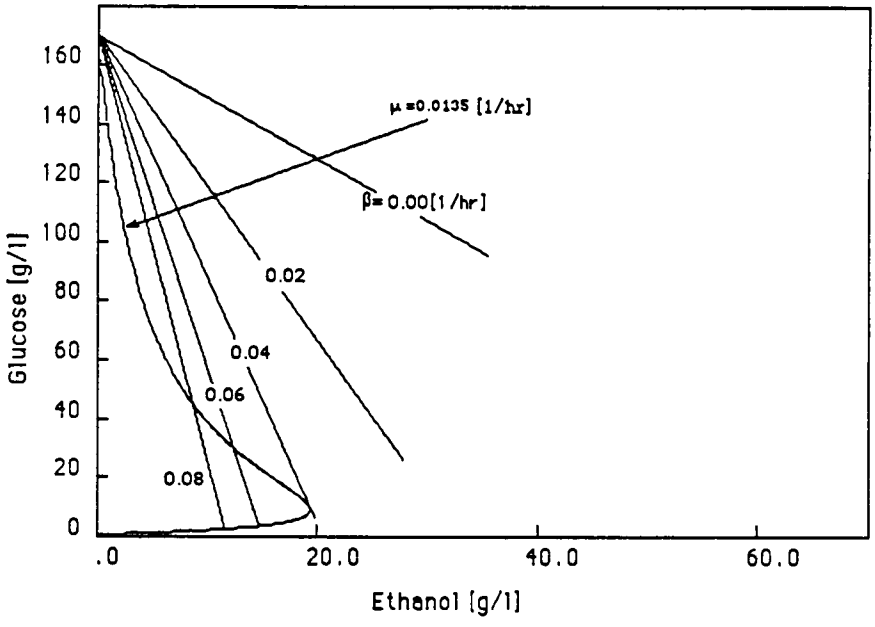


Figure 5.1.10 Stripping Effect on Steady State for Combined Product-Substrate Inhibition Kinetics: to be operated at higher substrate concentration and dilution rate (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8[g/l] and S_F=170 [g/l].)

C. Stability Analysis

While multiple steady states appear in the product-substrate inhibition case, the local stability as well as regional stability become an important issue. The former tells us whether the system can return to the steady state after a sufficiently small disturbance while the latter reveals the proper initial conditions for start-up to reach the desired steady state. The system equations for stability investigation are the governing equations of a continuous GSEF system, which are repeated here, for clarity.

$$\frac{dX}{dt} = (\mu - D)X \qquad (3.1.1)$$

$$\frac{dP_L}{dt} = \sigma X - (\beta + D)P_L \quad ; \; P_F = 0 \qquad (3.2.3)$$

$$\frac{dS}{dt} = \upsilon X + D(S_F - S) \qquad (3.1.8)$$

With the kinetic model given in equations (5.1.1), (5.1.2) and (5.1.3), equations (3.2.3) and (3.1.8) can be rearranged into a way such as:

$$\frac{dP_L}{dt} = (\frac{\mu}{Y_1} + \omega)X - (\beta + D)P_L \qquad (5.1.5)$$

$$\frac{dS}{dt} = -(\frac{\mu}{Y_2} + m)X + D(S_F - S) \qquad (5.1.6)$$

in which

$$Y_1 = \frac{1}{\alpha} \qquad (5.1.7)$$

$$Y_2 = \frac{Y_{p/s} Y_{x/s}}{\alpha Y_{x/s} + Y_{p/s}} \qquad (5.1.8)$$

$$m = \frac{\omega}{Y_{p/s}} \qquad (5.1.9)$$

where Y_1 and Y_2 are the product formation and substrate utilization yield factors that are associated with cell growth; m and ω are nongrowth-associated maintenance factors.

(i) Steady State(s) and Local Stability

Using the system equations (3.1.1), (5.1.5) and (5.1.6), the Jacobian matrix, equation (3.7.7), becomes

$$J=\begin{bmatrix} 0 & \phi X & -bX \\ -\left(\frac{D}{Y_2}+m\right) & -\frac{\phi X}{Y_2}-D & \frac{bX}{Y_2} \\ \frac{D}{Y_1}+\omega & \frac{\phi X}{Y_1} & -\frac{bX}{Y_1}-(D+\beta) \end{bmatrix}_{\text{steady state}} \qquad (5.1.10)$$

in which $\frac{\delta\mu}{\delta S}=\phi$ and $\frac{\delta\mu}{\delta P_L}=-b$ (b>0 because of product inhibition) and at steady state where μ=D. It is possible to solve the eigenvalues of the above equation, but it is extremely difficult and, furthermore, there is no easy conclusion can be drawn from the complicated solution. However, by using symbolic manipulation language MACSYMA, the calculation of Routh-Hurwitz criteria (equation 3.7.11) results in (D70, D72, D73 in Appendix B)

the necessary conditions:

$$a_1=\frac{\phi X}{Y_2}+\frac{bX}{Y1}+2D+\beta>0 \qquad (5.1.11)$$

$$a_3=\frac{\phi XD^2}{Y_2}+\frac{\phi X\beta D}{Y_2}+\frac{bXD^2}{Y_1}+bXD\omega+\phi XDm+\phi X\beta m>0 \qquad (5.1.12)$$

the sufficient condition:

$$a_1a_2\text{-}a_3 = \frac{4\phi bDX^2}{Y_1Y_2} + \frac{\phi b\beta X^2}{Y_1Y_2} + \frac{\phi b\omega X^2}{Y_2} + \frac{\phi^2 mX^2}{Y_2} + \frac{4\phi D^2X}{Y_2} + \frac{4\phi\beta DX}{Y_2} +$$

$$\frac{\phi\beta^2X}{Y_2} + \frac{2\phi^2DX^2}{Y_2^2} + \frac{\phi^2\beta X^2}{Y_2^2} + \frac{b^2\omega X^2}{Y_1} + \frac{\phi bmX^2}{Y_1} + \frac{4bD^2X}{Y_1} +$$

$$\frac{3b\beta DX}{Y_1} + \frac{2\beta^2DX^2}{Y_1^2} + bD\omega X + b\beta\omega X + \phi DmX + 2D^3 + 3\beta D^2 + \beta^2D > 0$$

(5.1.13)

where ϕ, b and X are evaluated at each steady state, the subscript "ss" is eliminated for simplicity.

In the necessary conditions, equations (5.1.11) and (5.1.12), and the sufficient condition, equation (5.1.13), all the quantities in those equations are positive except ϕ which may vary, depending on the specific growth rate on substrate. If $\phi > 0$, the local stability is naturally ensured. However, there is also a range of negative values of ϕ which satisfy the Routh-Hurwitz criteria. A plot of ϕ as a function of dilution rate for various values of β together with the parameters in Tables 5.1.1 and S_F=125 [g/l] is presented in Figure 5.1.11. The solid lines indicate the ϕ-value satisfying the local stability regions, equations (5.1.11)-(5.1.13), and the dotted lines are for local instability regions. In the analysis of substrate inhibition case, Agrawal et al. (1982), Van den Heuvel and Zoetemyer, (1982) and Chi and Howell (1976) have concluded that $\frac{\delta\mu}{\delta S} = \phi > 0$ is the necessary and sufficient conditions for the local stability. Van den Heuvel and Beeftink (1988) extended this result to a combined product-substrate inhibitions case, in which they have neglected some range of the

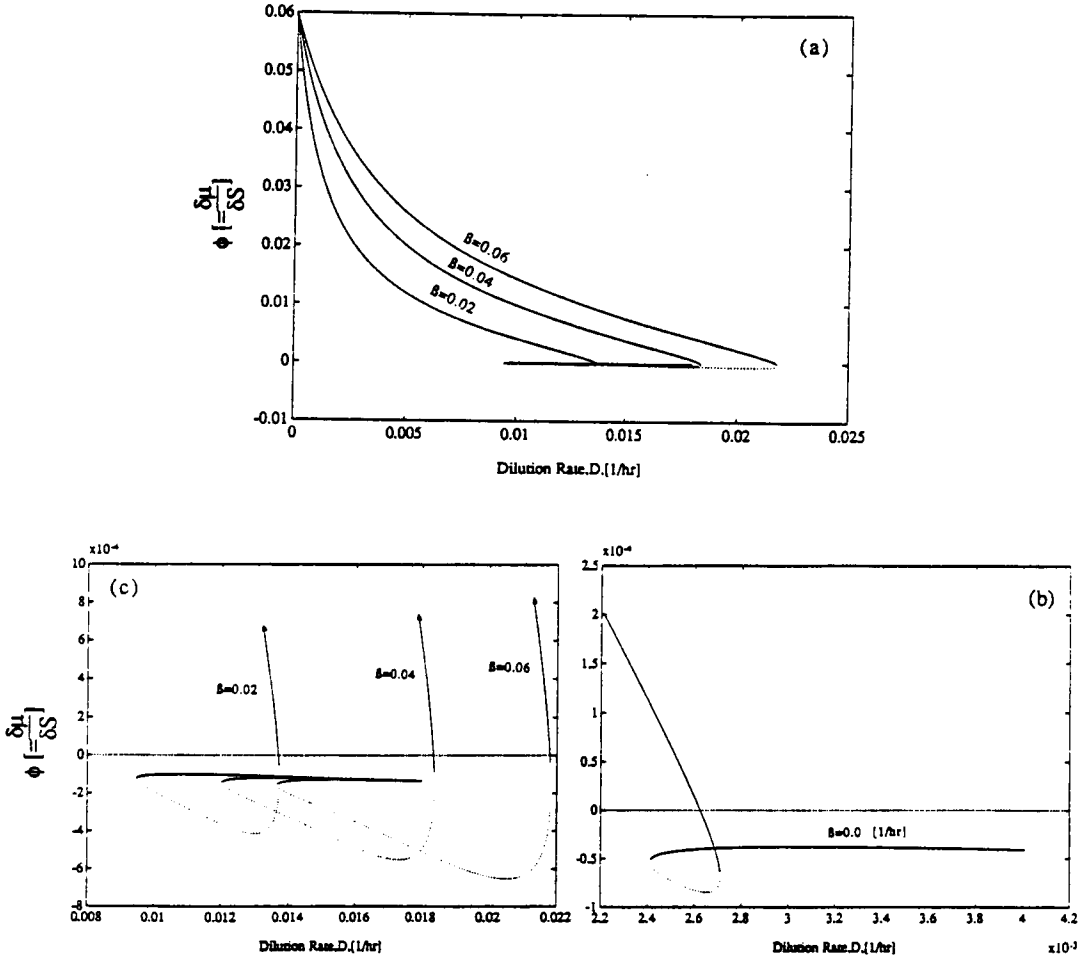


Figure 5.1.11 The Local Stability Criteria for the Value of ϕ [$= \frac{\delta\mu}{\delta S}$] as a Function of Dilution Rate; Figures (b) and (c) are the enlargement of bifurcation region of Figure (a). (where solid lines [-] represent stability and dotted lines [...] represent instability; Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{Si}=8 [g/l] and S_F=125 [g/l].)

negative values of ϕ still satisfying the criterion.

