SIMULTANEOUS FERMENTATION AND SEPARATION USING PERVAPORATION TECHNIQUE OF ACETONE-BUTANOL-ETHANOL FERMENTATION: A MATHEMATICAL APPROACH

A Thesis Submitted In Partial Fulfillment

Of the requirement for the degree of

Bachelor of Technology

Biotechnology

By

Amit Samal

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Department Of Biotechnology and Medical Engineering

National Institute of Technology, Rourkela

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Under guidance of

Prof. (Dr.) G.R. Satpathy

Department Of Biotechnology and Medical Engineering

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Rourkela

CERTIFICATE

This is to certify that the thesis entitled, **"Simultaneous Fermentation and separation using Pervaporation Technique of Acetone-Butanol-Ethanol Fermentation: A Mathematical Approach"** submitted by Amit Samal in partial fulfillment of the requirement for the award of bachelor of technology degree in Biotechnology Engineering at National Institute of Technology, Rourkela (Deemed University) is an authentic work carried out by him under my supervision and guidance. To the best of my knowledge the matter embodied in the thesis has not been submitted to any other University/Institute for award of any Degree/Diploma.

Date:

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Finally I would like to express my heartily thanks to my batch mates and others in the department for their help and support.

Amit Samal

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Abstract

Inhibition due to accumulation of products at higher concentration or toxic levels is considered one of the major problems in the simultaneous fermentation of acetone-ethanol and butanol. Butanol inhibits the process after a certain level of concentration. Various techniques are available for separation of butanol but pervaporation is most sought after as it is more effective and efficient. Biochemistry of ABE fermentation helps us understand the overall mechanism and pH dependency of the process. Here we consider the overall process of fermentation and pervaporative separation represented in form of simple mathematical model to explain its intricacies. The set of differential equation were solved using Berkeley Madonna 8.0 version differential equation solver. The inhibition constant for the process was determined and rp factor for including pervaporation effect into the model was taken into account. It was found inhibition constant has an important role in the overall process and also rp factor had pronounced effect on the concentration of products and substrate in the process. Minor modification in rp factor lead to large variation in the fermentation dynamics. After several trial runs it was concluded rp factor is dependent on the type of membrane considered for pervaporation. Apart from this the biomass concentration in both models reached similar level a fact supporting both the models.

Keywords: ABE fermentation, Pervaporation, Berkeley Madonna, Butanol.

Chapter 1

Introduction

1.1 Overview:

Fermentation is process of using microorganisms like bacteria and fungi to make various products which are useful for humans. Products prepared by the process of fermentation have found useful application in various industries ranging from pharmaceutical, food processing to production of chemicals and fuels. Production of chemicals and fuels are one such lucrative field especially when it's obtained from a renewable resource. But unfortunately in general products obtained from fermentation are present in very low concentration. Not only this conventional techniques used for separation are inefficient, energy consuming and commercially less viable. So emphasis has been laid on development of economically viable processes for product recovery from fermentation.

Acetone-Butanol-Ethanol or ABE fermentation is a process which uses microorganism to produce acetone, butanol and ethanol from a carbohydrate source like starch [9]. This method was used to produce acetone during the Second World War [9]. It is an anaerobic process. The maximum concentration of products in the process does not exceed 20g/l in the batch reactor and the weight ratio of the three products i.e. acetone, butanol and ethanol is in ratio of 3:6:1. Generally uses the clostridia class of bacteria for fermentation process. *Clostridium acetobutylicum* and C*lostridium beijerinckii* has been generally used for fermentation. Out of the 20g/l of pure solvent, butanol is about 8-13g/l in concentration and this causes increase in cost of recovery through various recovery processes. So the low concentration not only increases the cost of product separation ,at the same time large volume for downstream processing is required along with waste water treatment occurring from the overall system. Conventional methods like distillation are not suitable as butanol is less volatile than water. So as an alternative to

distillation other techniques have been developed. All these techniques focus on reducing the butanol inhibition and enhance the solvent productivity along with higher utilization of sugar so that cost of the process can be reduced.

Apart from this cost of the process can be reduced by implementing the following:

- Using butanol resistant strains of bacteria for the process.
- Optimizing and developing simultaneous production and recovery.

But recent development in the process has seen a shift from fermentative process to product recovery from petroleum based process due to cost consideration. So process development involving fermentation has received more importance and new breakthroughs have been seen in this field. Use of continuous flow reactors in the fermentation has led to problems as follows:

- Recovery of Product from dilute solutions
- Large volume of effluent from the reactors for disposal.

Recycling of the effluent is considered one method to actually minimize this and also increase the sugar utilization, thus increasing the product concentration. But associated problems of product inhibition have been seen in the overall scheme.

Various recovery process used in ABE fermentation are as follows:

- Adsorption
- Pervaporation
- Gas stripping
- Liquid-liquid extraction
- Reverse osmosis

- Membrane distillation
- Hybrid extraction
- Salt induced Phase separation
- Chemical Recovery

Pervaporation is seen as one of the most sought after techniques for separation [4]. Apart from that to describe the whole process of ABE fermentation several mathematical models [1] have been proposed which focuses on various aspects like product inhibition concentration, P^H inhibition [3]etc. Here we are considering few of the proposed models and introducing the pervaporation effect in it.

Chapter 2

ABE Fermentation

2.1 Overview of ABE fermentation

ABE is the acronym for Acetone-Butanol-Ethanol Fermentation generally used in industry to produce solvents like butanol etc. using microorganisms. Mostly the solvent producing strains of Clostridium species is used in the process. The process was made viable in around the late early 90's with introduction of new approach in the process. But the process dates back to the Second World War during 1939-1945 [9]. After the end of war American firms saw the industrial potential of ABE fermentation and hence Commercial Solvents Corporation of Maryland [9] was set up. But after the war production from fermentation saw a gradual decline due to introduction of more cheaply and economical methods like processing of the hydrocarbons, hence the contribution of fermentation in 1960 virtually ceased in United States and Great Britain [9]. Another reason for the decline in United States was the increase use of molasses as cattle feed. Generally the microorganisms used played a lot of significance in the overall process. *C.acetobutylicum* is generally used but other strains like *C. aurantibutyricum* is also used. In the latter case instead of acetone iso-propyl alcohol production occurs along with butanol. Recently isolated strain of *C. tetanomorphum* produces equal amount of butanol and ethanol, but no other product. So if we consider ABE fermentation its importance can be gauged from the fact that it produces three solvents i.e. acetone, ethanol and butanol. Apart from the solvents gases also evolve which include hydrogen and carbon dioxide as a part of the byproducts of the whole process.

2.2 Biochemistry and Physiology of ABE fermentation

As seen in a batch culture solvent producing strain of the microbe produces hydrogen, carbon dioxide, acetate and butyrate in the initial period of production or the initial growth phase. During this phase PH of the medium decreases with increase in acidic conditions. When the culture enters into the stationary phase the cells undergo change in their metabolism and solvent production takes place. Also the PH of the culture and solvent production acts as a major factor in the industrial production of the products involved as it affects the economy of the process. Hence the biochemistry of the overall process is considered very important, as it gives us various parameters which can be optimized so that production technology can be developed in a better way.

We can classify the biochemical compounds into three divisions which are a part of the ABE fermentation as follows [9]:

- Adenosine Phosphates: These are high energy yielding molecules and provide energy during various reactions.
- Nicotinamide Adenine Dinucleotide phosphate
- Nicotinamide adenine dinucleotide

Various Biochemical Pathways which are responsible for the whole process are as follows:

- Embden-Mererhof-Parnas pathway
- Pentose-Phosphate-Pathway
- Entner-Doudroff Pathway

The hexose sugar are generally metabolized using the EM pathway with overall conversion of 1 mole of hexose sugar into 2 mole of pyruvate with overall production of 2 mole of ATP and 2 mole of NADH (reduced). Apart from these clostridia strains of solvent producing type utilize the PPP way or the pentose phosphate pathway which converts pentose sugar into pentose-5-phospate, later converted into fructose-6-phospate and glyceraldehyde-3-phospate, thus finally yielding 5 mole of ATP and 5 mole of NADH.

