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SOLVENT PRODUCTIVITY IMPROVEMENT IN ACETONE-BUTANOL FERMENTATION BY TWO-STAGE CONTINUOUS SYSTEM

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พิมพ์ต้นฉบับบทคัดย่อวิทยานิพนธ์ภายในกรอบสีเขียวนี้เพียงแผ่นเดียว

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วิทยานิพนธ์นี้ศึกษาการประยุกต์ใช้ระบบต่อเนื่องแบบสองขั้นตอน ในการเพิ่มอัตราผลผลิตตัวทำละลายในกระบวนการหมักแอซีโตน-บิวทานอล ของเชลล์จุลินทรีย์ Clostridium acetobutylicum ATCC 824 โดยใช้สารละลายน้ำตาลกลูโคสที่ความเข้มข้น 50 กรัม/ลิตร ค่าความเป็นกรด-เบส (pH) 4.8 และอุณหภูมิในการหมัก 35°ซ. จากการศึกษาพบว่าที่อัตราการป้อนสารอาหารต่อปริมาตรถังหมักขั้นตอนที่หนึ่งและ สอง (dilution rates) เท่ากับ 0.17 และ 0.057 ต่อชั่วโมงตามลำดับ เป็นอัตราที่เหมาะสมในการหมักที่มีอัตราผลผลิตตัวทำละลายสูงสุดที่ 0.63 กรัม/ลิตร-ชั่วโมง คิดเป็น 42% ของระบบต่อเนื่องแบบขั้นตอนเดียว ในขณะที่ความเข้มข้นของตัวทำละลายเป็น 1.7 เท่า ทำให้คุ้มค่าในการกลั่นแยกผลิตภัณฑ์มากกว่า เมื่อประยุกต์ใช้ระบบการกรองเซลล์จุลินทรีย์แบบไมโครฟิลเตรชัน ประกอบกับถังหมักขั้นตอนที่สอง พบว่าได้อัตราผลผลิตตัวทำละลายเป็น 3.7 เท่าของระบบต่อเนื่องแบบขั้นตอนเดียว และ เป็น 8.9 เท่าของระบบต่อเนื่องแบบสองขั้นตอนที่ไม่มีการกรองเซลล์จุลินทรีย์ ที่อัตราการป้อนสารอาหารต่อปริมาตรถังหมักขั้นตอนที่หนึ่ง และ สองเท่ากับ 0.17 และ 0.55 ต่อชั่วโมง ตามลำดับ ในขณะที่ความเข้มข้นของตัวทำละลายใกล้เคียงกับระบบต่อเนื่องแบบสองขั้นตอนที่ไม่มีการกรองจุลินทรีย์ ดังนั้นระบบต่อเนื่องแบบสองขั้นตอนที่ประกอบกับระบบการกรองเซลล์จุลินทรีย์แบบไมโครฟิลเตรชัน จึงเป็นระบบที่เหมาะสมในการเพิ่มอัตราผลผลิตตัวทำละลาย

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Two-stage continuous system was applied to improve solvent productivity in acetone-butanol fermentation of Clostridium acetobutylicum ATCC 824. In this system, the medium concentration, pH, and temperature were kept constant at 50 g/l glucose, 4.8, and 35°C, respectively. The optimum dilution rates of 0.17 hr^{-1} in the first stage and 0.057 hr^{-1} in the second stage were obtained from the experiment. The maximum solvent productivity was 0.63 g/l-hr, which was 42% of that obtained from the single-stage continuous system, while the solvent concentration was increased by 1.7 times, which was more suitable for separation. By coupling this system with microfiltration in the second stage, the solvent productivity was increased to 3.7 times that of the single-stage continuous system, or 8.9 times that of the two-stage continuous system without microfiltration. The optimum dilution rates for the first stage and the second stage were 0.17 hr^{-1} and 0.55 hr^{-1}, respectively, and the solvent concentration was close to that of the two-stage continuous system without microfiltration. Then the most suitable system, which was applied to improve solvent productivity, was the two-stage system coupling with microfiltation.

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CHAPTER I

INTRODUCTION

Butanol has been produced by both fermentation processes and petrochemical processes. With the advent of petrochemical processes and low cost petrochemical feedstocks, the acetone-butanol fermentation process became economically unattractive. However, in the future, the increased cost of petrochemical feedstocks and the desire to produce a methanol cosolvent for gasoline due to the energy shortage crisis have stimulated the investigation for the improvement of acetone-butanol fermentation.

Butanol is used not only as a reactant and a solvent in many industries but also as a fuel in mixture with either petrol or diesel. It also has a potential use as a cosolvent in Methanol/Petrol mixture (1,2) to prevent phase separation at low temperature. The advantages of butanol over methanol as a liquid fuel extender are its low vapor pressure, its low miscibility with water and its complete miscibility with diesel even at low temperature (Table 1.1).