To elucidate the effects of the stripping factor, ß, on the multiple steady states, numerical results of steady state solutions for substrate concentration, S, ethanol concentration in broth, P_L, cell concentration, X, and productivity, Ψ, as a function of dilution rate (D) with S_F=125 [g/l], K_{si}=8 [g/l] and ß=0.0 (0.02) 0.06 [1/hr] are presented in Figures 5.1.12-5.1.15. Those solutions as a function of stripping factor, ß, with three dilution rates, 0.0135, 0.01 and 0.006 [1/hr] are also presented in Figures 5.1.16-5.1.19. In these figures, the solid lines are used to represent the stable steady regions and the dotted lines are for unstable steady region. In the three nontrivial steady states case, the middle one is always unstable while the other two with lowest and highest (optimal) substrate utilization are stable. An introduction of stripping seems to extend the range of operating variables on the multiple steady states. For the cases discussed, the operating variables are the dilution rate (D) and the stripping factor (ß). If the optimal steady state is "approachable", it is advantageous to use the GSEF system in the product-substrate inhibition case.

When a dilution rate is increased gradually from a very low value to a critical values (e.g. D=0.0184 [1/hr] for ß=0.04 [1/hr]), a jump (discontinuity) in a steady state is observed. While the dilution rate is decreased slowly from a high value, a discontinuity is also observed at another critical value (e.g. D=0.0122 [1/hr] for ß=0.04 [1/hr]). This phenomenon is often termed as the hysteresis effect (Forment and Bischoff, 1979; Aris, 1965), which can be annoying. For example, the system is difficult to operate around D=0.003 [1/hr]

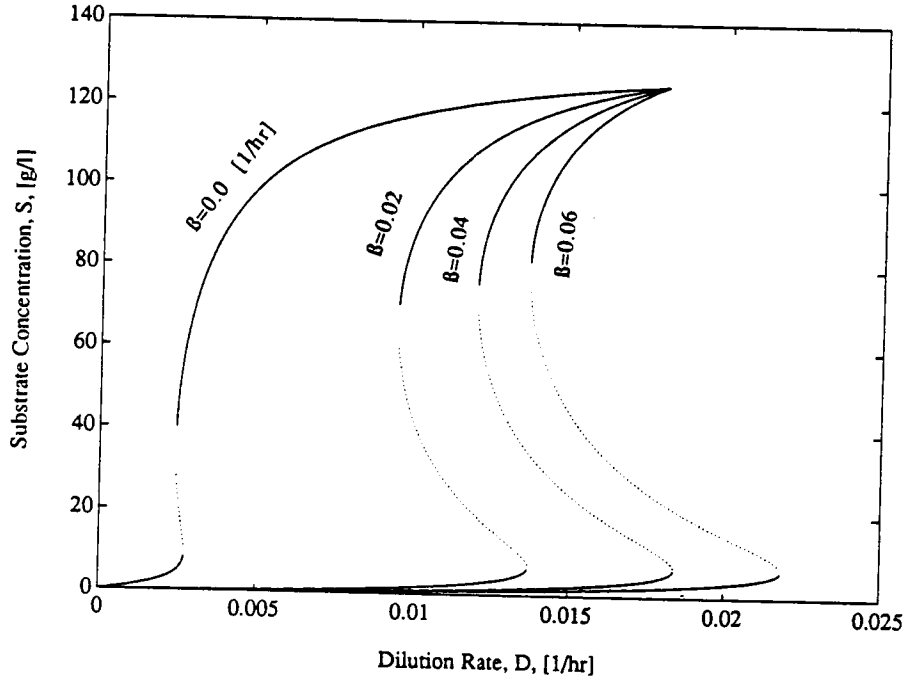


Figure 5.1.12 Effect of Stripping Factor on Steady State Substrate Concentration as a Function of Dilution Rate for Combined Product-Substrate Inhibition Kinetics (where solid lines [-] represent stability and dotted lines [...] represent instability; Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l])

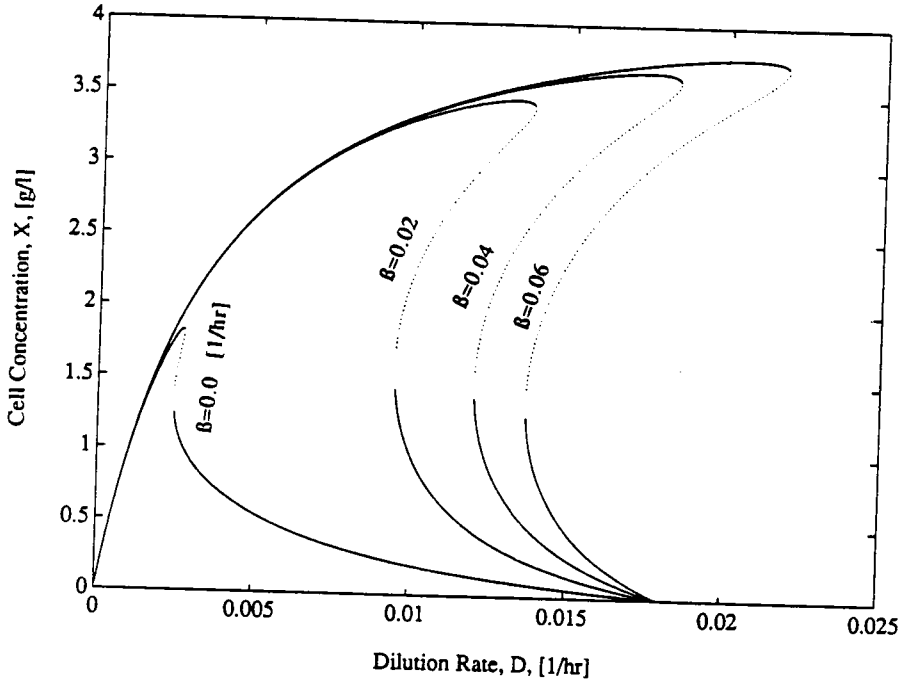


Figure 5.1.13 Effect of Stripping Factor on Steady State Cell Concentration as a Function of Dilution Rate for Combined Product-Substrate Inhibition Kinetics (where solid lines [-] represent stability and dotted lines [...] represent instability; Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{Si}=8 [g/l] and S_F=125 [g/l].)

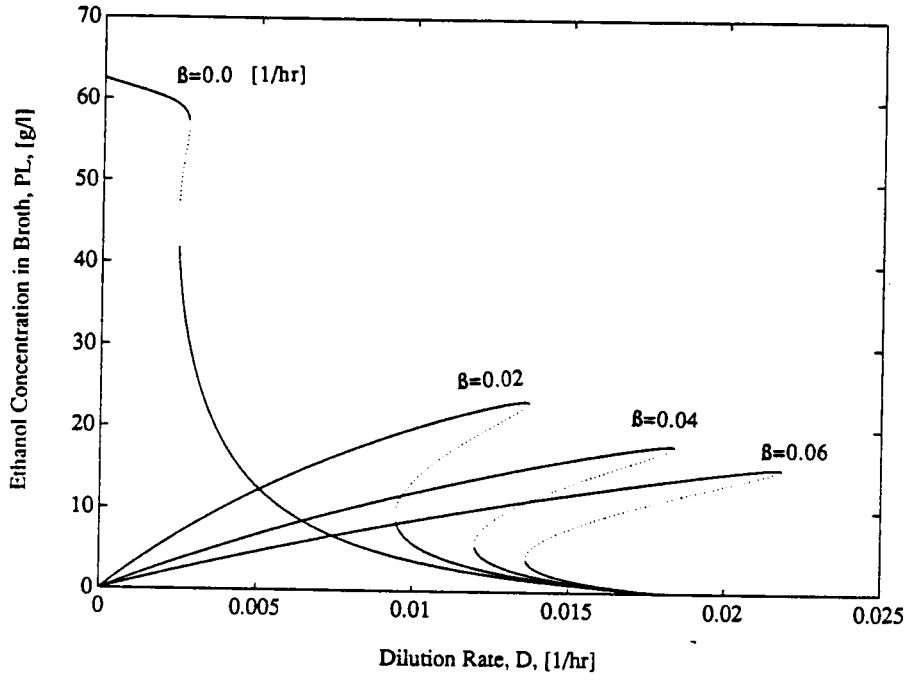


Figure 5.1.14 Effect of Stripping Factor on Steady State Ethanol Concentration in the Broth as a Function of Dilution Rate for Combined Product-Substrate Inhibition Kinetics (where solid lines [-] represent stability and dotted lines [...] represent instability; Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

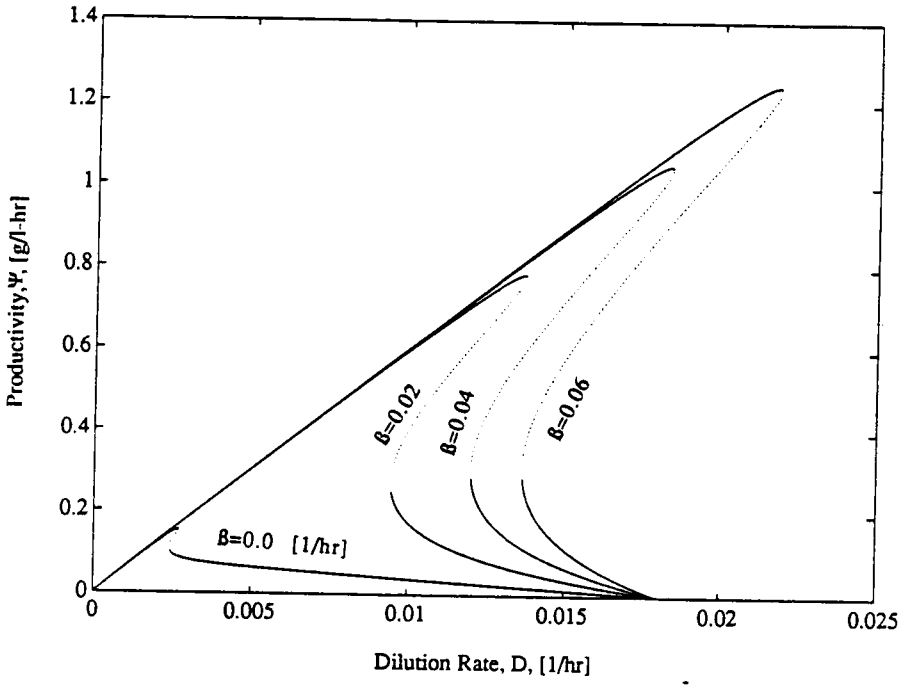


Figure 5.1.15 Effect of Stripping Factor on Steady State Productivity as a Function of Dilution Rate for Combined Product-Substrate Inhibition Kinetics (where solid lines [-] represent stability and dotted lines [...] represent instability; Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

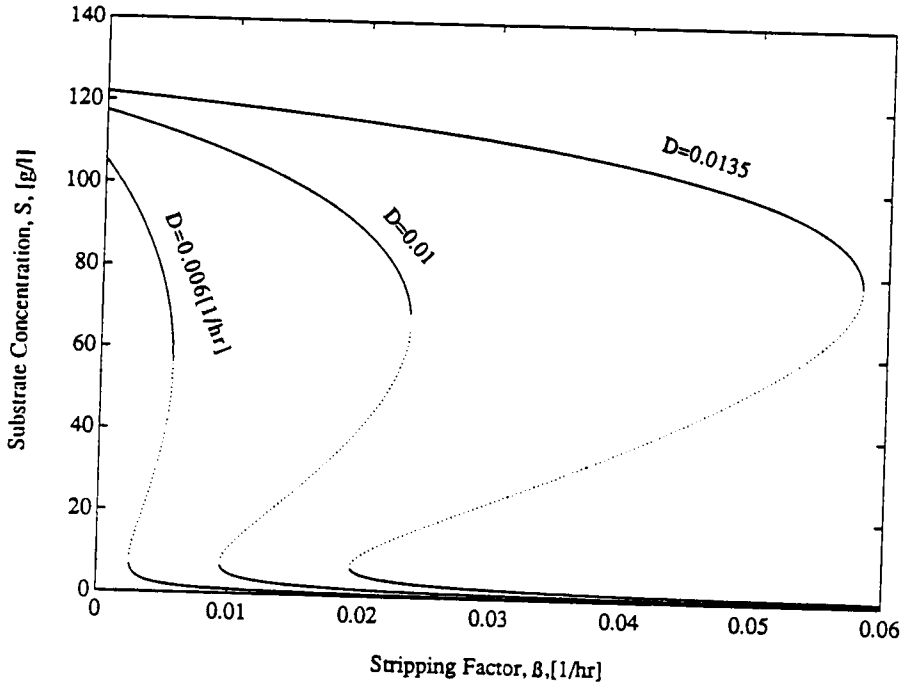


Figure 5.1.16 Effect of Dilution Rate on Steady State Substrate Concentration as a Function of Stripping Factor for Combined Product-Substrate Inhibition Kinetics (where solid lines [-] represent stability and dotted lines [...] represent instability; Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