The pyruvate produce during glycolysis is broken in the presence of pyruvate ferredoxin oxidoreductase and coenzyme A (CoA) to yield carbon dioxide, acetyl-CoA, and reduced ferredoxin. Acetyl-CoA produced by phosphoroclastic cleavage is considered the most important intermediate in the fermentation pathways involving both acid and solvent production.

2.2.1 Production of Butanol

During simple batch culture fermentation using glucose as substrate it has two stages of fermentation [9]. Categorization of done as

- Acidogenic phase: it is the initial phase where decrease in PH occurs due to increase in acid content.
- Solventogenic Phase: Occurs during the stationary phase marked by accumulation of butanol.

C.acetobutytcium has been observed to grow exponentially on sugar substrates at a pH of 5.6 or higher and produces butyrate, acetate, CO_2, H_2 as the important products. Electron balance is maintained in the overall process of fermentation. Thus 2.4 mole of Hydrogen production requires 1 mole of hexose sugar as said earlier accompanied by production of 0.4 mole acetate

and 0.8 mole butyrate. Bulk of 0.4 mole hydrogen results from the transfer of electron from NADH. As a result of increase in acid content the pH decreases between 4.0-4.5. With the onset of stationary phase in the life of the bacterial strain used net uptake of acetate and butyrate occurs from the fermentation broth and results in formation of butanol and ethanol. Sometimes iso-propyl alcohol is produced which depends on the type of strain used for the fermentation process.

The following change occurs when the phase changes from acidogenesis to solventogenesis:

- The alcohol production pathway requires 2 mole of extra NADH for each mole of alcohol generated.
- Decrease in ATP yield per mole of glucose used in fermentation with increase in accumulation of acetate and butyrate.
- Hydrogen is reduced to 1.4 mole per mole of glucose used.

Acetone production in many of the species of clostridia it has been seen no extra production of ATP. As the solvent phase goes on decrease in acid production is observed and acetone accumulation increases over the period of time.

2.2.2 Solvent Toxicity

During the whole batch process of fermentation it has been observed metabolism of the strain of microorganism occurs till the concentration of the solvent reaches 20g/l, thereafter the cell metabolism ceases or decreases. Out of the solvent produced butanol is the most toxic and in ABE fermentation it is the only one product produced till the inhibitory concentration. Solvent

production stops when the butanol concentration reaches 13g/l as seen in industrial processes. The inhibitory effect of butanol was observed to be enhanced in cells grown on xylose, and

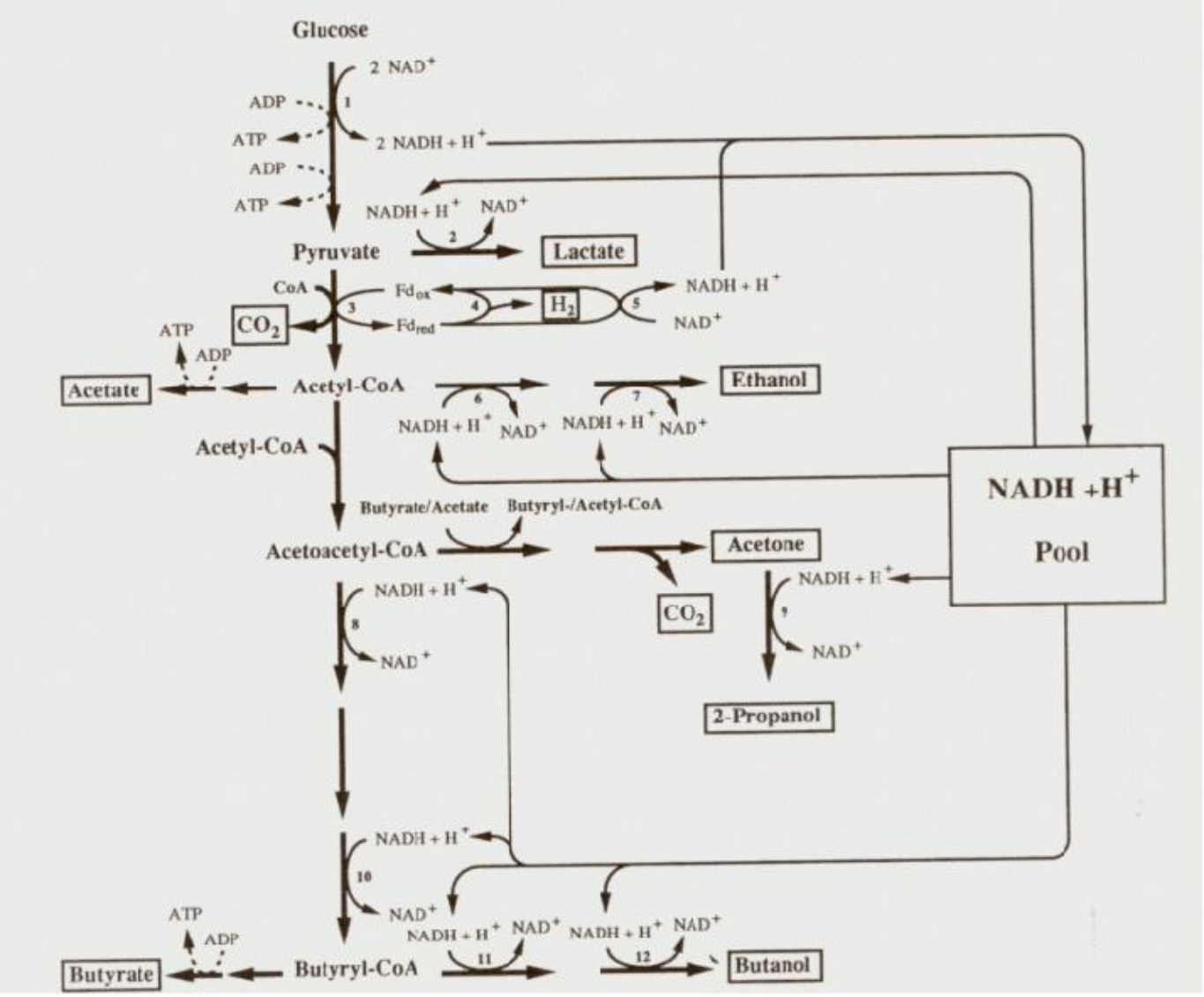

Fig 2.1 Butanol Production

growth was inhibited totally when 8 g of butanol per liter was added to the medium [8] .Presence of acetone and ethanol are also inhibitory in nature but at a significantly higher concentration

like 40g/l and hence inhibition due to acetone and ethanol is considered insignificant as compared to that of butanol.

2.2.3 Microorganisms used for ABE fermentation [9]

We can list down the following microorganisms which are used for ABE fermentation process.

- C.tetanomorphum
- C.puniceum
- C.auratinbutyricum
- C.acetobutylicum
- C.sachharolyticum
- C.thermohydrosulfuricum
- C.thermosachharolyticum
- C.beijerinckii
- C.sachharoperbutylacetonium
- C.thermocellum

2.2.4 Substrates used for Fermentation in Industrial Process [9]

Non cellulosic Substrates

Apart from maize, rye, wheat, millet other sources like potato, rice, jawari, bajra etc. has been also used as substrate to a varying degree of success in industry. Some of non-cellulosic substrates include:

- Jerusalem artichokes
- Cheese whey
- Algal biomass
- Apple pomace

Substrates derived from Lignocellulose

Some of the lignocellulose substrates are as follows:

- Pentose sugar
- Sulfite waste liquor
- Hydrolysates

2.3 Limitations of ABE batch Fermentation [9]

The batch ABE fermentation process suffers from a number of major shortcomings, few of them are explained below:

(i) The process relies on the utilization of renewable carbohydrate substrates such as maize and molasses. But the market economics of these raw materials increased, after the Second World War and was more expensive than the use of petrochemical methods.