The old fermentation processes were batch processes with low productivity and solvent concentration because of an end product inhibitory effect and a long fermentation time. However, the solvent productivity can be increased by using a single-stage continuous culture; but its disadvantages are that the dilution rate limits the specific growth rate and that butanol still inhibit solvent productivity for it has not yet been separated from the broth.

There are two techniques used to overcome these disadvantages. One is to use a cell recycling in order to increase cell concentration in the fermenter. Therefore, the dilution rate does not limit the specific growth rate. Another is to use a two-stage system in order to separate acidogenic and solventogenic phases from each other to prevent butanol inhibitory effect. Since only cells in the second stage are exposed to the high solvent concentration, the

two-stage process can be expected to increase the longevity of the continuous process at high solvent productivity as a result of the continuous feeding of fresh cells to the second stage.

The objective of this work :

The principal aim of the study was to determine the optimum condition for producing solvent in acetone-butanol fermentation by using the two-stage continuous system.

The scope of this work :

1. Setting up the experimental apparatus.
2. Determining the optimum operating condition of the two-stage continuous system.
3. Comparing the solvent productivity of the two-stage continuous to those of the single-stage continuous and the batch systems.
4. Comparing the solvent productivity of the two-stage system with cell recycling to that of the two-stage without cell recycling at the same condition.
5. Comparing the solvent productivity of the two-stage continuous system to those of other systems.

TABLE 1.1 Characteristics of chemically pure fuels.*

Fuel	Molecular weigth	Specific gravity	Boiling point (C)	Vapor pressure at 37.7 C (psi)	Combustion energy (kJ/kg)	Latent heat (kJ/kg)	Solubility (parts in 100 parts water)	Stoichiometic air fuel ratio
Methanol	32	0.79	65	4.60	23864.80	1170.00	infinite	6.5
Ethanol	46	0.79	78	2.20	30610.60	921.10	infinite	9
Butanol	74	0.81	117	0.30	36681.00	432.60	9	11.2
Octane	114	0.70	210	1.72	48264.50	360.50	insoluble	15.2
Hexadecane	240	0.79	287	3.46	47264.30	-	insoluble	15

* Data from Comprehensive Biotechnol, 3 (1985) : 915-931.

CHAPTER II

LITERATURE REVIEW

2.1 Acetone- butanol Fermentation

2.1.1 The Fermentation Process

In an industrial acetone-butanol fermentation, butanol, acetone and ethanol are produced by selective bacterial fermentation (Table 2.1) of carbohydrate-containing materials (Table 2.2, 2.3). It is necessary to give the raw material some kind of preliminary treatment. When corn is used as the source of carbohydrate, the germ (24 % oil) is removed and sold as coproduct, and karnels are ground to a coarse meal. The ground meal is diluted with water to a sugar concentration of 6 to 8 percent approximately, sterilised, cooled to 30°C and pumped to a fermenter where it is inoculated with a culture of bacteria (Clostridium acetobutylicum) and allowed to ferment for 48 to 72 hours at 37°C (Table 2.4). Protein nutrients and an alkaline buffer to control the pH are also added to improve yields. It is essential to sterilise all the fermenters, pipes, pipe connections and equipments where grain or butanol organisms may come into contact, since contamination in this fermentation is usually a very serious matter that may cause considerable losses.

After fermentation is completed, the completely fermented medium is generally termed "beer". The beer may contain 1.7 to 2.4% mixed solvent, depending on the initial carbohydrate concentration and on temperature and culture used. The ratio of acetone, butanol and ethanol will also be varied. The beer may be temporarily stored in a large holding vessel prior to distillation. The beer is first run through a beer still which strips off the solvent. When a 50% solvent mixture is stripped overhead, distiller slop is removed as bottoms. This slop is dried and sold as a high protein meal coproduct. It is a source of riboflavin and other components of the vitamin B complex to be used as animal feed. Another by-product is a mixture of carbondioxide and hydrogen evolved during the fermentation.

The mixed-solvent vapor from the beer column are then separated by a batch fractionating column from which three fractions (acetone, ethanol and butanol) are removed overhead, leaving water as bottoms. The acetone and ethanol fraction, containing about 15% water , is led to a column from which vapors containing 70% butanol and 30% water are removed overhead. On condensation, two layers are formed. The top layer (80% butanol and 20% water) is returned to the butanol column , and the bottom layer (4% butanol and 90% water) is returned to the beer column.

The yield of mixed solvents is approximately 30% by weight base on the sugar content. Solvent yield ratio are 60-65% butanol, 30-35% acetone and 5-10% ethanol by weight. Figure 2.1 and 2.2 are the flow diagram of acetone-butanol fermentation. The biochemical pathway and scheme for the butylalcohol fermentation are shown in Figure 2.3 and 2.4, respectively.