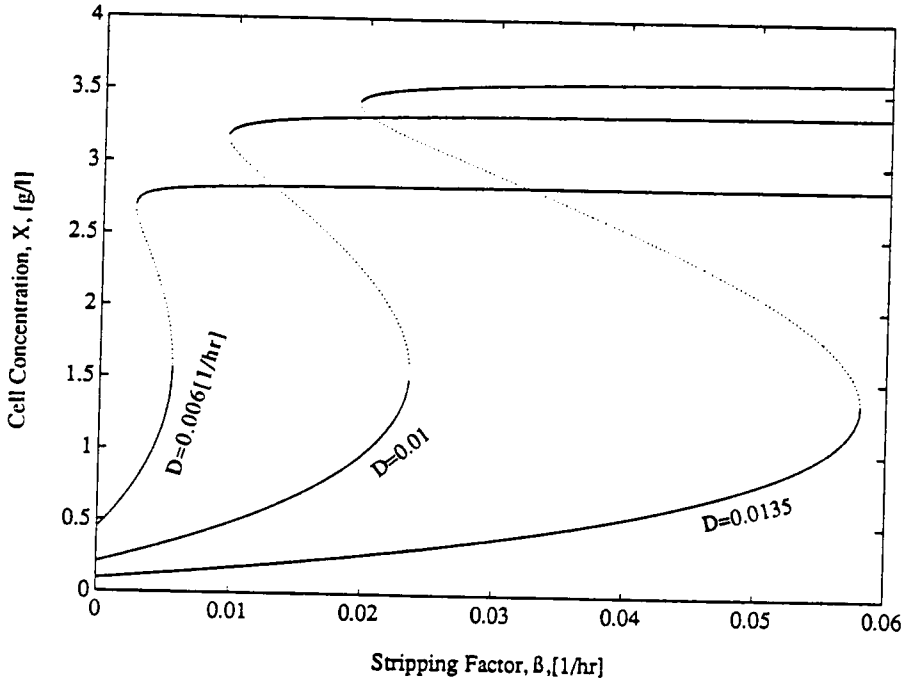


Figure 5.1.17 Effect of Dilution Rate on Steady State Cell Concentration as a Function of Stripping Factor for Combined Product-Substrate Inhibition Kinetics (where solid lines [-] represent stability and dotted lines [...] represent instability; Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

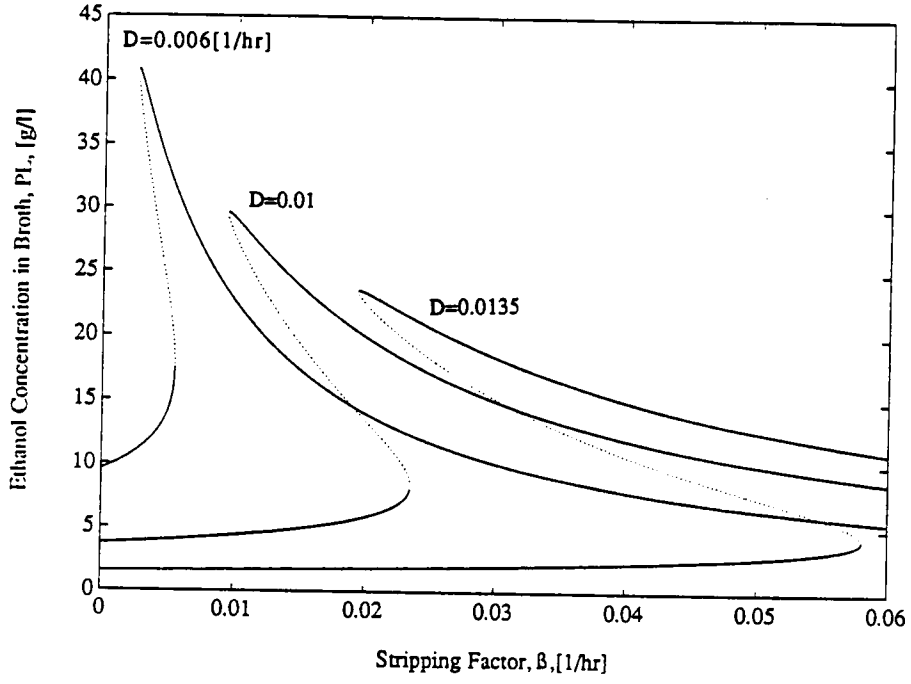


Figure 5.1.18 Effect of Dilution Rate on Steady State Ethanol Concentration in the Broth as a Function of Stripping Factor for Combined Product-Substrate Inhibition Kinetics (where solid lines [-] represent stability and dotted lines [...] represent instability; Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

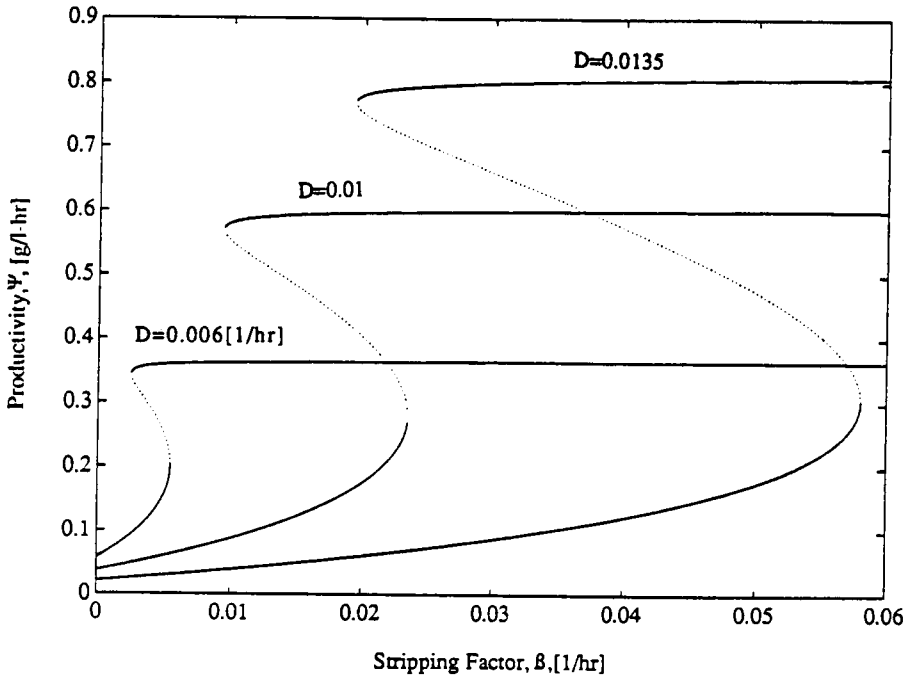


Figure 5.1.19 Effect of Dilution Rate on Steady State Productivity as a Function of Stripping Factor for Combined Product-Substrate Inhibition Kinetics (where solid lines [-] represent stability and dotted lines [...] represent instability; Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

without stripping (Figures 5.1.12-5.1.15) because of the hysteresis effect and inevitable internal noise of dilution rate.

The hysteresis effect is also observed for the case where the stripping factor is a continuous variable. In this case, the system is difficult to operate at D=0.006 [1/hr] around ß=0.005 [1/hr] (Figures 5.1.16-5.1.19). The difficulties in operating a gas stripping system by Mulholland (1984) may be due to the hysteresis effect and improper choice of operating variables. It is emphasized again that the hysteresis effect is associated with multiple steady states which are resulting from the product-substrate inhibition kinetics. To those who have not realized the possibility of multiple steady states in the couple inhibition kinetics (Nanba et al., 1983; Ierusalimsky, 1967), this may provide them with a cautionary note.

Also, the global stability is discussed in section (iii) for an effect of the initial conditions on the "approachability" of the optimal stability.

(ii) Special Case: m=0

When the maintenance factor (m=0) for substrate utilization is negligible, the eigenvalues of equation (5.1.10) can be solved analytically as follows:

$$\lambda_1 = -D \tag{5.1.14}$$

$$\lambda_2 = -\xi + \sqrt{\xi^2 - \zeta} \tag{5.1.15}$$

$$\lambda_3 = -\xi - \sqrt{\xi^2 - \zeta} \tag{5.1.16}$$

in which $$\xi = \frac{D + \frac{\phi X}{Y_2} + \frac{b X}{Y_1} + \beta}{2} \tag{5.1.17}$$

and $$\zeta = \frac{\phi X}{Y_2}(D+\beta) + bX\left(\frac{D}{Y_1}+\omega\right) \tag{5.1.18}$$

The necessary and sufficient conditions for the local stability are that the real parts of the roots are negative. For λ_1, the dilution rate is always positive and the stability is assured. For and λ_2 and λ_3 the conditions for the local stability are as follows:

(1) $\xi^2 < \zeta$, the eigenvalues λ_2 and λ_3 are two complex numbers. The system oscillates and the local stability requires $\xi > 0$.

(2) $\xi^2 = \zeta$, the eigenvalues λ_2 and λ_3 are two real equal roots. The system does not oscillate and the local stability requires $\xi > 0$.

(3) $\xi^2 > \zeta$, the eigenvalues λ_2 and λ_3 are two real unequal roots and the local stability requires both $\xi > 0$ and $\zeta > 0$.

In summary , the necessary and sufficient conditions for the local stability are:

$$\xi = \frac{D + \frac{\phi X}{Y_2} + \frac{b X}{Y_1} + \beta}{2} > 0, \text{ i.e. } \quad D + \frac{\phi X}{Y_2} + \frac{bX}{Y_1} + \beta > 0 \qquad (5.1.19)$$

$$\text{and} \quad \zeta = \frac{\phi X}{Y_2}(D+\beta) + bX\left(\frac{D}{Y_1}+\omega\right) > 0. \qquad (5.1.20)$$

In equations (5.1.19) and (5.1.20), all the quantities are positive except ϕ. The range of ϕ that satisfies the stability criterion for this case is also presented in Figure 5.1.20.