(ii) Butanol is highly toxic to biological systems even at low concentrations, thus recovery of these low concentrations is very expensive by traditional method of recovery and extraction like distillation.

(iii) The whole process of fermentation has its own internal limitation like the whole concentration of solvent, thus the production ratio is considered undesirable.

(iv)Fermentation process is very complex and is susceptible to contamination by unwanted substances thereby reducing the overall process efficiency and output.

(v) Overall fermentation process produces large volumes of effluent and treatment of these effluents and safe discharge is a major concern for the plant operators and affects the overall economics of the process.

2.4 Result of study of ABE fermentation

The following are the important conclusion we draw from the study of ABE fermentation physiology and biochemical aspects:

- Understanding the product tolerance aspect during fermentation
- Substrate utilization aspect.
- Inhibition of the metabolic pathway and its manipulation for the increase in production of the products.

Chapter 3

Insight into Pervaporation

3.1 Introduction

Pervaporation can be defined as one of the methods of separation using the principles of permeation or selective separation through membranes [4]. The membranes can be porous as well as non-porous. It is one of the alternative methods for separation in different process including fermentation. Generally the following points make it more attractive method to adopt:

- Cost effective, economical
- Advantage in performance for separation of azeotropes.
- Heat sensitive products can be easily separated using this method.

Pervaporation technique can be used in following things like azeotropic separation, dehydration, waste water treatment, butanol separation from ABE fermentation etc. [4].

The membrane is selective and helps in distinguishing between two phases of the feed liquid and vapor phase. The targeted product is separated via vaporization method. Here the partial pressure governs or acts as the driving force for the feed to be separated into permeate and retentate. Theory behind pervaporation can be described as:

- Sorption
- Diffusion
- Desorption

Sorption and diffusion are regulated by the effect of temperature, pressure and nature of the functional group or the buildup of the membrane structure.

3.2 Characteristics of Pervaporation

Characteristics can be broadly described as following [4]:

- Energy consumption is low or minimal compared to other process.
- The product targeted or the permeate should be volatile in nature at standard operating environment.
- Pervaporation doesn't depend on liquid-vapor equilibrium.
- Contamination probability is less.

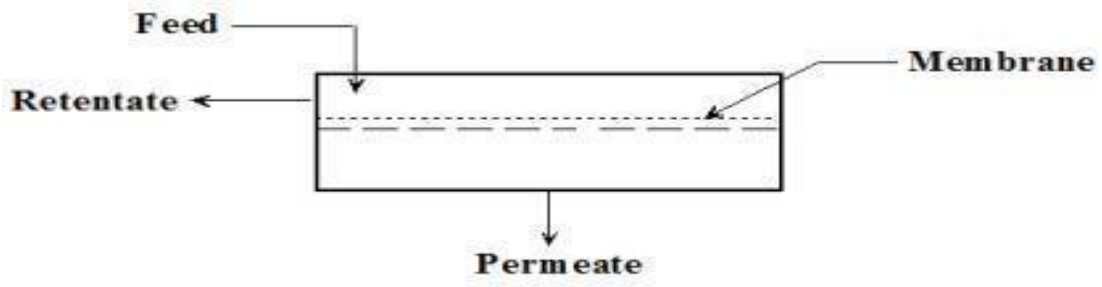

Fig 3.1 Schematic representation of pervaporation

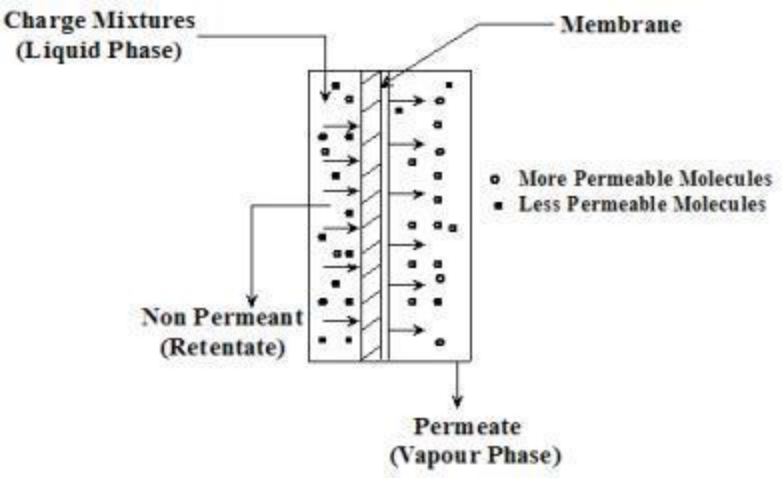

Fig 3.2 Schematic representation of liquid Pervaporation

3.3 Types of pervaporation [4]

Pervaporation can be classified as the following:

- Batch Pervaporation: This has great flexibility, but has to maintain a tank acting as buffer.
- Continuous pervaporation: Consumes very little energy during the whole process. Generally preferred in liquid-vapor separation.

3.4 Membranes used for Pervaporation

Classification of membranes [4] used in the process of pervaporation can be done on basis of separation property.

- Hydrophilic membranes: These membranes are used in removal of water from the organic solutions or the feed concerned. These membranes have glass transition temperature above room temperatures. Polyvinyl alcohol is one such example of hydrophilic membrane.
- Organophilic Membranes: These membranes are used to recover or extract organic compounds from the mixture. Generally made up of elastomeric materials. Glass transition temperature of these membranes is below room temperature. Styrene Butadiene rubber, nitrile butadiene rubber are few examples of this class of membranes.

Few examples of types of membrane used are as follows:

- Polydimethylsiloxane(PDMS) [10]
- Silicone membrane
- Silicate silicone membrane [7]
- Styrene butadiene rubber(SBR)
- Poly(ether block amide) (PEBA)
- Polytetraflouroethylene (PTFE)

Out of these most commonly used is PDMS as an organophilic membrane for separation in pervaporation technique. This polymer has a very large free volume and is selective to organic compounds permeation.

Rate of permeation varies from membrane to membrane and this would prove to be an important factor during the modeling as the reciprocating factor or coefficient of separation would be assumed to be membrane specific.

3.5 Industrial Application of Pervaporation

- Waste water treatment having higher levels of organic material [5].
- Developing pollution control application [5].
- Recovery of important compounds during process side streams.
- Separation of 99.5 % pure ethanol-water solutions [6].
- Harvesting of organic substances from fermentation broth.

Chapter 4

Model

4.1 Basic model without Pervaporation

The following model [1] describes the acetone-butanol fermentation reflecting the biochemical dynamics of the process and also the physiological aspects. Biosynthesis of solvents by *C.acetobutylicum* can be described by many steps. Glucose is utilized by the culture by glycolytic steps. Butanol as the final product is formed via production of butyrate. Apart from these important factors that control the production by strains of the microorganism used depend on the pH, inhibition by solvents like butanol, sporulation.

We know from fundamental of biochemical kinetics

$$\mu = Y_{X/S} Q g(S)$$

Where $Y_{X/S}$ is maximum macroscopic yield coefficient and g(S) function depends on environmental factors, generally taken as unity.

4.1.1 Assumption [1]

- Glucose acted as the limiting source for carbon as well as energy.
- Butanol is the major inhibitory product. It inhibits cell growth, sugar transport through membrane and synthesis of solvents and acids.
- Acetic and butyric acids are the intermediate products in the synthesis of the solvents.
- Concentration of acids is not high enough to cause a major inhibition of the cellular activities
- The solvents produced are neutral compounds and their transport across the cellular membrane takes place by diffusion.