TABLE 2.1 Microorganisms which can produce butanol by fermentation.*

U.S. patent no.	Name of bacteria	Substrate	Solvent ratios (%)			
			Butanol	Ethanol	Acetone	Isopropanol
1,725,083	Bacillus saccharobutyllicum-beta	Inverted molasses and $CaCO_3$	75	-	3	35
1,908,361	Cl. saccharobutylicum-gamma	Blackstrap molasses and $CaCO_3$	65-80	-	18-34	1-2
1,922,921	Cl. sacharobutyl-acetonicum	Blackstrap molasses, corn gluten and $(NH_4)2\ SO_4$	64	-	36	-
2,017,572	Cl. viscifaciens	Inverted molasses and $CaCO_3$	66	-	3	31
2,050,219	Cl. saccharoacetobutylicum-beta and gamma	Cane molasses and degraded protien such as ammonia, steep water or distillery slop	68-73	1-3	26-32	-
2,063,448	Cl. propyl butylicum	Inverted molasses, NH_3 and $CaCO_3$	69-70	-	4-17	14-28 (mixture isopropyl and ethyl)
2,073,125	Cl. invertoacetobutylicum	Louisianna molasses (inverted) and ammonium salt or alkalies	66-70	2-3	27-31	-
2,089,522	Cl. saccharoacetobutylicum	Louisianna molasses $(NH_4)2SO_4$ and $CaCO_3$	68-73	1-3	26-32	-
2,132,039	Cl. propyl butylicum-alpha	Inverted molasses, $(NH_4)2SO_4$, $CaCO_3$, K_2HPO_4 and $MgSO_4$	65-70	-	5-10	16-26
2,139,108	Cl.saccharobutyl-acetonicum-liquefaciens-gamma and delta	Blackstrap molasses, $(NH_4)2SO_4$, $CaCO_3$ and P_2O_5	58-74	2-6	24-36	-
2,139,111	Cl.saccharobutyl-acetonicum-liquefaciens-gamma and delta	Cuban molasses, $(NH_4)2SO_4$, $CaCO_3$ and P_2O_5	60-69	3-4.5	26-35	-
2,147,487	B. butacone	Blackstrap molasses and animal and vegetable protein	65	-	28	-
2,169,246	Cl. celerifactor	Inverted molasses, ammonia and $CaCO_3$	60	2	38	-
2,195,629	Cl. granulobacter acetobutylicum	Molasses, corn gluten, ammonium salt and $CaCo_3$	60-75	1-10	25-30	-
2,219,426	Cl. saccharobutyl-isopropyl-acetonicum-beta	Cane and beet molasses,$(NH_4)2SO_4$ and $CaCO_3$	60-85	-	15-40	0.1-4
2,308,837	Cl. esdisonii	Cuban blackstrap,$(NH_4)2SO_4$, $CaCO_3$ and NH_4OH	75-76	4-6	17-20	-
2,420,998	Cl. amylosaccharobutyl-propylicum	Inverted molasses, $(NH_4)2SO_4$, $CaCO_3$ and P_2O_5 or NH_4OH and P_2O_5	65-72	Trace	2-4	26-32
2,430,791	Cl. saccharoacetoperbutylicum	Inverted molasses, $(NH_4)2SO_4$, $CaCO_3$ and P_2O_5 or NH_4OH and P_2O_5	60-76	2-7	18-25	-

* Data from Microbiol Technology, 1 (1979) : 187-209.

TABLE 2.2 Raw material which can be used as carbon source in acetone-butanol fermentation.*

Raw material	Microorganism	Fermentation time (hr)	% Production yield	total solvent (g/l)	Solvent (g/l)		
					Butanol	Acetone	Ethanol
Whey	Cl. acetobutylicum P262	39	42	9.5	7	2.5	-
Corn starch	Cl. acetobutylicum No.105	72	26.7	13.3	7.2	4.3	1.8
Molass	Cl. acetobutylicum P262	30-36	31-32	16.18	A*	A*	A*
Tapioca Starch	Crostridium No. 8P-2	25-30	29.22	14.03	9.82	3.95	0.25
Tapioca Starch	Cl. butylicum NRRL B592	68.5	27.83	14.63	9.51	4.86	0.26

A* = no reported data

* Data from Master's Thesis "Production of Acetone-butanol from enzyme hydrolysate of water hyacinth", Graduate School, Chulalongkorn University, (1989)

TABLE 2.3 Sugars which can be used as carbon source in acetone-butanol fermentation.*

Substrate	Microorganism	Fermentation time (hr)	% Production yield	total solvent (g/l)	Solvent (g/l)		
					Butanol	Acetone	Ethanol
Glucose	Cl. acetobutylicum ATCC 824	96	32	20.8	15	4.5	1.3
Glucose	Cl. butylicum NRRL B592	110	28.2	14.1	A*	A*	A*
Glucose	Cl. acetobutylicum P262	58	32	12.7	9	3.4	0.3
Xylose	Cl.acetobutylicum ATCC 824	144	28	14.1	8.9	3.9	1.3
Arabinose	Cl.acetobutylicum ATCC 824	99	29	16.5	10.5	4.5	1.5
Lactose	Cl. acetobutylicum P 262	96	38	9.5	6.7	2.6	0.2
Galactose	Cl. acetobutylicum P262	42	31	10	7.1	2.7	0.2

A* = no reported data

* Data from Master's Thesis "Production of Acetone-butanol from enzyme hydrolysate of water hyacinth", Graduate School, Chulalongkorn University, (1989)

2.1.2 Culture Methods

The continued transfer of a seed culture in the usual media results in the culture becoming sluggish, with corresponding decreased yield of solvents.