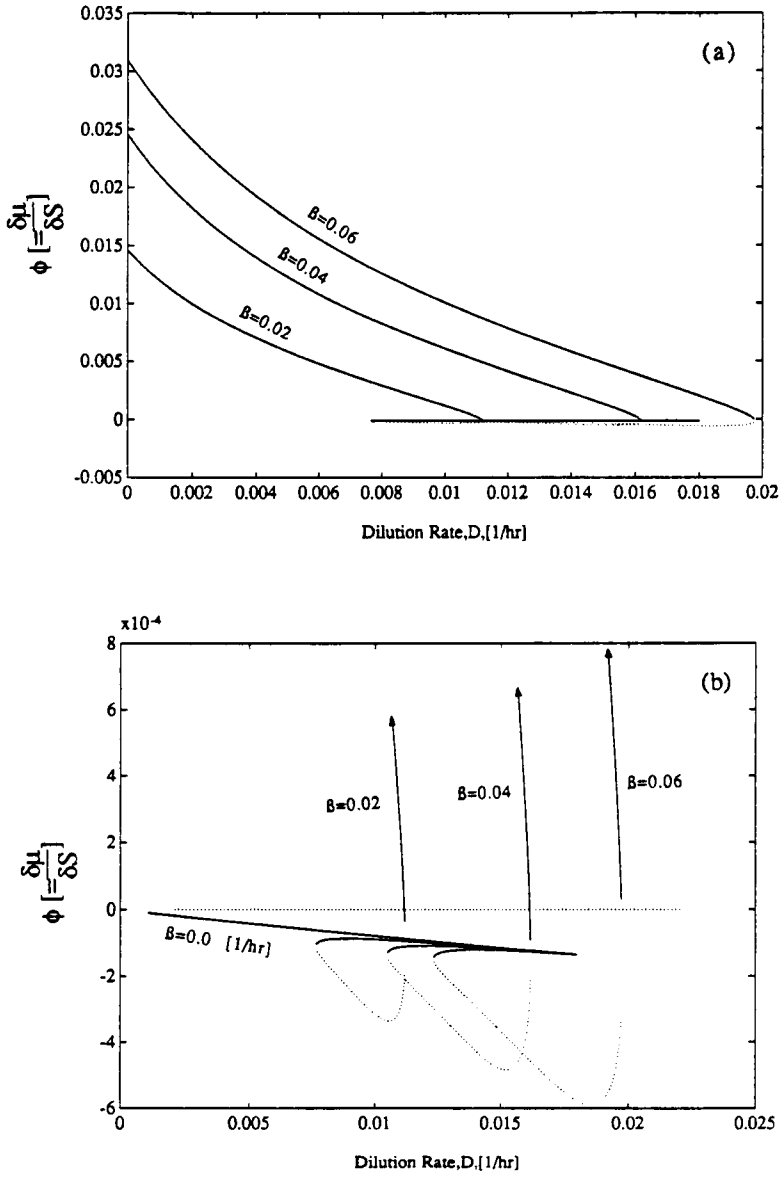


Figure 5.1.20 The Local Stability Criteria for the Value of ϕ [$= \frac{\delta\mu}{\delta S}$] as a Function of Dilution Rate in the Case of m=0; Figure (b) is the enlargement of bifurcation region of Figure (a). (where solid lines [-] represent stability and dotted lines [...] represent instability; Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{Si}=8 [g/l] and S_F=125 [g/l].)

(iii) Effect of Initial Concentrations on Global Stability

An extensive simulation of the global transient behavior for the case of combined product-substrate inhibitions given by equations(3.1.1), (5.1.5), (5.1.6) and (5.1.1) was performed by using SIMNON (a nonlinear system equations solver which is presented in Appendix C). The operating parameters used were ß=0.04 [1/hr], D=0.0135 [1/hr] and S_F=125 [g/l] together with the kinetic parameters in Table 5.1.1 (K_{si}=8 [g/l]) which has three nontrivial steady states as shown in Figure 5.1.8. The simulations were carried out with systematic scanning of all the possible initial conditions, i.e. X(0), P_L(0), and S(0) to see the proper initial (start-up) conditions that would lead the system to optimal steady state. Figures 5.1.21.A, B, C and D are the sample results for X(0)=0.5, 1.0, 1.5 and 2.0 [g/l], respectively. In Figure 5.1.21.A-(i), various S(0)'s from 0 to 20 [g/l] are used with P_L(0)=0 [g/l] in which only S(0)=0 and 5 [g/l] lead the system to the optimal steady state (bottom one) while others move to the other steady state. When P_L(0) is increased to 1.0 [g/l], only the one with S(0)=0 [g/l] moves to optimal steady state as shown in Figure 5.1.21.A-(ii). Further increase of P_L(0) such as P_L(0)=5 and 10 [g/l] in Figure 5.1.21.A-(iii) and (iv), there is no S(0) can drive the system to the optimal steady state. The similar pattern is observed for X(0)=1.0, 1.5, 2.0 [g/l] in Figure 5.1.21.B to D, i.e. for a larger P_L(0), the smaller of the feasible region of S(0) which could lead the system to the optimal steady state. Thus, the attraction region diagrams for the conditions given in the simulation were searched and presented in Figure 5.1.22, in which the area under the curve is the attraction region for the optimal steady state. With an increase in initial cell concentration, the attraction region increases, too. Therefore, it is concluded that higher cell concentration and lower substrate and product concentrations will favor the

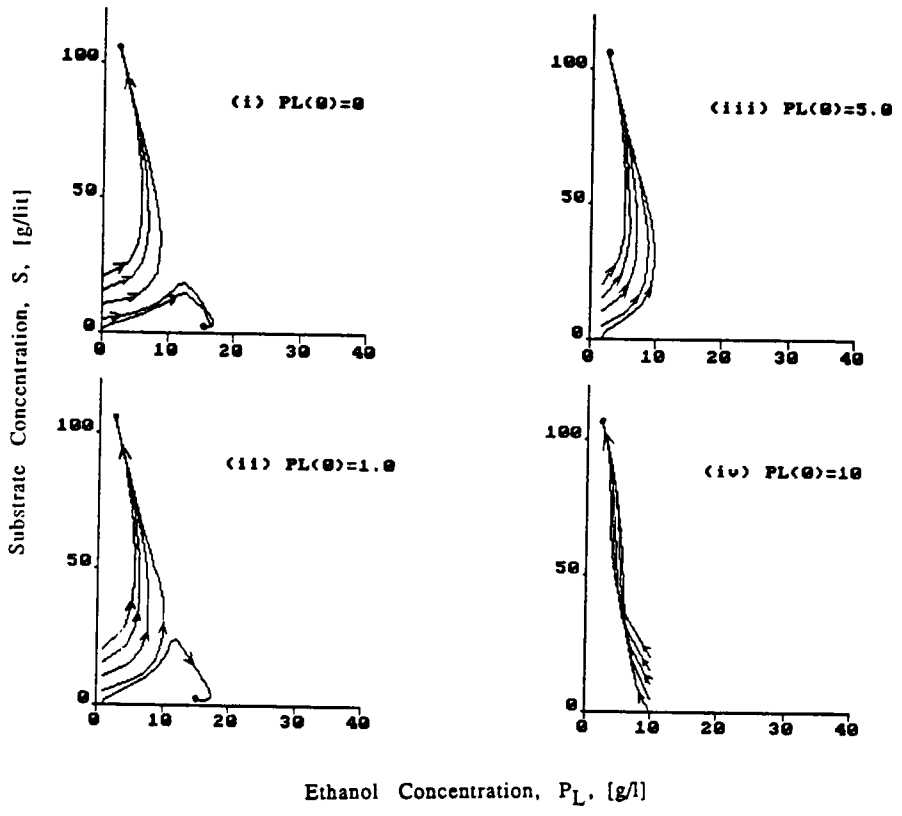


Figure 5.1.21.A Phase Diagram Between Initial Concentration of Ethanol in Broth and Initial Concentration of Substrate Showing the Transient Behavior of a GSEF system with Combined Product-Substrate Inhibition Kinetics for D=0.0135 [1/hr], ß=0.04 [1/hr] and X(0)=0.5 [g/l] (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

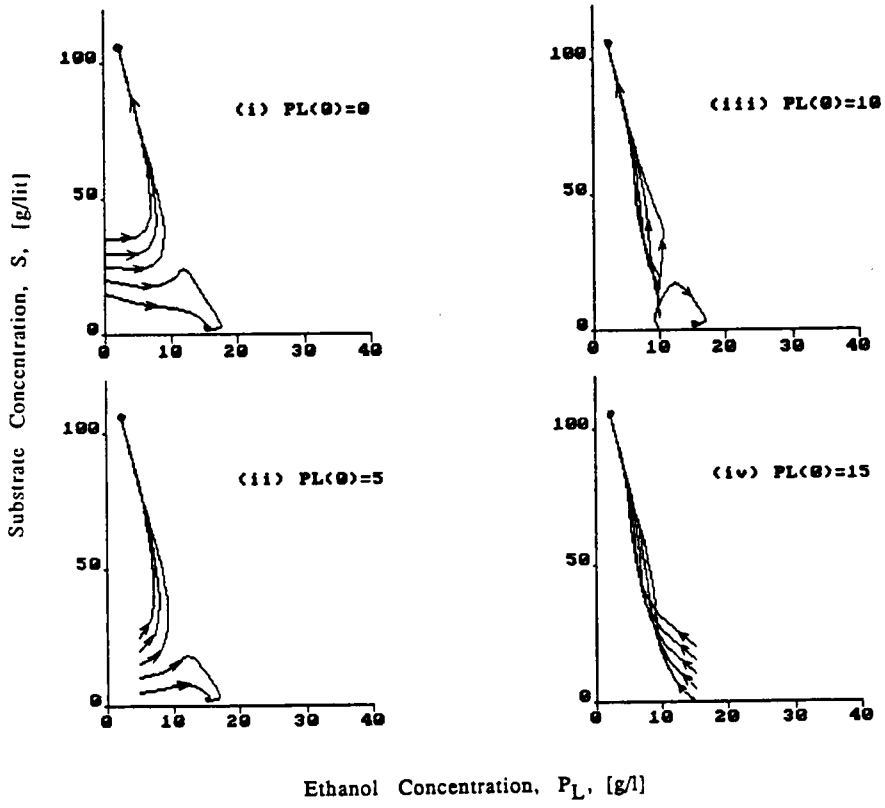


Figure 5.1.21.B Phase Diagram Between Initial Concentration of Ethanol in Broth and Initial Concentration of Substrate Showing the Transient Behavior of a GSEF system with Combined Product-Substrate Inhibition Kinetics for D=0.0135 [1/hr], ß=0.04 [1/hr] and X(0)=1.0 [g/l] (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

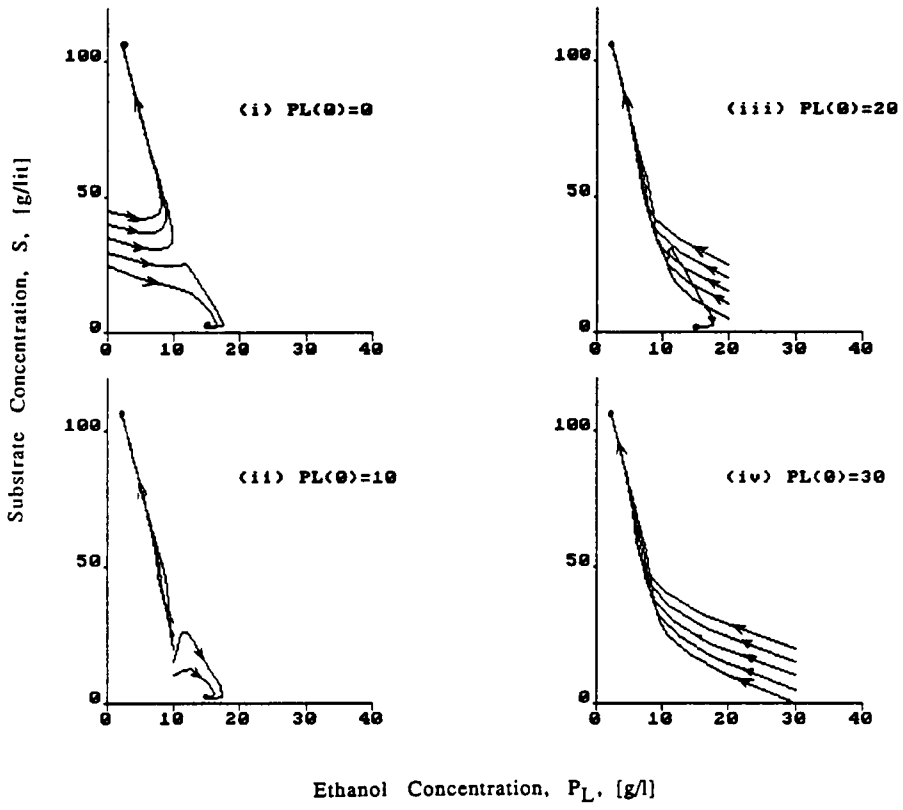


Figure 5.1.21.C Phase Diagram Between Initial Concentration of Ethanol in Broth and Initial Concentration of Substrate Showing the Transient Behavior of a GSEF system with Combined Product-Substrate Inhibition Kinetics for D=0.0135 [1/hr], ß=0.04 [1/hr] and X(0)=1.5 [g/l] (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