Describing the model proposed by J.Votruba, B.Volesky and L.Yerushalmi is as follows:

$$\frac{dX}{dt} = d\ y-1\ X - K2XB \quad (1)$$

$$\frac{dy}{dt} = \quad K1S\ \ \frac{Ki}{Ki+B}\ \ - d\ y-1 \quad y \quad (2)$$

$$\frac{dS}{dt} = -k3SX - K4\ \ \frac{S}{Ks+S}\ \ X \quad (3)$$

$$\frac{dBA}{dt} = K5S\ \ \frac{Ki}{Ki+B}\ \ X - K6\ \ \frac{BA}{BA+Kba}\ \ X \quad (4)$$

$$\frac{dB}{dt} = K7SX - \frac{0.841dBA}{dt} \qquad (5)$$

$$\frac{dAA}{dt} = K8\ \ \frac{S}{S+Ks} \quad \frac{Ki}{Ki+B}\ \ X - K9\ \ \frac{AA}{AA+Kaa} \quad \frac{S}{S+Ks}\ \ X \quad (6)$$

$$\frac{dA}{dt} = K10\ \ \frac{S}{S+Ks}\ \ X - \frac{0.484dAA}{dt} \quad (7)$$

$$\frac{dE}{dt} = K11\ \ \frac{S}{S+Ks}\ \ X \quad (8)$$

$$\frac{dCO2}{dt} = K12\ \ \frac{S}{S+Ks}\ \ X \quad 9$$

$$\frac{dH2}{dt} = K13\ \ \frac{S}{S+Ks}\ \ X + K14SX \quad (10)$$

This basic ten equation describe the ABE fermentation in the simplest possible way. Here initial condition is taken as y(0)=1, which depicts that the inoculum is in its stationary phase. In ABE

fermentation, a linear relationship w.r.t substrate is taken along with the product inhibition according to Yerusalimski-Monod type.

4.2 Updated Model of the Batch Acetone-Butanol Fermentation

Previous models were unable to explain the inhibition by the accumulation of metabolites and thus were unable to simulate the acidogenic culture dynamics occurring due to lower pH level. This model proposed by A.K Srivastava and B.Volesky [3] takes into account an inhibition term and pH dependent terms to describe the biochemical kinetics.

4.2.1 Assumption [3]

- Glucose is the only limiting substrate in the batch process.
- There is no process limitation by the nitrogen source.
- The culture inhibition is by the accumulating metabolic products.
- Only undissociated forms of acetic and butyric acids gain reentry into the cell and get converted to acetone and butanol respectively. Dissociated form of acids accumulates in the fermentation broth as extracellular products.
- Ethanol, acetoin and lactic acid are synthesized from C-substrate only.
- The culture pH is known and controlled at a constant value throughout the modeled period. The model has to be reidentified to describe the culture dynamics under different pH conditions.

So the model equation can be described as given below:

$$\frac{dS}{dt} = \frac{-\frac{1}{Yx}\ dX}{dt} - \sum \frac{\frac{1}{Ypi}\ dPi}{dt} - keX \quad (11)$$

$$\frac{dPi}{dt} = \frac{midX}{dt} + niX \ (12)$$

Replacing equation 12 in equation 11 we get replacing dX/dt by μX yields the substrate consumption rate as expressed in the later equation below. We get by replacement

$$k1 = \frac{1}{Yx} + \sum \frac{mi}{YPi} \quad (13)$$

$$k2 = ke + \sum \frac{ni}{YPi} \quad (14)$$

Rates of undissociated acids can be described as follows

$$ACID_{undiss} = (\text{Total acid} * 10^{(pKa-pH)}) / (1+10^{(pKa-pH)})$$

For BA ,pKa =4.82 and AA .pKa=4.76. pH was maintained at 6.0.

$$P = AA + BA + B \quad 15$$

$$f(I) = (1-(P/Pmax)^n) \ (16)$$

$$\mu=\mu_{max}(S/S+Ks)(f(I)) \quad (17)$$

$$-rs = k1\mu X + k2X \ (18)$$

$$-rs' = k1'\mu X + k2'X \ (19)$$

$$rBA = k3rs - k4 \ \frac{BAundiss}{BAundiss+Kba} \ \frac{S}{S+Ks} \ X \ (20)$$

$$rB = k5rs + k4 \ \frac{BAundiss}{BAundiss + Kba} \ \frac{S}{S + Ks} \ X \ (21)$$

$$rAA = k6rs - k7 \ \frac{AAundiss}{AAundiss + Kaa} \ \frac{S}{S + Ks} \ X \ 22$$

$$rA = k8rs + k7 \ \frac{AAundiss}{AAundiss + Kaa} \ \frac{S}{S + Ks} \ X \ (23)$$

$$rE = k9rs \ (24)$$

$$rAT = k10rs \ (25)$$

$$rLA = k11rs \ (26)$$

This model describes the effect of pH on the culture kinetics. From the study of above two models we concluded that inhibition effect can be decreased by regulating the pH as well as concentration of butanol which acts as the product inhibition. So introduction of the pervaporation can be done along with the criterion to decrease the butanol concentration below the inhibitory level by selective removal by the pervaporative membrane.

Chapter 5

Solution to Model

5.1 Basic Model without Pervaporation

The given set of differential equation obtained was solved using the Berkeley Madonna 8.0 version solver. The conditions taken were chosen to get the best possible result and all parameters mentioned were in accordance to conditions or environment variables mentioned in papers.

The solution Code as mentioned in Berkeley Madonna solver is as follows:

METHOD RK4

STARTTIME = 0

STOPTIME=80

DT = 0.02

K1=0.009

K2=0.0008

K3=0.0255

K4=0.6764

K5=0.0135

K6=0.1170

K7=0.0113

K8=0.7150

K9=0.1350

K10=0.1558

K11=0.0258

K12=0.6139

K13=0.0185

K14=0.00013

Ki= 0.11

Ks=2.0

Kba=0.5

Kaa=0.5

d=0.56

init y = 1

init x= 0.15

init s=100

init ba=0

init aa=0

init b=0

init a=0

init e=0

init c=0

init h=0

x' = (d*(y-1)*x)-(K2*x*b)

y' = ((K1*s*(Ki/(Ki+b))-(d*(y-1))))*y

s' = (-K3*s*x)-(K4*(s/(Ks+s)))

ba' = ((K5*s*Ki*x)/(Ki+b))-((K6*ba*X)/(ba+Kba))

b' = K7*s*x - (0.841*ba')

aa' = ((K8*s*Ki*x)/((s+Ks)*(Ki+b)))-((K9*aa*s*x)/((aa+Ks)*(s+Ks)))

a' = (K10*s*x)/(s+Ks)-(0.484*aa')

e' = (K11*s*x)/(Ks+s)

c' = (K12*s*x)/(s+Ks)

h' = ((K13*s*x)/(s+Ks))+ (K14*s*x)

The above code made the simulation for the fermentation process by plotting the concentrations of biomass, butanol and substrate with time.

5.2 pH dependent Model

Thought the study of pH dependent model was only to understand the significance of the pH regulation we solved it using the solver, this gave an insight into the product inhibition due to change in environmental parameters.

METHOD RK4

STARTTIME = 0

STOPTIME=80

DT = 0.02

P = aa + ba + b

f = (1-((P)/(Pmax))^n)

u = (umax)*(f)*((s)/(s+Ks))

s' = -K1*u*x- K2*x

s1' = -K11*u*x-K12*x

ba' = K3*s'- K4*((baund)/(baund+Kba))*((s)/(s+Ks))*x

b' = K5*s' + K4*((baund)/(baund+Kba))*((s)/(s+Ks))*x

aa' = K6*s' - K7*((aaund)/(aaund+Kaa))*((s)/(s+Ks))*x

a'= K8*s' + K7*((aaund)/(aaund+Kaa))*((s)/(s+Ks))*x

e'= K9*s'

at' = K10*s'

la'=K13*s'

baund = ((ba)*(10)^(4.82-6.0))/(1+(10)^(4.82-6.0))

aaund = ((aa)*(10)^(4.76-6.0))/(1+(10)^(4.76-6.0))

init x=1.0

init s=55

init a=0

init aa=0

init ba=0

init b=0

init e=0

init at=0

init la=0

init s1=0

Pmax = 17.1

n = 3.75

umax = 0.4809

K1=19.41

K2=0.000022

K11=2.95

K12=0.71

K3=0.688

K4=1.618

K5=0.0173

K6=0.179

K7=0.078

K8=0.0016

K9=0.0145

K10=0.0317

K13=0.036

Ks=10.69

Kba=5.18

Kaa=1.358

x'= 0.56*(y-1)*x-0.0008*x*b

y'=(0.09*s*((0.11)/(0.11+b))-0.56*(y-1))*y

init y=1

This model was a trial run just to infer on the already derived explanation as mentioned in the papers regarding the updated model of Acetone-Butanol fermentation kinetics.