It has been shown that the most prodigious producers of solvents are in general the most heat resistant. " Heat Shocking " is a method wherein the vegetative cells and the weaker spores of a culture are destroyed. By subjecting a culture of the organism, in which the development of spore has been favored, to a temperature of 100°C for 1 to 2 minutes, heat shocking is effected.

The treatment bears a direct relation to the temperature employed, the size and nature of the tube containing the seed culture and the characteristics of medium. If one is to use a thin-walled wafer tubing and a very small amount of the culture, it is obvious that neither the time of shocking nor the temperature should be excessive , since even the resistant spores may be destroyed.

Alternate heat shocking and successive subculturing are commonly used to activate a culture. The medium containing the seed culture is allowed to stand at room temperature for a few days to encourage sporulation. New medium is inoculated from the spore-containing culture and heat shocked. The surviving spores are permitted to germinate under favorable conditions and subcultures are made successively at daily intervals for 4 to 7 days. At the end of this, the culture is again permitted to stand at room temperature to encourage spore formation. The cycle is then repeated heat-treatment, repeated subculturing, rest for sporulation, heat shocking, and so on.

2.1.3 Optimum Conditions for Fermentation

Temperature - The most favorable temperature range is 37°C to 42°C. Since the neutral solvents, especially acetone, are volatile at these temperatures it is necessary to take precautions to avoid losses during the fermentation process.

Oxygen Relationship - Since the organism best suited to the production of acetone and butanol are anaerobic in nature, the highest yields will be obtained when anarobiosis is maintained.

pH - Growth may be obtained in corn mashes between a pH of 4.7 and 8 by the butanol organisms, but there is a low production of solvent at both of the extremes. The pH range of 5 to 7 is satisfactory in most cases.

2.1.4 Uses of Products and By-Products of Acetone-butanol Fermentation

Butanol - Butanol is used primary in the manufacturing of lacquers which are utilized on automobiles, rayon, detergent, brake fluids, amine for gasoline additive. It is also used as a solvent for fats, waxes, resins, rubber, plastics, shellac and varnish. Large quantities of butanol and its derivatives are used in other industrial processes.

Acetone - Acetone is used mostly as a solvent for fats, oils, waxes, resins, rubber plastics, lacquers, varnishes. Moreover, it is used in the manufacturing of artificial silk and leather, photographic film, airplane dopes, rubber cement and other products.

Ethanol - Ethanol is used in pharmaceuticals industrials and is also used for chemical material synthesis such as ether or chloroform.

Gases - The gases , consisting of 60% CO_2 and 40% H_2 , have been used for synthesizing both methanol and ammonia. When the two gases are separated, the hydrogen can be used in chemical synthesis as burned for energy or used as an industrial chemical and the carbondioxide is often converted to dry ice.

Solid Residues - The acetone-butanol fermentation produces a beer which contain riboflavin, B complex vitamins, protein and certain unknown growth factors in relatively large concentrations. After drying, the solid residues are being used as a vitamin supplement for feed stuffs.

The mass balance of fermentation material is shown in Table 2.4, 2.5 and the Figure 2.4 .