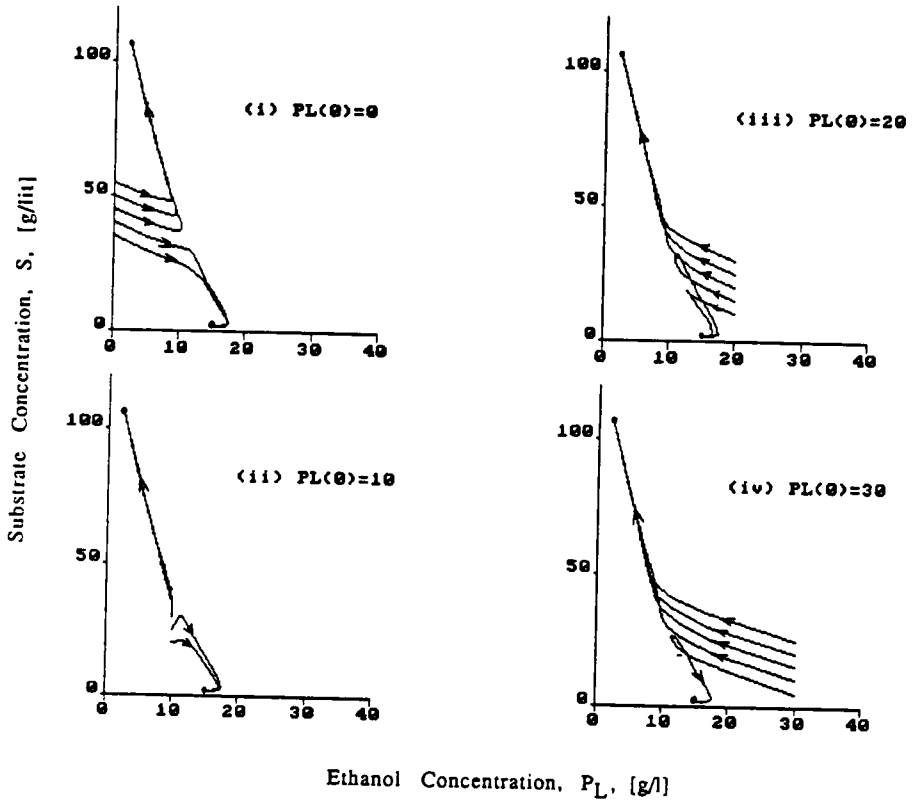


Figure 5.1.21.D Phase Diagram Between Initial Concentration of Ethanol in Broth and Initial Concentration of Substrate Showing the Transient Behavior of a GSEF system with Combined Product-Substrate Inhibition Kinetics for D=0.0135 [1/hr], ß=0.04 [1/hr] and X(0)=2.0 [g/l] (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

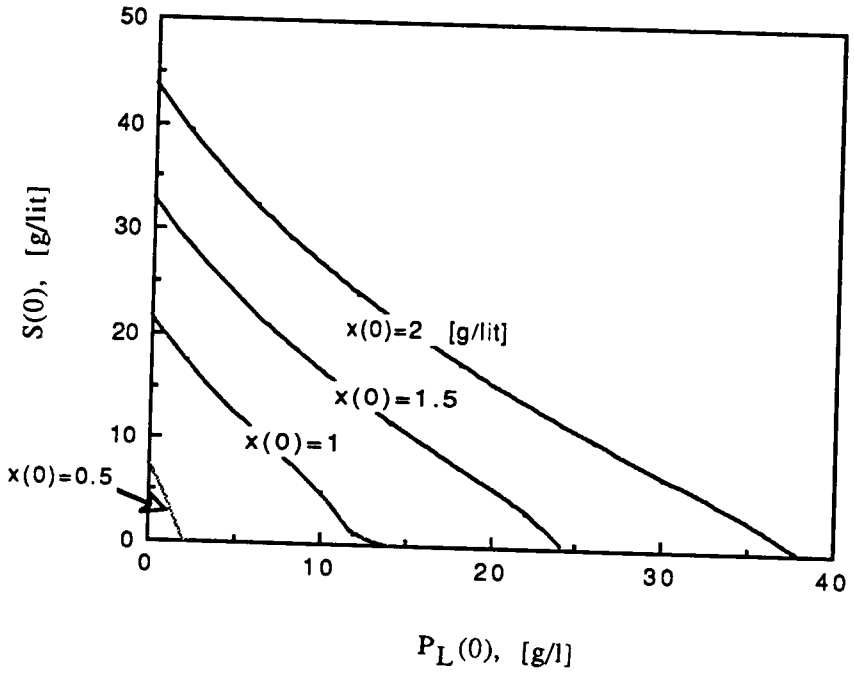


Figure 5.1.22 Attraction Region for the Optimal Steady State with Various Initial Cell Concentrations for D=0.0135 [1/hr] and ß=0.04 [1/hr] (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

optimal steady state. Because the system has three independent variables, X, P_L, and S which are coupled together, the results of Figure 5.1.22 can be constructed in a three dimensional graph, X-P_L-S space, as Figure 5.1.23. The figure shows a surface that divides the whole space into two regions, while the region under the surface is the attraction region for the optimal steady state.

This conclusion may be used to explain the advantageous result Ramalingaham and Finn (1977) obtained for their vacuum fermentation system. They found that by adding yeast cream to the vacuum fermentation system it moves the system to "good fermentation rate" and also remains stable for "a long period of time". Because the optimal steady state can be reached only if the the condition is under the curve shown in Figure 5.1.22. Increasing the cell concentration would enlarge the attraction region which make the optimal steady state "approachable".

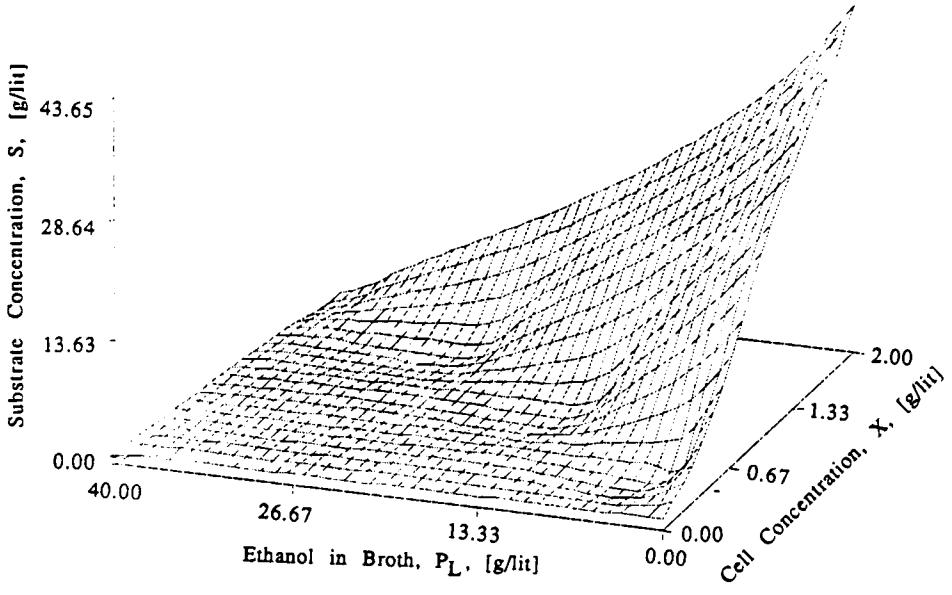


Figure 5.1.23 Attraction Space for the Optimal Steady State in Three Dimensional Graph for D=0.0135 [1/hr] and ß=0.04 [1/hr] (Kinetic model and parameters are presented in equations (5.1.1)-(5.1.3) and Table 5.1.1 with K_{si}=8 [g/l] and S_F=125 [g/l].)

5.2 Batch GSEF System

The continuous GSEF system has shown to have the advantages: (1) that the cell concentration increases with increase in the stripping factor, (2) that the substrate consumption increases with the stripping factor, (3) that the ethanol concentration in broth decreases with the stripping factor, (4) that the ethanol productivity increases with increase with stripping factor. However, in a batch reactor, the advantages may not always exist.

The governing equations for batch GSEF system can be obtained form a CSTR case with D=0 (equations (3.1.1), (3.1.8) and (3.2.3) with P_F=0), that is

$$\frac{dX}{dt} = \mu X \tag{5.2.1}$$

$$\frac{dS}{dt} = \upsilon X \tag{5.2.2}$$

$$\frac{dP_L}{dt} = \sigma X - \beta P_L \tag{5.2.3}$$

By using the model of Ghose and Tyagi as equations (5.1.1), (5.1.2) and (5.1.3) and rearranging equations (5.2.2) and (5.2.3) in a more general form ($\sigma = \frac{\mu}{Y_1} + \omega$, $\upsilon = -(\frac{\mu}{Y_2} + m)$) as that in section 5.1.C, one might have

$$\frac{dP_L}{dt} = (\frac{\mu}{Y_1} + \omega)X - \beta P_L \tag{5.2.4}$$

$$\frac{dS}{dt} = -(\frac{\mu}{Y_2} + m)X \tag{5.2.5}$$

The parameters Y_1, Y_2, m and ω are the same as described in section 5.1.C.

The total production rate ($\frac{d\Sigma}{dt}$) of ethanol in this batch GSEF system is the sum of production rate in the fermentation broth and the ethanol removal rate by the stripping gas. Thus, from equation (5.2.4), one obtains

$$\frac{d\Sigma}{dt} = \frac{dP_L}{dt} + \beta P_L = (\frac{\mu}{Y_1} + \omega)X \qquad (5.2.6)$$

Division of (5.2.6) by (5.2.5) gives

$$-\frac{d\Sigma}{dS} = \frac{\frac{\mu}{Y_1} + \omega}{\frac{\mu}{Y_2} + m} = H[\mu(S,P_L;\beta)] \qquad (5.2.7)$$

Equation (5.2.7) is the infinitesimal ratio of total product produced to the substrate consumed, and this ratio can be defined as a function of $H[\mu]$ in which μ is function of S, P_L as well as ß. Therefore, the task to maximize the total product becomes to maximize the $H[\mu]$ with respect to stripping factor, ß.

Whether an enhancement of specific growth rate, μ, due to the stripping, will increase the total product or not can be revealed by differentiation of H respect to μ.

$$\frac{dH}{d\mu} = \frac{\Delta}{(\frac{\mu}{Y_2} + m)^2} \qquad (5.2.8)$$

$$\text{where} \quad \Delta = \frac{m}{Y_1} - \frac{\omega}{Y_2} \qquad (5.2.9)$$

Therefore, the effect of variational specific growth rate, μ, on total product produced can be discussed in three cases.