5.3 Basic Model with Pervaporation

Here we consider the basic model by introducing the pervaporation parameter (rp). It must be taken into account the rp [10] can be defined in accordance to the membrane characteristics. Later on we have taken another function in describing the rp factor.

METHOD RK4

STARTTIME = 0

STOPTIME=80

DT = 0.02

K1=0.009

K2=0.0008

K3=0.0255

K4=0.6764

K5=0.0135

K6=0.1170

K7=0.0113

K8=0.7150

K9=0.1350

K10=0.1558

K11=0.0258

K12=0.6139

K13=0.0185

K14=0.00013

Ki= 0.08

```
rp = 0.004*b^3.5411*0.0628*0.75

Ks=2.0

Kba=0.5

Kaa=0.5

d=0.56

v=1.5

init y = 1

init x= 0.15

init s=100

init ba=0

init aa=0

init b=0

init a=0

init e=0

init c=0

init h=0

x' = (d*(y-1)*x)-(K2*x*b)

y' = ((K1*s*(Ki/(Ki+b))-(d*(y-1))))*y

s' = (-K3*s*x)-(K4*(s/(Ks+s)))

ba' = ((K5*s*Ki*x)/(Ki+b))-((K6*ba*X)/(ba+Kba))

b' = K7*s*x - (0.841*ba')-((rp)/v)

aa' = ((K8*s*Ki*x)/((s+Ks)*(Ki+b)))-((K9*aa*s*x)/((aa+Ks)*(s+Ks)))

a' = (K10*s*x)/(s+Ks)-(0.484*aa')-((rp)/v)
```

e' = (K11*s*x)/(Ks+s)

c' = (K12*s*x)/(s+Ks)

h' = ((K13*s*x)/(s+Ks))+ (K14*s*x)

5.4 Model considering modified rp factor

The rp factor [10] taken above is simple one, here we have tried to incorporate and exponential function in order to describe the pervaporation effect in the overall process.

METHOD RK4

STARTTIME = 0

STOPTIME= 80

DT = 0.02

K1=0.0090

K2=0.0080

K3=0.0255

K4=0.6764

K5=0.0136

K6=0.1170

K7=0.0113

K8=0.7150

K9=0.1350

K10=0.1558

K11=0.0258

Ki=0.8333

Ks=2.0

Kba=0.5

Kaa=0.5

d=0.56

z=0.063

Tm=80

x'= d*(y-1)-K2*b

y'= ((K1*S*Ki)/(Ki+b)-d*(y-1))*y

s'=-K3*s*x-(K4*s*x)/(Ks+s)

ba'= (K5*s*Ki*x)/(Ki+b)- (K6*ba*x)/(Kba+ba)

b'= K7*s*x - 0.841*ba' - (rp*Bp)/V - (b*rp)/V

aa' = (K8*s*Ki*x)/((Ks+s)*(Ki+b))- (K9*aa*s*x)/((Kaa+aa)*(Ks+s))

a' = (K10*s*x)/(Ks+s)- 0.484*aa'-(rp*a)/V

e' = (k11*s*x)/(Ks+s)

rp = (0.680*z*(1000-b+200*b*e^(-b/10))*(1-(time)/Tm))/(2*10^5*e^(-b/10))

Bp = (2*10^5*b*e^(b/10))/(1000-b+2000*b*e^(-b/10))

init x=0.15

init y=1, init a=0

init s=100

init ba=0

init b=0

init aa=0

init e=0

V=1

Chapter 6

Result and Discussion

6.1 Result and Discussion

The codes as mentioned previously were run for the different values of substrate; biomass and RNA concentration and results were analyzed. The general trend of curves was obtained from is as follows:

6.1.1 Basic model without pervaporation

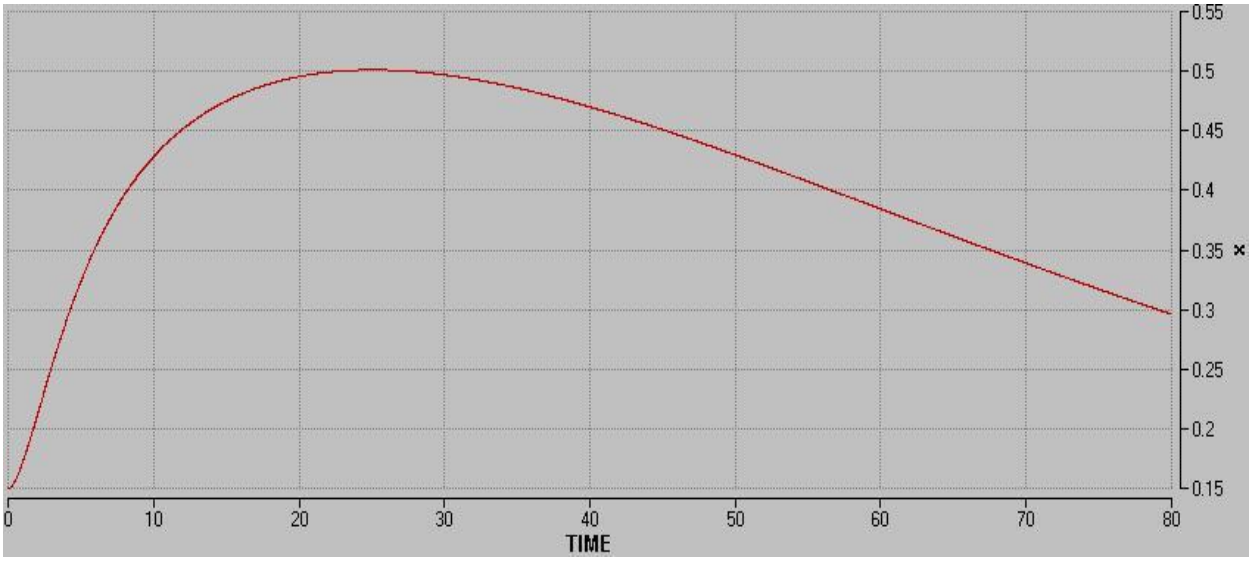

Fig 6.1 biomass variation with time

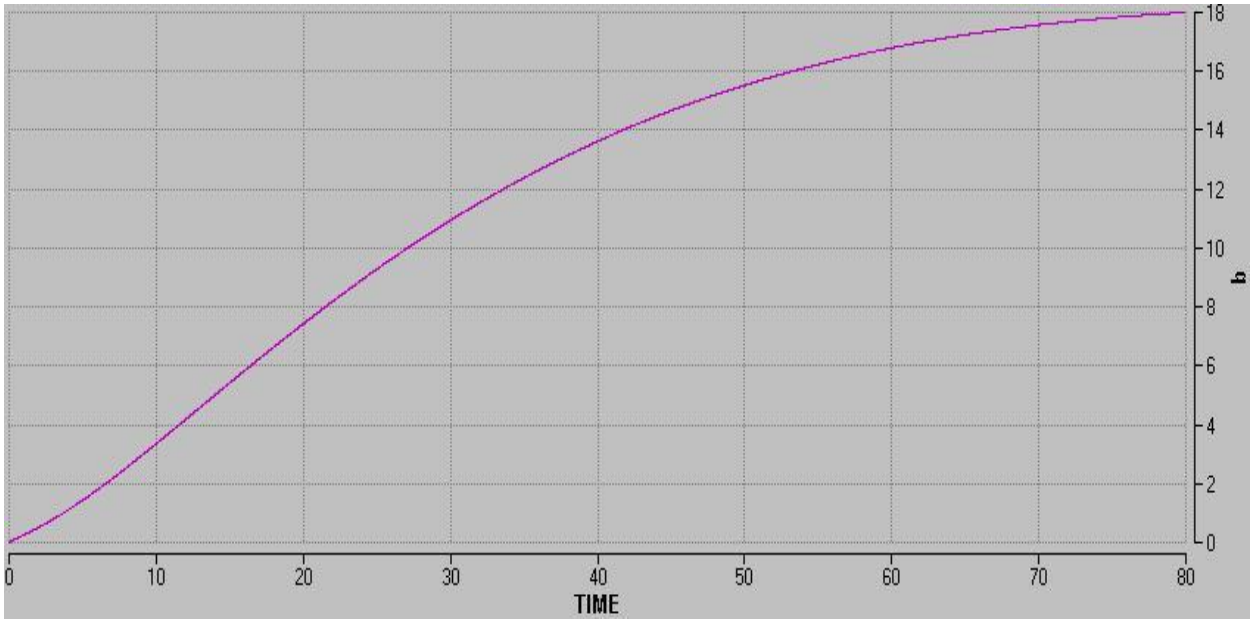

Fig 6.2 Butanol variation with time

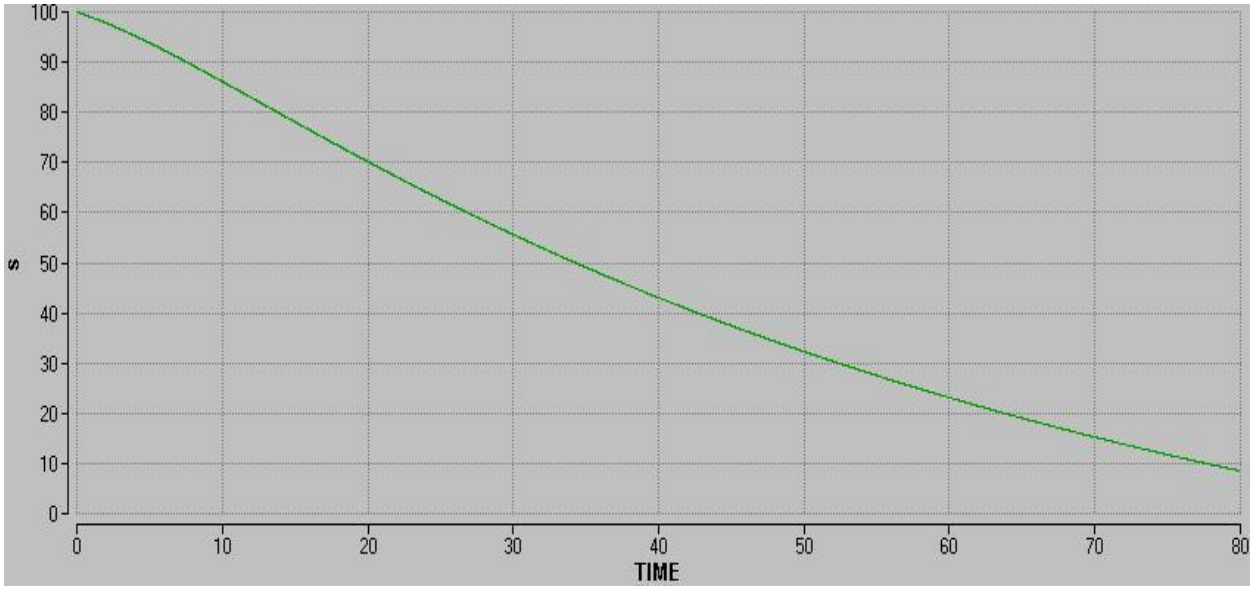

Fig 6.3 Substrate variation with time

6.1.2 Basic Model with Pervaporation

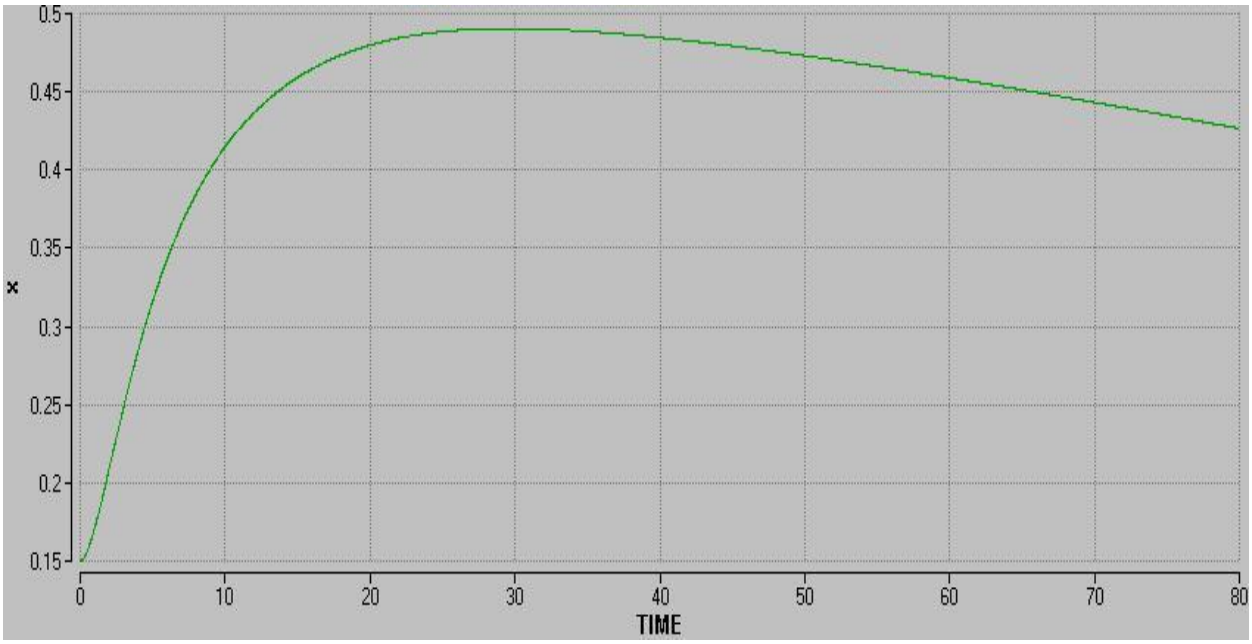

Fig 6.4 Biomass variation with time

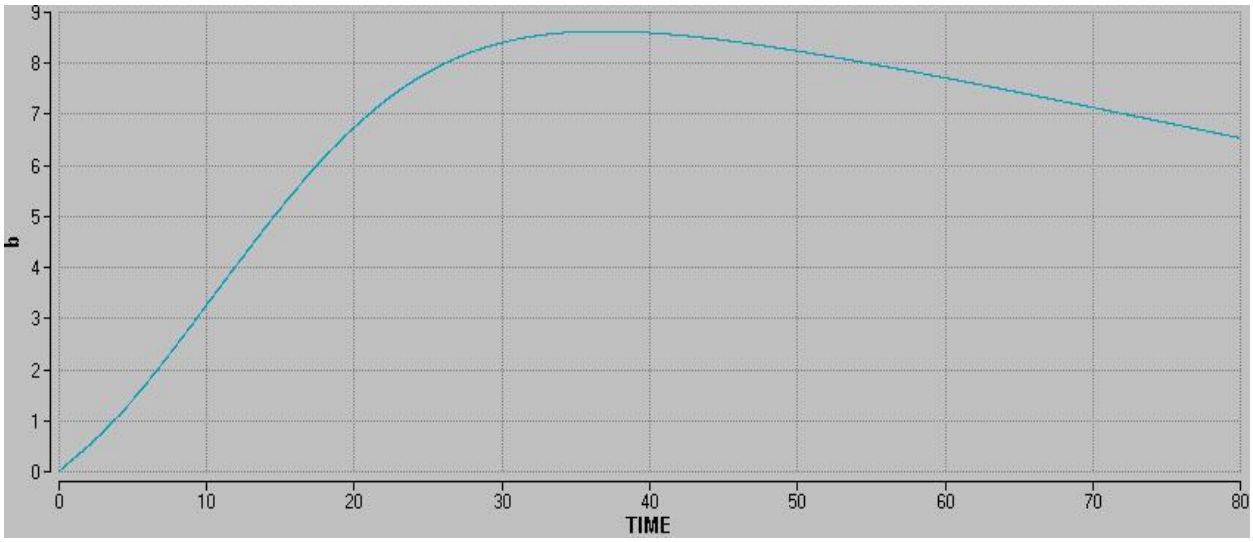

Fig 6.5 Butanol variation with time

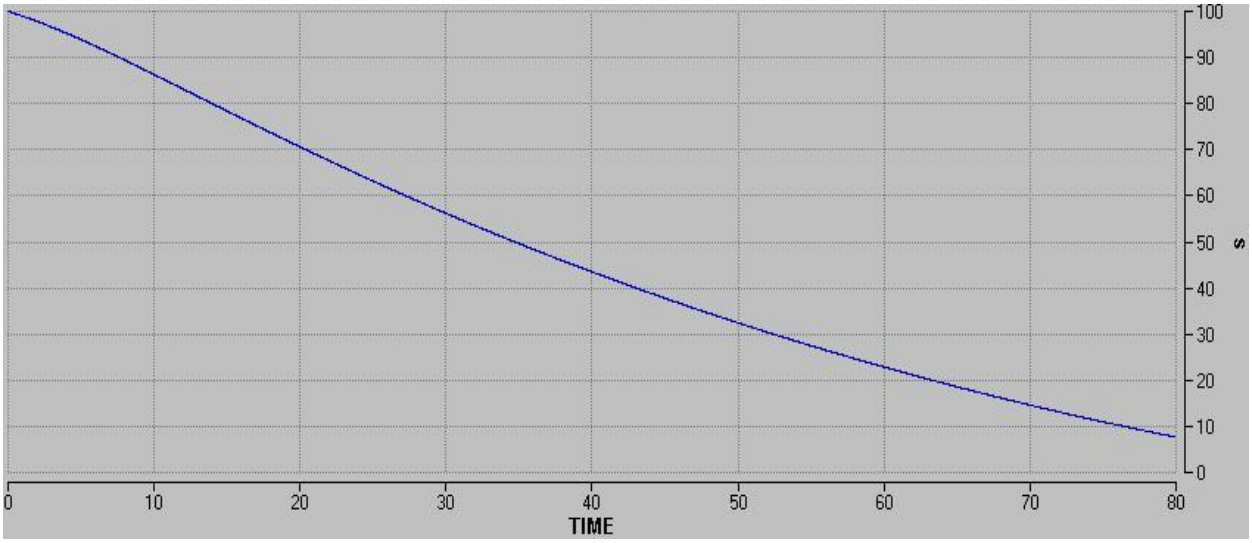

Fig 6.6 Substrate variation with time