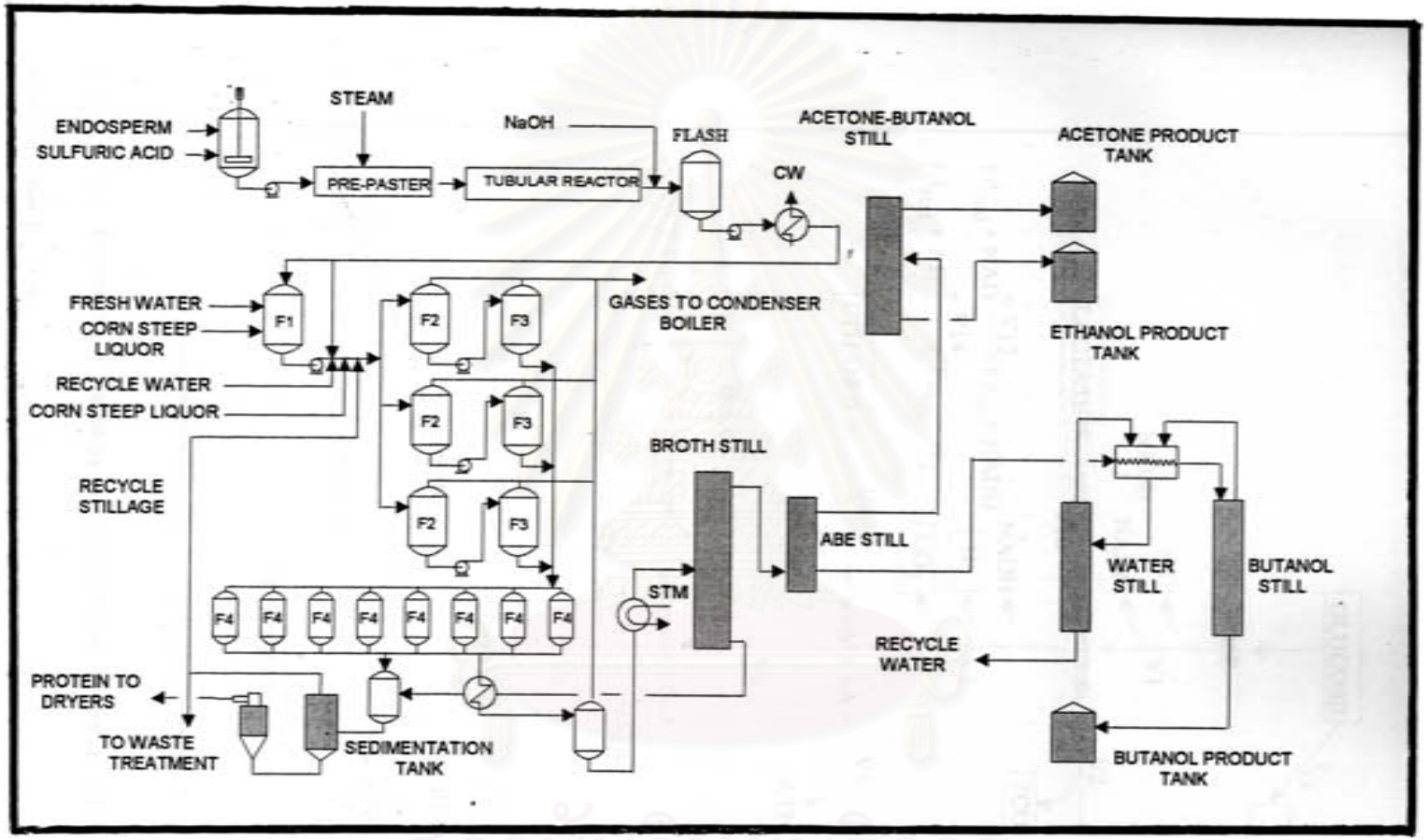

FIGURE 2.1 Process for n-butanol production fermentation.*

* Data from Biotechnol. Progress, 2 (March, 1986) : 23-28.