(a) When $\Delta = \frac{m}{Y_1} - \frac{\omega}{Y_2} > 0$, an increase in μ increases $H[\mu]$ and also increases the total product yield at the final time $\Sigma(t_f)$. The final time, t_f, is defined as the time when substrate is completely consumed. Therefore, *in situ* product removal is favored with higher β-values.

(b) When $\Delta = \frac{m}{Y_1} - \frac{\omega}{Y_2} = 0$, $H[\mu]$ is always a constant regardless the change of μ and the total product yield at the final time $\Sigma(t_f)$ is independent of the β-value.

(c) When $\Delta = \frac{m}{Y_1} - \frac{\omega}{Y_2} < 0$, an increase in μ decreases $H[\mu]$ and also decreases the total product yield at the final time $\Sigma(t_f)$. Therefore, *in situ* product removal is unfavorable and the lowest value is required which is non-stripping case, β=0. However, in all three cases, the fermentation rate will be enhanced due to inhibition removal. That is, the fermentation time, t_f, decreases with the increase of stripping factor while the yield might be increased, decreased or no changed.

Advanced Continuous Simulation Language (ACSL, Appendix D) was used to calculate concentrations of total product, product in the broth, cell, and substrate in a batch system as a function of fermentation time for various β from equations (5.2.1), (5.2.4)-(5.2.6) and (5.1.1), which will permit evaluation of the characteristics of simulation results obtained. The parameters used in the simulation were presented in Table 5.2.1.

Figures 5.2.1-(a), (b), and (c) present the total product concentration (Σ) as a function of fermentation time with variation of β-value; (a) for the case when $\Delta > 0$, (b) for the case when $\Delta = 0$, and (c) for the case when $\Delta < 0$,

Table 5.2.1

The Numerical Parameters Used in Equations (5.2.1), (5.2.4)-(5.2.6) and (5.3.1) for Simulation

case	ω	m	Δ
(a) $\Delta > 0$	0.1	0.6	5
(b) $\Delta = 0$	0.0	0.0	0
(c) $\Delta < 0$	0.4	0.4	-5

$$\Delta = \frac{m}{Y_1} - \frac{\omega}{Y_2}$$

β = 0.00 (0.04) 0.16*

S_F= 100 [g/lit]

These values are taken from Sevely et al. (1980) except Y_2.

μ_m	=	0.3	[1/hr]	K_s	=	5.0	[g/lit]
K_p	=	4.5	[g/lit]	P_{max}	=	85.0	[g/lit]
Y_1	=	0.08	[-]	Y_2	=	0.04	[-]
K_{si}	=	∞	[g/lit]				

* The number in the parentheses between two numbers is an increment.

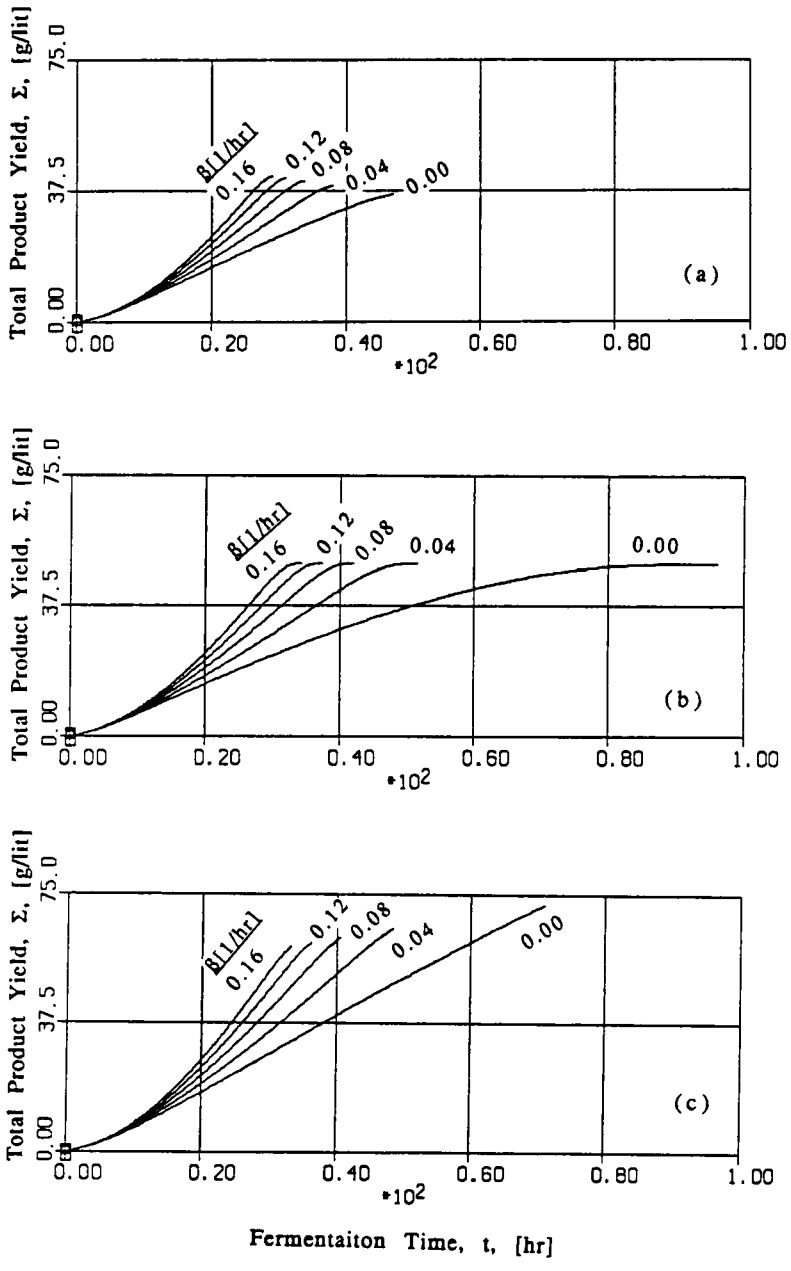


Figure 5.2.1 Effect of Stripping Factor on Total Product Yield for a Batch GSEF System (a) $\Delta > 0$, (b) $\Delta = 0$, (c) $\Delta < 0$ (Kinetic model and parameters are presented in equations (5.2.1), (5.2.4)-(5.2.6) and (5.2.6), and Table 5.2.1.)

respectively. Figure 5.2.1-(a) demonstrates the total product increases with increase in the stripping factor, Figure 5.2.1-(b) shows the total product is independent of the stripping factor, and Figure 5.2.1-(c) illustrates the total production decreases with increase in the stripping factor. However, in all three cases the fermentation time is reduced by increasing the stripping factor.

Figures 5.2.2-(a), (b), and (c) demonstrate the product concentration (P_L) in the broth as a function of fermentation time with various values of ß for each control case. The concentration decreases with increase in the stripping factor for all three cases and the fermentation time is reduced by increasing the stripping factor. With the stripping, the concentration becomes convex having a maximum for all three cases.

Figures 5.2.3-(a), (b), and (c) illustrate the cell concentration (X) as a function of fermentation time with various values of the stripping factor for each control case. In cases (a) and (c), the concentrations increase with increase in the stripping factor and the concentration for case (b) is independent of the stripping factor. However, for all three cases, the fermentation time is reduced by increasing the stripping factor.

Figures 5.2.4-(a), (b), and (c) show the substrate concentration as a function of fermentation time with variation in the β-value for each control case. The fermentation time is reduced by increase in the stripping factor for all three cases.

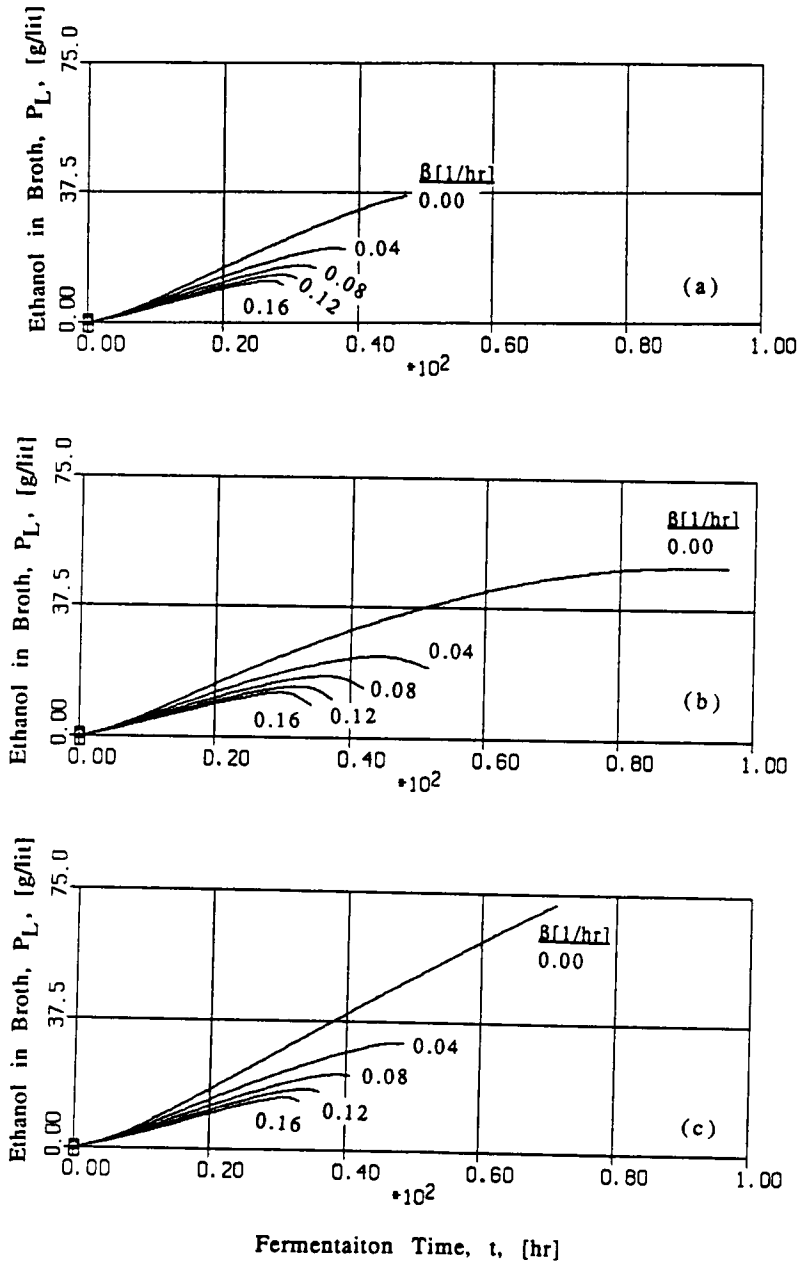


Figure 5.2.2 Effect of Stripping Factor on Ethanol Concentration in the Broth for a Batch GSEF System (a) $\Delta > 0$, (b) $\Delta = 0$, (c) $\Delta < 0$ (Kinetic model and parameters are presented in equations (5.2.1), (5.2.4)-(5.2.6) and (5.2.6), and Table 5.2.1.)