In this trend we have taken a simple linear function to describe rp factor and this overall trend in fermentation variation. We observe that after introduction of rp factor the the butanol concentration was decreased which indicates the toxicity level decreases hence and desired explanation to check product inhibition.

6.1.3 pH dependent Model

From pH dependent model result we get an illustrative comparison of the model-predicted culture dynamics and experimental data for an acidogenic culture at pH of 6.0 where other models fail to explain.

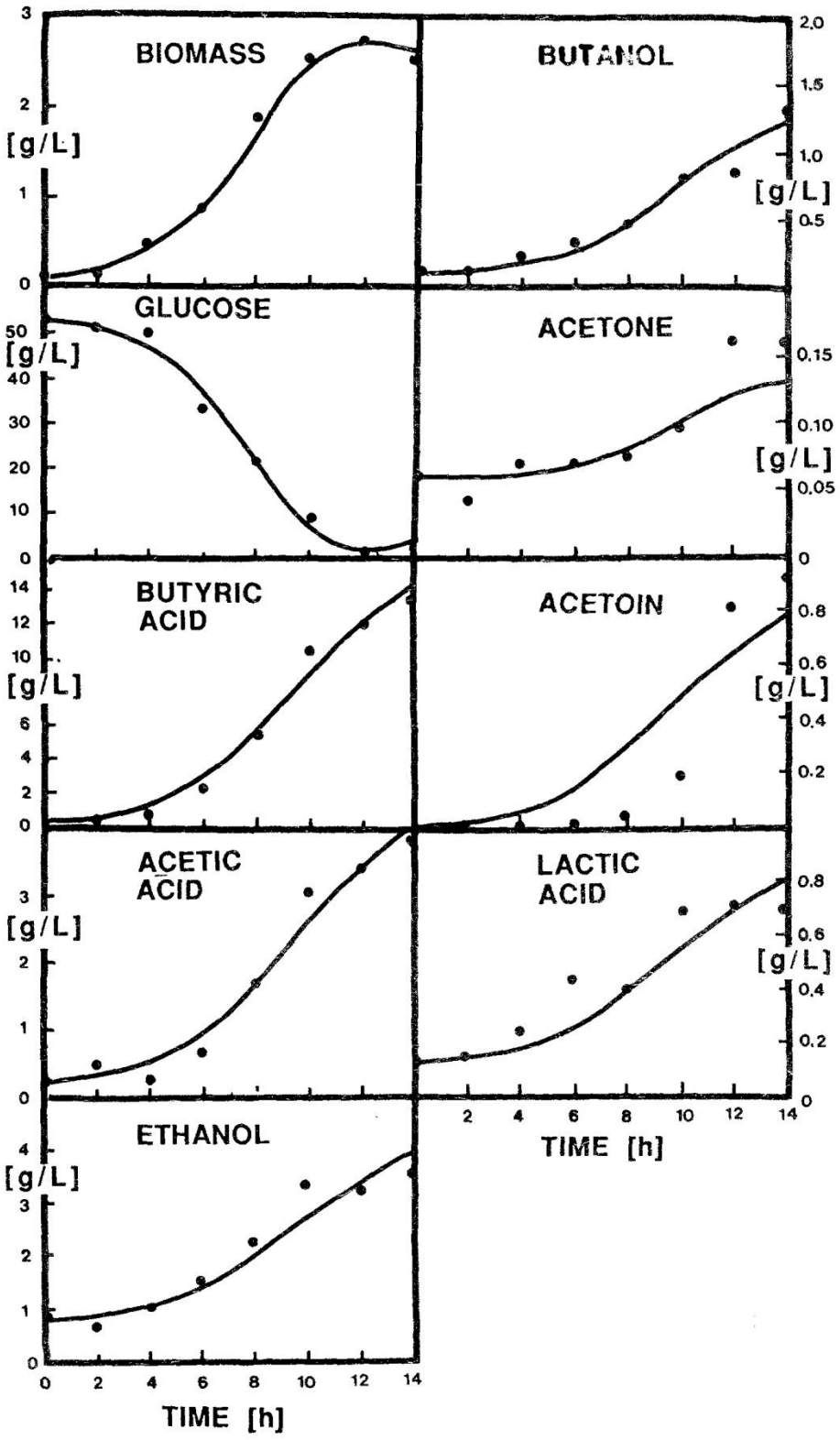

Fig 6.7 Comparison of modeled culture dynamics with experimental data points [3].