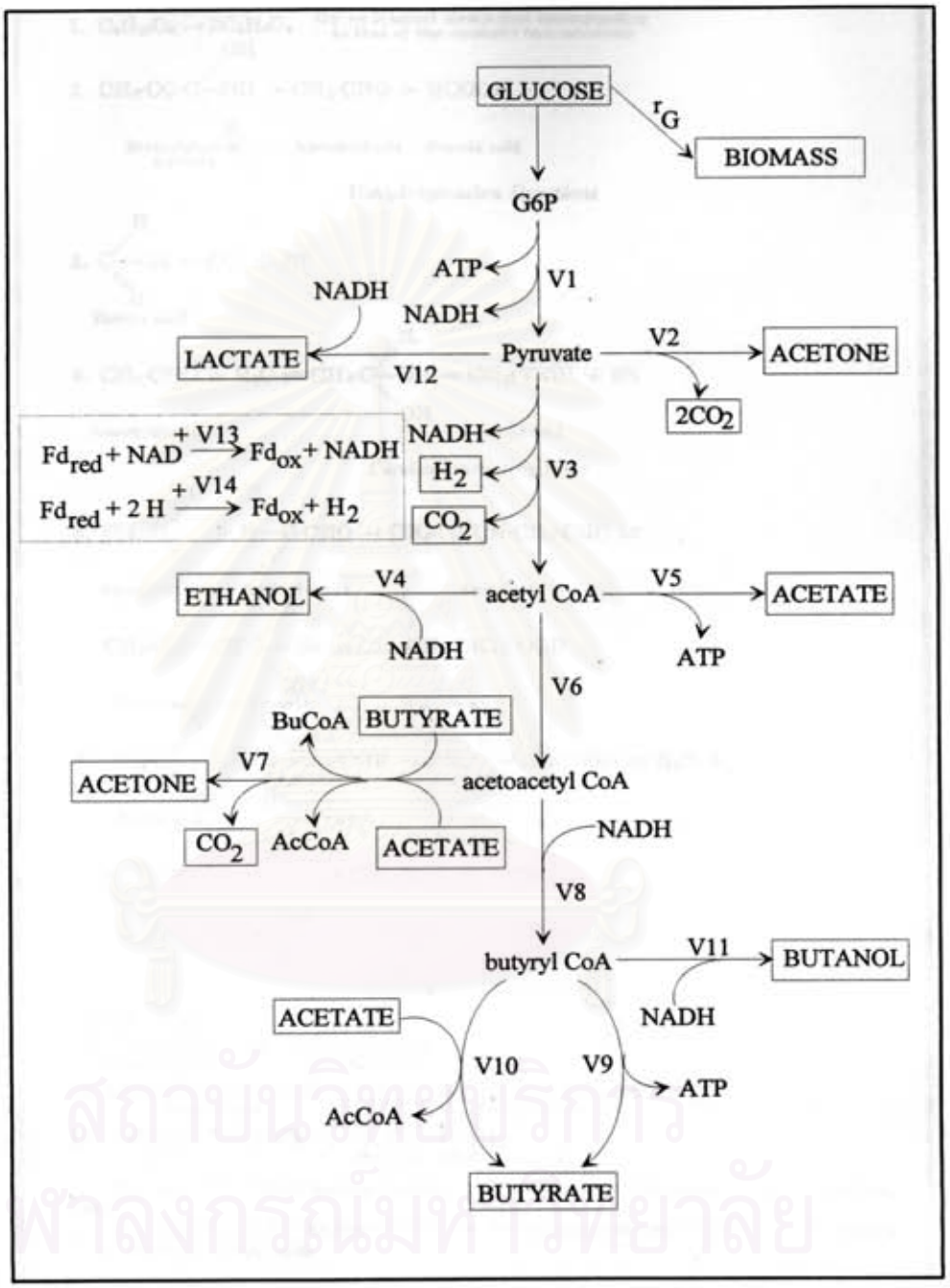

FIGURE 2.2 Biochemical pathway for conversion of sugar into organic solvents by Cl. acetobutylicum.*

* Data from Biotechnol. Progress, 3 (September, 1987) : 156-157.