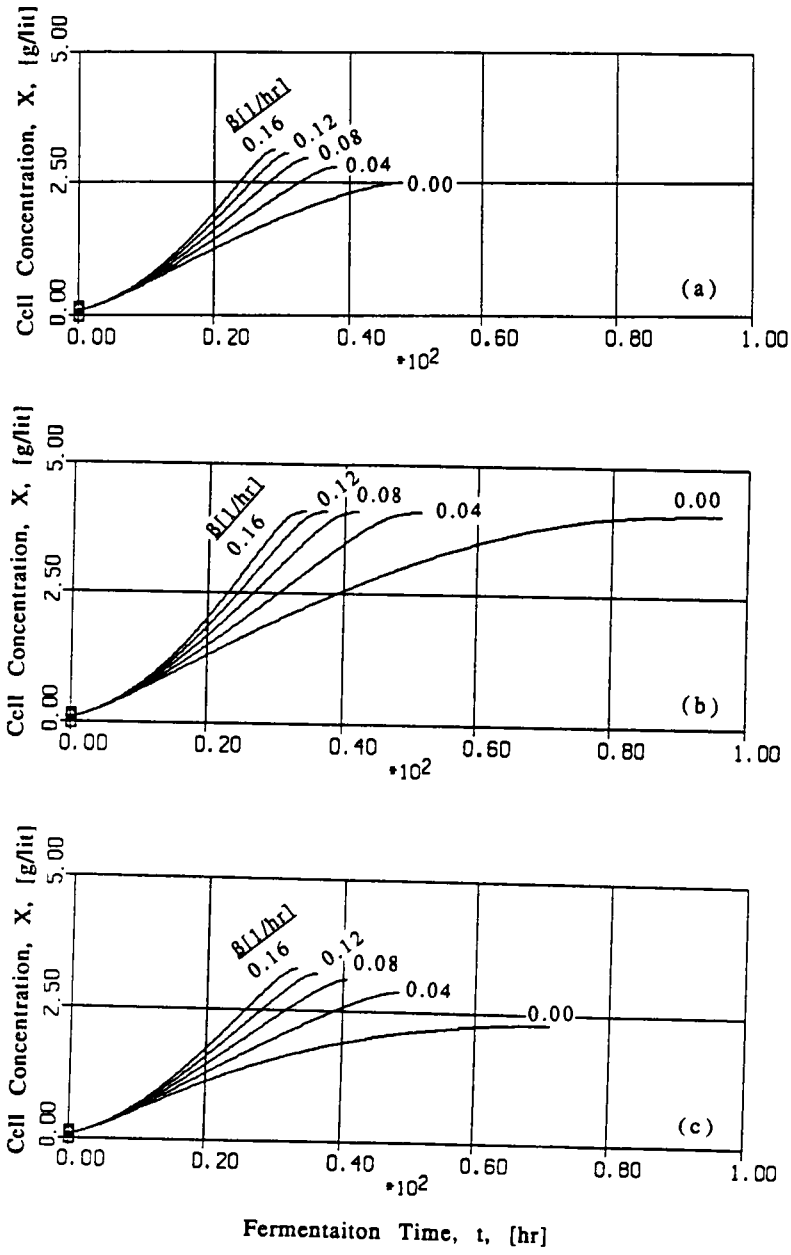


Figure 5.2.3 Effect of Stripping Factor on Cell Concentration for a Batch GSEF System (a) $\Delta > 0$, (b) $\Delta = 0$, (c) $\Delta < 0$ (Kinetic model and parameters are presented in equations (5.2.1), (5.2.4)-(5.2.6) and (5.2.6), and Table 5.2.1.)

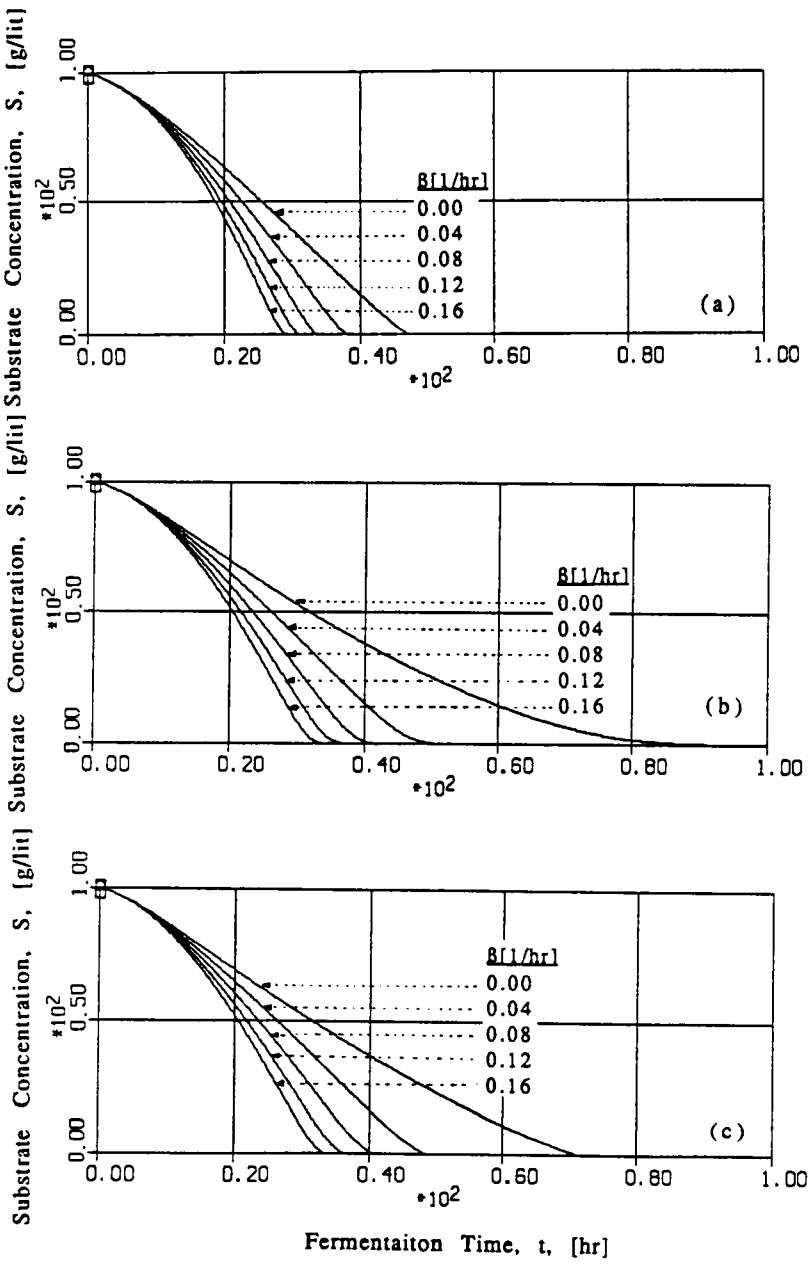


Figure 5.2.4 Effect of Stripping Factor on Substrate Concentration for a Batch GSEF System (a) $\Delta > 0$, (b) $\Delta = 0$, (c) $\Delta < 0$ (Kinetic model and parameters are presented in equations (5.2.1), (5.2.4)-(5.2.6) and (5.2.6), and Table 5.2.1.)

The physical meaning of this criterion might be easier to explain by rescaling of substrate and total product as $S'=Y_2 \cdot S$ and $\Sigma' = Y_1 \cdot \Sigma$. Thus, equations (5.2.5) and (5.2.6) become

$$\frac{dS'}{dt} = -(\mu + Y_2 \cdot m)X \qquad (5.2.10)$$

$$\frac{d\Sigma'}{dt} = (\mu + Y_1 \cdot \omega)X \qquad (5.2.11)$$

In viewing these two equations, one might imagine that there two mechanisms in both substrate utilization and product formation, i.e. growth-associated (μ in both equations) and nongrowth-associated ($Y_2 \cdot m$ and $Y_1 \cdot \omega$) mechanisms. While nongrowth mechanism consumes more substrate and produces less product ($Y_2 \cdot m > Y_1 \cdot \omega$ which leads case (a), $\Delta = \frac{m}{Y_1} - \frac{\omega}{Y_2} > 0$), elimination of the effect of nongrowth mechanism is preferred because maximum product formation is our objective. That is, increasing growth mechanism, to make the nongrowth mechanism comparably less significant, will increase the total product. The same reasoning could apply to case (b) and case (c). However, whether the gain in productivity will compensate the additional separation cost due to the *in-situ* product removal depends on the values of the product as well as the method used, which is truly an economical issue.

Although, the economics of selective ethanol removal show very little advantages over batch system (Maiorella et al. 1984). This simple analysis reveals somewhat an interesting point, i.e. an *in-situ* removal of inhibitor (therefore, to enhance the cell growth) from batch fermentation system does not always result in higher total product or cell concentration. Therefore, it is not surprising to see an organic extraction of 2,3-butanediol produced by *Klebsiella oxytoca* (ATCC 8724) fermentation results in a cell yield of 0.10 [g/g

D-xylose], and a 2,3-butanediol yield of 0.32 [g/g-D-xylose] which is similar to that obtained in control (solventless) experiments (Eiteman and Gainer, 1989). Moreover, Groot et al. (1989) has reported lower yield (0.33 & 0.34 [Kg alcohol/ Kg glucose]) for batch *in-situ* removal fermentations compared with conventional one (0.37 [Kg alcohol/Kg glucose]) using *Clostridium* sp. DSM 2152 while substrate consumption rate was increased threefold for *in-situ* product recovery. Similar observation of lower yield (0.27 [g solvent/g lactose vs 0.39 g [solvent/g lactose]) for an *in-situ* removal batch fermentation system was reported by Ennis et al. (1986) when lactose utilization rate was doubled.

Extension of this viewpoint might clarify the question raised by Lacis and Lawford (1989) about whether higher initial sugar concentration in a batch ethanol fermentation will have higher yield in term of sugar consumption. *Thermoanaerobacter ethanolicus* (ATCC 31550)-glucose system has shown the ratio for the ethanol/glucose was greater for an initial glucose concentration of 20 [g/l] compared with 10 [g/l] (Kannan and Mutharasan, 1985), while *Thermoanaerobacter ethanolicus* (ATCC 31938)-xylose system was found that the ratio of ethanol/xylose was greater with an initial xylose concentration of 4 [g/l] compared to 12 or 31 [g/l] (Lacis and Lawford, 1985). But *Z. mobilis* ZM4 (Lee et al. 1980) shows no significant difference in yield (ethanol/glucose) for various initial glucose concentrations from 100 to 300 [g/l]. Higher initial sugar concentration usually implies high growth rate (with exception of substrate inhibition), however, does not result in better ethanol yield necessarily. It depends on the relative yield contributions from growth-associated and nongrowth-associated mechanisms.

The apparent yield factor of a continuous system at steady state ($\mu = D$) might be obtained by the definition given in equation (3.6.3) with the

generalized kinetic (i.e. $\sigma = \frac{\mu}{Y_1} + \omega, \upsilon = -(\frac{\mu}{Y_2} + m)$) used in equations (5.1.5) and (5.1.6) as,

$$Y_{a.p/s} = \frac{\frac{D}{Y_1} + \omega}{\frac{D}{Y_2} + m} \qquad (5.2.12)$$

which is similar to equation (5.2.7), therefore, the effect of dilution rate on ethanol yield could be positive or negative or there could be no effect that results in the same criterion ($\Delta = \frac{m}{Y_1} - \frac{\omega}{Y_2}$ >0, <0, or =0). This explains another question raised by Lacis and Lawford (1989) concerning the lack of consistent pattern of growth rate effect (i.e. dilution rate effect) on ethanol yields in viewing the following various reports: a large negative effect for glucose-limited *C. thermohydrosulfuricum*, a slight negative effect on xylose-limited *C. thermohydrosulfuricum*, and large negative effect on nitrogen-limited with either glucose or xylose as the energy source (Zeikus et al. 1981); no effect for a glucose-limited *Thermoanaerobium brockii* variant; and a positive effect for glucose-limited *T. brockii* DSM 1457 (Sconnleitner, Fiechter and Giovannini, 1984). That is, the apparent yield factor is the combined effect of growth-associated mechanism and nongrowth-associated mechanism. Whether an increase in dilution rate will increase the apparent yield factor really depends the relative yield contribution from both mechanisms.