6.1.4 Model Considering modified rp factor

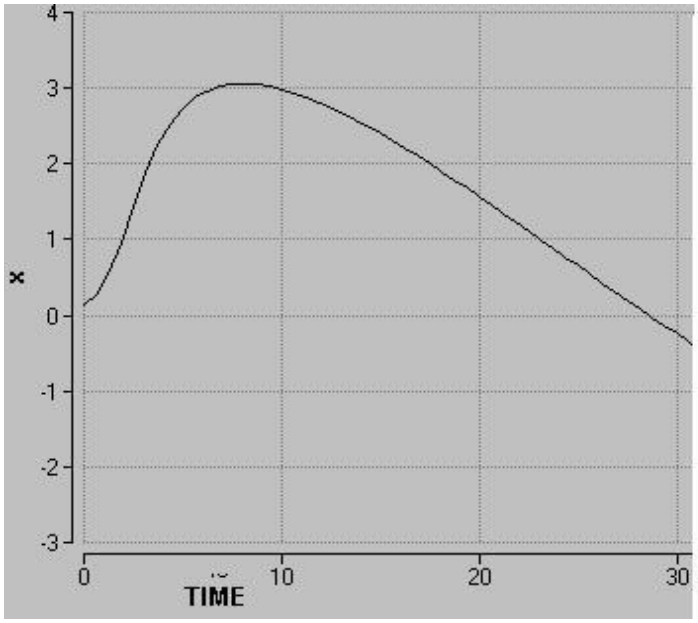

Fig 6.8 Biomass variation with time

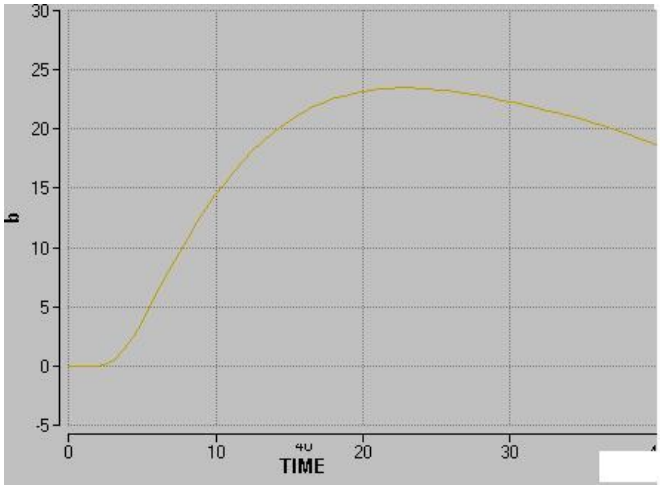

Fig 6.9 Butanol Variation with time

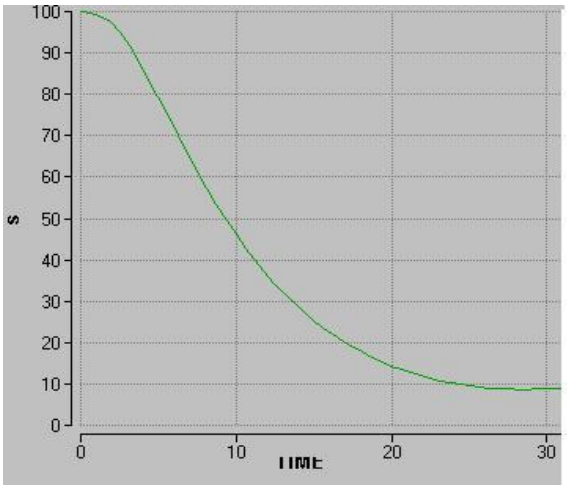

Fig 6.10 Substrate variation with time

6.1.5 Result from Experimental Data

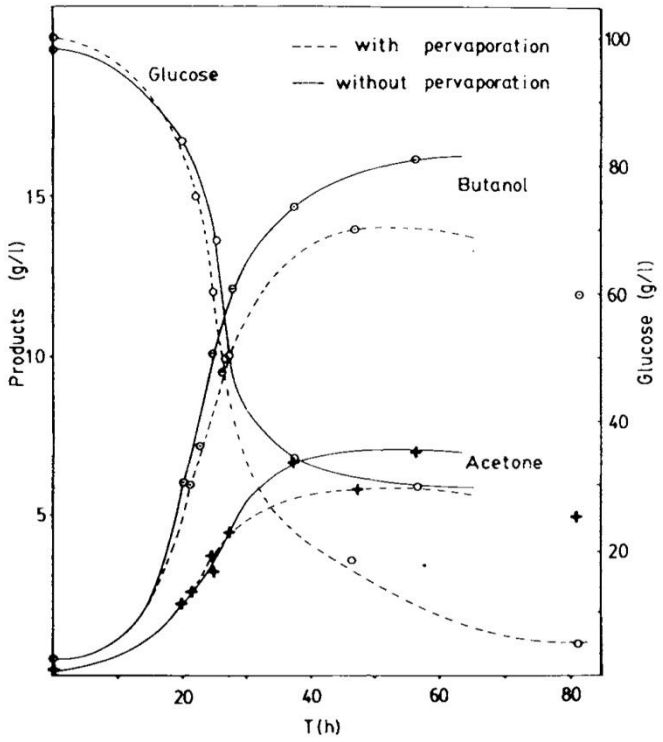

Fig 6.11 Comparison of Kinetics. The initial Glucose concentration was 100 g/l [2].

It has been observed from the work of Larrayoz and Puigjaner [2] the following experimental results were obtained from the fermentation process with and without pervaporation [2]. With the above simulation to approximate the experimental model we varied the inhibition constant in the second model to obtain an approximation. While the change in rp factor into an exponential function in the other model prediction though it was able to simulate the process but not to the desired level indicating it required more optimization, but it variation trend was with expectation. So varying the inhibition constant we found an optimum value by trial and error and curve fit as Ki = 0.035.

Chapter 7

Conclusion

7.1 Conclusion

The following conclusion can be drawn from the project:

- pH has a profound effect the culture kinetics.
- Inhibition constant has profound effect on the culture dynamics like biomass production and substrate consumption rate, and this has obvious relation with pH as well as inhibition due to butanol toxicity.
- Pervaporation decreases product inhibition due to decrease in butanol level and better separation of products.
- No change in production of ethanol, acetone, carbon-dioxide and hydrogen in both the basic cases with some changes in production level of the solvents were seen.
- The rp factor in case 1 was considered from paper but it factors like membrane scaling was not taken into account, but the next rp factor based on exponential function(case 2) was more closer to explain the process in a general approach. But the second rp factor needs further optimization and correction.

Nomenclature

Symbol	Meaning	Units
X	Biomass concentration	g/l
y	Marker of the physiological state of the culture	Dimensionless
S	Substrate concentration	g/l
B	Butanol concentration	g/l
AA	Acetic acid concentration	g/l
BA	Butyric acid concentration	g/l
A	Acetone concentration	g/l
E	Ethanol concentration	g/l
Ki	Inhibition constant	g butanol/l
AAundiss	Concentration of undissociated acetic acid	g/l
BAundiss	Concentration of undissociated butyric acid	g/l
Y_X	Yield of biomass w.r.t substrate	g.biomass/g.subs
Y_{Pi}	Yield of product w.r.t substrate	dimensionless
k1	Constant in eqn. 13	g.subs/g.biomass
k2	Constant in eqn. 14	g.subs/g.biomass
f(I)	Inhibiton function	Dimensionless
μ	Specific growth rate	h^{-1}
Kaa	Acetic acid saturation constant	g/l
Kba	Butyric acid saturation constant	g/l
Ks	Monod saturation constant	g/l
n	Empirical constant	Dimensionless
pKa	Equilibrium constant	Dimensionless
AT	Acetoin concentration	g/l
k1’	Constant in eq. 19	g.subs/g.biomass
k2’	Constant in eqn. 19	g.subs/g.biomass.h

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