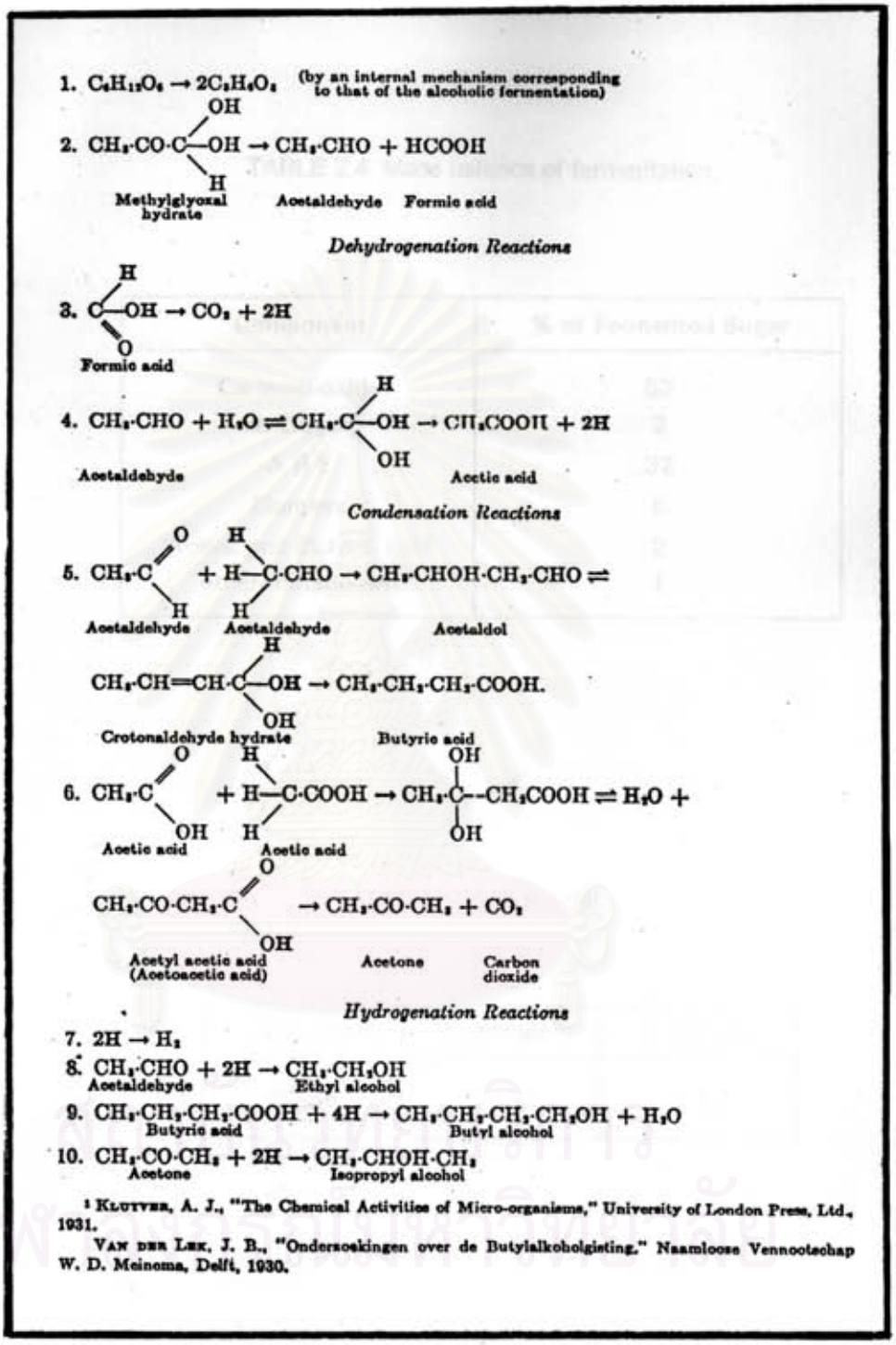

FIGURE 2.3 The scheme for the butanol fermentation.*

* Data from The Chemical Activities of Microorganisms, (1931).

TABLE 2.4 Mass balance of fermentation.

Component	% of Fermented Sugar
Carbondioxide	57
Hydrogen	2
A B E	32
Biomass	6
Acetic and Butyric acid	2
other metabolism	1

TABLE 2.5 Solvent ratio.

% n-Butanol	% Acetone	% Ethanol
60-65	30-35	5-10

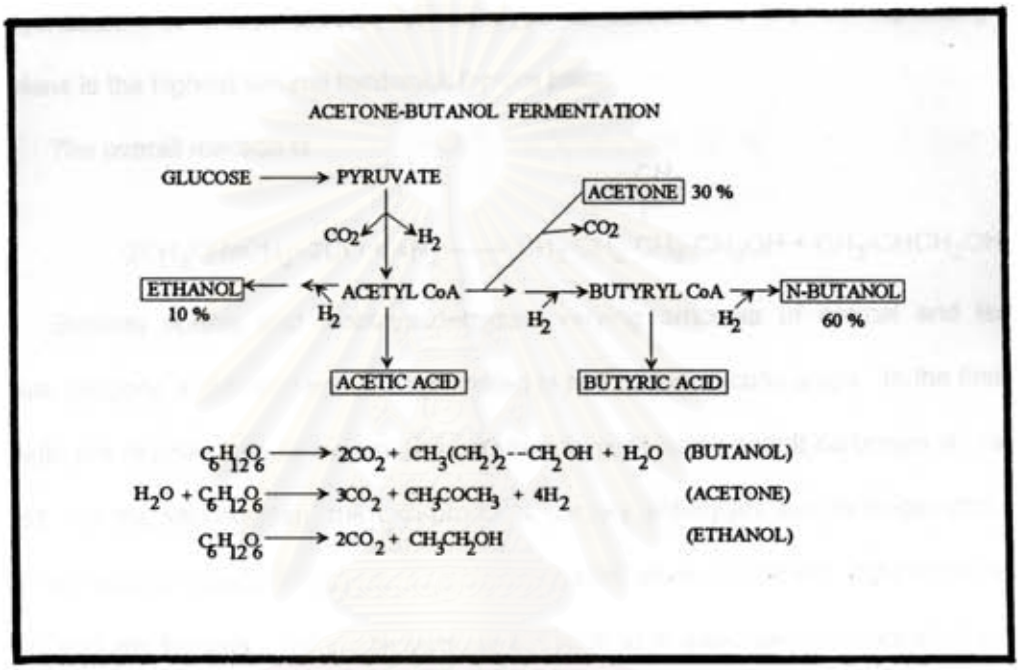

FIGURE 2.4 Glucose balance of acetone-butanol fermentation.*

* Data from Encyclopedia of Chemical Processing and Design.

2.1.5 The Petrochemical Processes

In the United States, 70% of butanol is made by the oxoprocess. The oxo or hydroformylation process has been developed into a 3 MMt/y industry. Most of the aldehydes produced are either reduced to alcohols directly or subjected to alcohol condensation prior to hydrogenation. A limited amount of aldehyde is oxidized to the corresponding acid. Propylene is the highest volume feedstock for oxo plant.

The overall reaction is

$$2CH_3CH{=}CH_2 + 2CO + 4H_2 \longrightarrow CH_3\text{-}CH_2\text{-}CH_2\text{-}CH_2OH + CH_3\text{-}\underset{\displaystyle CH_3}{\underset{|}{C}}H\text{-}CH_2OH$$

Besides normal and isobutylaldehydes, varying amounts of normal and isobutyl alcohols, propane and heavy ends are produced in two major reaction steps. In the first step, the olefin are reacted with hydrogen and carbonmonoxide using cobalt carbonyls as catalyst to yield. In the second step, the oxo-products, largely aldehydes are hydrogenated using supported metallic cobalt as catalyst. The hydrogenated product is caustic mashed to remove organic acid and formats. The crude alcohols are purified in a two tower fractionation system. The process is shown in Figure 2.5.