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The following conclusions were drawn from this work:

(1) The mass transfer effect in the batch stripping experiments was negligible in the experimental range, which means that the equilibrium is established rapidly. The Henry's law constants are obtained from the stripping experiments at 35°C, 50°C and 60°C. They are to be used in the estimation of ethanol removal rate associated with the stripping factor, ß, in the GSEF system. Also, a simple stripper-condenser experiment at 60°C was performed which demonstrated the advantages of the GSEF system both in the ethanol removal to alleviate the product inhibition and in the product concentration in the overhead condenser.

(2) A thermophilic anaerobic bacterium, *Clostridium thermosaccharolyticum* (ATCC 31925, HG-6), was evaluated to determined the feasibility of incorporating into the GSEF system. However, this strain of bacteria has shown to be incapable of growing at high xylose concentration. Consequently, the ethanol concentration produced is also too low to demonstrate the alleviation of the product and of the product-substrate inhibitions by the gas stripping.

(3) In a continuous GSEF system with either product inhibition kinetics or combined product-substrate inhibition kinetics, the cell concentration, productivity, substrate consumption increase with an increase in the stripping factor and the ethanol concentration in the broth decreases with an increase in the stripping factor. The enhancement of productivity is mainly

due to an increase in the cell concentration and the substrate utilization in the system.

(4) For the product inhibition case, there is always one (nontrivial) steady state provided that the dilution rate is less than the maximum growth rate. However, in the combined product-substrate inhibition case, there are possibly three nontrivial steady states, and the hysteresis effect is also observed. When multiple steady states arise, the one with the best substrate utilization (optimal) is always stable. The attraction region of the optimal steady state suggests that the higher cell concentration and the lower substrate and ethanol concentrations are in favor of the system to move toward the optimal steady state. In this case, the removal of inhibitory product has been shown to increase the range of operation variables in dilution rate and feed substrate concentration.

(5) In a batch GSEF system with the product inhibition case, the removal of inhibitory product in broth is not always in favor of the total yield. A criterion is obtained to distinguish three different cases (increase, decrease or no effect to the total yield).

6.2 Recommendations for Future Study

The following recommendations for future study are made:

(1) Although the thermophilic bacterium, *Clostridium thermosaccharolyticum* (ATCC 31925, HG-6), tested in this study is unable to reach the desired objectives, the potentials of the GSEF system are numerous. The use of mesophiles or thermo-tolerant yeasts in the GSEF system is suggested.

(2) Fermentation products sufficiently volatile, such as acetone and butanol, are also worth investigating.

(3) Because of low recovery of ethanol (efficiency: 35%-65%) in the condenser, the recycling of stripping gas and fermentation gas is highly recommended in the future. Furthermore, the recycling of stripping gas can reduce the cost of gas supply and sterilization of gas as well as the risk of contamination.

(4) An optimization of the integrated reactor/separator-recovery system is very important in making the GSEF system economically feasible.

(5) Since the GSEF system offers a very simple operation, the independent variables such as gas flow rate, cooling water flow rate and gas recycling ratio etc., or process option such as periodical stripping need to be investigated further.

(6) A nonlinear behavior of systems with a product-substrate inhibition coupled with biochemical interactions warrants further investigation. Since a product-substrate inhibition case demonstrates multiplicity and hysteresis effect, this complicated nonlinear system which is a step closer to the reality

may provide some other important effects to elucidate the complexity of biological systems.

(7) Improvement of finding new strains of thermophilic bacteria as well as modifying existing strains becomes critically important for practical applications. For example, the maximum ethanol concentration reported for *Clostridium thermosaccharolyticum* is 11.2 [g/l] (Kersey and Lynd, 1989), for *T. ethanolisus* is 4.7 [g/l] (Lacis and Lawford, 1989) in continuous fermentations. These values are far below the yield of 100 to 200 [g/l] in the conventional yeast fermentations.

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Appendixes

Appendix A

Steady State Solution

I. Steady-State Solution for the Product Inhibition

From equation (3.4.2) X (the subscript "ss" indicating the steady state has been eliminated for simplicity) is:

$$X = (S\text{-}S_F)\frac{D}{\upsilon} \qquad (A1)$$

From equation (3.4.2) and (3.4.3) by eliminating X, one has

$$S = AP_L + S_F \qquad (A2)$$

where

$$A = (1 + \frac{\beta}{D})\frac{\upsilon}{\sigma} \qquad (A3)$$

Substituting equation (A2) into Equation (4.3.1) and rearranging, one has

$$C_1 P_L^2 + C_2 P_L + C_3 = 0 \qquad (A4)$$

in which C_1, C_2 and C_3 are constant given by

$$C_1 = A(1 + B) \qquad (A5)$$

$$C_2 = A(BK_p - P_{max}) + S_F(1 + B) + BK_s \qquad (A6)$$

$$C_3 = BKp(K_s + S_F) - P_{max}S_F \qquad (A7)$$

where the quantity B is defined as:

$$B = \frac{\mu P_{max}}{\mu_m K_p} \qquad (A8)$$

Since the system is at steady state, all the quantities in A and B are constants, thus A and B are all also constants.

II. Steady-State Solution for the Substrate-Product Inhibition

The equivalence of equation (A4) for this case is a third order algebraic equation in S, which is

$$S^3 + C_4S^2 + C_5S + C_6 = 0 \qquad (A9)$$

in which

$$C_4 = K_{si} + e + \frac{b}{D} \qquad (A10)$$

$$C_5 = K_{si}e + d - \frac{ab}{D} \qquad (A11)$$

$$C_6 = d\,e \qquad (A12)$$

where

$$a = AP_{max} + S_F \qquad (A13)$$

$$b = \frac{K_{si}\mu_m K_p}{P_{max}} \qquad (A14)$$

$$d = K_{si}\,K_s \qquad (A15)$$

$$e = AK_p - S_F \qquad (A16)$$

The quantity A is already given in equation (A3).

Appendix B

MACSYMA Output

```
(C62) J;

                        [ MU - D    MUS X          MUP X        ]
                        [                                       ]
(D62)                   [    V      VS X - D        VP X        ]
                        [                                       ]
                        [    TQ      TQS X     TQP X - D - BETA ]
(C63) I;

                                    [ 1  0  0 ]
                                    [         ]
(D63)                               [ 0  1  0 ]
                                    [         ]
                                    [ 0  0  1 ]
(C64) DETERMINANT(J-LA*I);

                                                                  2
(D64) (MU - LA - D) ((TQP X - LA - D - BETA) (VS X - LA - D) - TQS VP X )

 + MUP X (TQS V X - TQ (VS X - LA - D)) - MUS X

 (V (TQP X - LA - D - BETA) - TQ VP X)
(C65) RATCOEF(-D64,LA,3);

(D65)                                  1
(C66) RATCOEF(-D64,LA,2);

(D66)                    (- VS - TQP) X - MU + 3 D + BETA
(C67) RATCOEF(-D64,LA,1);

                                  2
(D67) (TQP VS - TQS VP) X  + ((MU - 2 D - BETA) VS - MUS V + (MU - 2 D) TQP

                                - MUP TQ) X + (- 2 D - BETA) MU + 3 D  + 2 BETA D
                                                                    2
(C68) RATCOEF(-D64,LA,0);

(D68) (((D - MU) TQP + MUP TQ) VS + ((MU - D) TQS - MUS TQ) VP

 + (MUS TQP - MUP TQS) V) X  + (((D + BETA) MU - D  - BETA D) VS
                          2                    2

 + (- D - BETA) MUS V + (D MU - D ) TQP - D MUP TQ) X + (- D  - BETA D) MU + D
                               2                          2                  3

         2
 + BETA D
(C69) D66*D67-D68;

(D69) ((- VS - TQP) X - MU + 3 D + BETA)
```

```
((TQP VS - TQS VP) X^2 + ((MU - 2 D - BETA) VS - MUS V + (MU - 2 D) TQP

- MUP TQ) X + (- 2 D - BETA) MU + 3 D^2 + 2 BETA D)

- (((D - MU) TQP + MUP TQ) VS + ((MU - D) TQS - MUS TQ) VP

+ (MUS TQP - MUP TQS) V) X^2 - (((D + BETA) MU - D^2 - BETA D) VS

+ (- D - BETA) MUS V + (D MU - D^2) TQP - D MUP TQ) X - (- D^2 - BETA D) MU - D^3

 - BETA D^2
(C70) EXPAND(EV(D66));

                                A X   B X
(D70)                           --- + --- + 2 D + BETA
                                Y2    Y1
(C71) EXPAND(EV(D67));

          2 A D X   A BETA X   2 B D X                2
(D71)     ------- + -------- + ------- + B W X + A M X + D  + BETA D
            Y2         Y2        Y1
(C72) EXPAND(EV(D68));

          2                        2
        A D  X   A BETA D X   B D  X
(D72)   ------ + ---------- + ------ + B D W X + A D M X + A BETA M X
          Y2         Y2        Y1
(C73) EXPAND(EV(D69));

      4 A B D X^2    A B BETA X^2    A B W X^2    A^2 M X^2    4 A D^2 X   4 A BETA D X
(D73) ---------- + ----------- + -------- + ------- + -------- + ------------
        Y1 Y2          Y1 Y2         Y2         Y2          Y2           Y2

        2          2    2     2          2    2    2          2        2
  A BETA  X   2 A  D X    A  BETA X    B  W X    A B M X    4 B D  X
+ -------- + --------- + ---------- + ------- + -------- + --------
     Y2            2           2         Y1         Y1         Y1
                  Y2          Y2

                      2    2
  3 B BETA D X   2 B  D X
+ ------------ + --------- + B D W X + B BETA W X + A D M X + 2 D^3 + 3 BETA D^2
       Y1              2
                      Y1

       2
+ BETA  D
```

Appendix C

SIMNON Simulation Program

```
CONTINUOUS SYSTEM gsef
STATE x s p
DER dx ds dp
dx=(mu-d)*x
ds=-((alpha/yps+1/yxs)*mu+omega/yps)*x+d*(sf-s)
dp=(alpha*mu+omega)*x-(d+beta)*p
mu=mum*s/(s+ks+s*s/ksi)*(1-p/pm)*(kp/(kp+p))
mum:0.3
ks:5
ki:4.5
pm:85
kp:4.5
yps:0.5
yxs:0.83
alpha:13
omega:0.05
ksi:8
beta:0.02
d:0.01
sf:125
x:2
p:0
s:0
end
```

Appendix D

ACSL Simulation Program

```
program    batch stripping system
initial
        constant mum=0.3, ks=5, ki=4.5,pm=85
        constant y1=0.04, m1=0.0, y2=0.08, m2=0.0, beta=0.0
        constant xic=0.1, sic=100, pic=0, totic=0.0
        constant cint=0.1, tmax=100
        l1..continue
end
dynamic
derivative
        x=integ(mu*x,xic)
        s=integ(-mu*x/y1-m1*x,sic)
        p=integ(mu*x/y2+m2*x-beta*p,pic)
        total=integ(mu*x/y2+m2*x,totic)
        mu=mum*s/(ks+s)*ki/(ki+P)*(1-p/pm)
end
        termt(t .ge. tmax .or. s .le. 0.1)
end
terminal
        call logd(.true.)
        beta=beta+0.04
        if(beta .le. 0.16) go to l1
end
end
```

VITA

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