2.2 Development of Acetone-butanol Fermentation Processes

2.2.1 Single-stage Continuous Fermentation

M. Fick, P. Pierrot and J. M. Engasser (1985) obtained a solvent productivity of 0.75 g/l-hr with an end concentration of 13 g/l solvent from a continuous culture of ATCC 824 (Clostridium acetobutylicum) on a complex medium containing 40 g/l glucose. This process was maintained for 2 months, at an optimum dilution rate of 0.06 hr^{-1}. For batch culture, butanol toxicity limits high product concentration and volumetric productivities. However, the continuous culture is usually limited by high concentration of butanol which has a strong toxic effect and inhibits cell growth.

2.2.2 Immobilization

M.Chirakarn and G.Goma (1984) reported a solvent productivity of 0.75 g/l-hr in a two-stage continuous process when an immobilization technique is used in the second stage fermenter. Spore and vegetative cells of Cl. acetobutylicum were immobilized in pored-valcano rock.

Ch.Frick and K.Schugerl (1986) studied a two-stage continuous fermentation with and without immobilized ATCC 824 in calcium algenate gel of the second fermenter for comparison between the two systems. The systems without immobilization obtained a solvent productivity of 1.93 g/l-hr with an end concentration of 15.4 g/l solvent while the systems with immobilization obtained a solvent productivity of 4.02 g/l-hr

The single-stage continuous process of butanol has been reported from immobilized Clostridium acetobutylicum (L.Haggtrom and N.Molin, 1980) in calcium algenate gel. These yields a solvent productivity of 0.48 - 0.64 g/l-hr. However, since butanol toxicity rapidly reduced cell activity within the immobilization matrix, extensive investigation will be required to establish economical working condition for such a process.

2.2.3 Microfiltration

The application of microfiltration (MF) to fermentation processes has been studied to improve productivity in acetone-butanol fermentation. Continuous ethanol fermentation, using crossflow microfiltration (CFM) to recycle cell back to the fermenter, has been reported to increase the biomass per unit volume, facilitate an increase in productivity. In MF and CFM, fermentation broth flows tangentially across the membrane surface with cell-free liquid permeating through the membrane. Accumulated cells are swept away from the membrane surface using a high recirculation flow rate. Concentration cells and a portion of the cell-free liquid stream are returned to the fermenter.

G.Albert and L.I.Engene (1986) studied butanol separation from acetone in broth by using microfiltation technique. This experiment was very satisfactory.

M.Minier, E.Feras, G.Goma and P.Soucallce (1986) improved productivity in acetone-butanol fermentation by coupling continuous fermentation with microfiltration. The membrane was a carbon tube with a microfiltering ceramic coat inside. With total recycle of biomass, a dry weight concentration of 125 g/l was obtained, which enchanced a solvent productivity of 4.5 g/l-hr at dilution rate of 0.33 hr^{-1}.

D.Schlote and G.Gottschalk (1986) improved a productivity of acetone-butanol fermentation by using cellulosetriacetate membrane to separate and recycle cells in a continuous fermentation of Clostridium acetobutylicum ATCC 824 under phosphate limitation ($0.74x10^{-3}$ molar) at dilution rate of 0.40 hr^{-1}. A solvent productivity of 4.1 g/l-hr was maintained for three months.

M.Fick, P.Pierrot and J.M.Engasser (1986) used a microfiltration which was coupled by continuous fermenter at dilution rate of 0.50 hr^{-1}, a biomass concentration of 20 g/l, a solvent productivity of 6.5 g/l-hr and an end concentration of 13 g/l solvent.

P.Muenduen (1989) studied application of microfiltration for improved productivity in continuous acetone-butanol fermentation. A multitubular ceramic microfilter was used to separate and recycle cells of Cl.acetrobutylicum ATCC 824. With total recycle of biomass, a dry weight concentration of 80 g/l and a solvent productivity of 6.06 g/l-hr were obtained at dilution rate of 0.55 hr^{-1} on a complex medium containing 42.4 g/l glucose.

However, the microfiltration technique is failure because of blockage of membrane when operation carried out for a long time.

2.2.4 Two-stage Continuous Fermentation

H.Bahl, W.Andersh and G.Gottschalk (1982) studied two-stage continuous acetone-butanol fermentation under phosphate limitation ($0.74x10^{-3}$ molar) and found the optimum condition to be dilution rate of 0.125 hr^{-1} at temperature of 37 °C in the first stage and dilution rate of 0.04 hr^{-1} at temperature of 33 °C in the second stage with an overall concentration of 16.87 g/l solvent.

C.Godin and J.M. Engasser (1988,1989,1990) used 2 litres fermenter as the first stage coupling with 6 litres fermenter as the second stage. The first stage was kept in a suitable condition for cell growth and the second stage for solvent production with pH 4.5 for both stages at dilution rate of 0.1 hr^{-1} and 0.04 hr^{-1}, respectively. An overall solvent productivity of 0.57 g/l-hr was obtained.

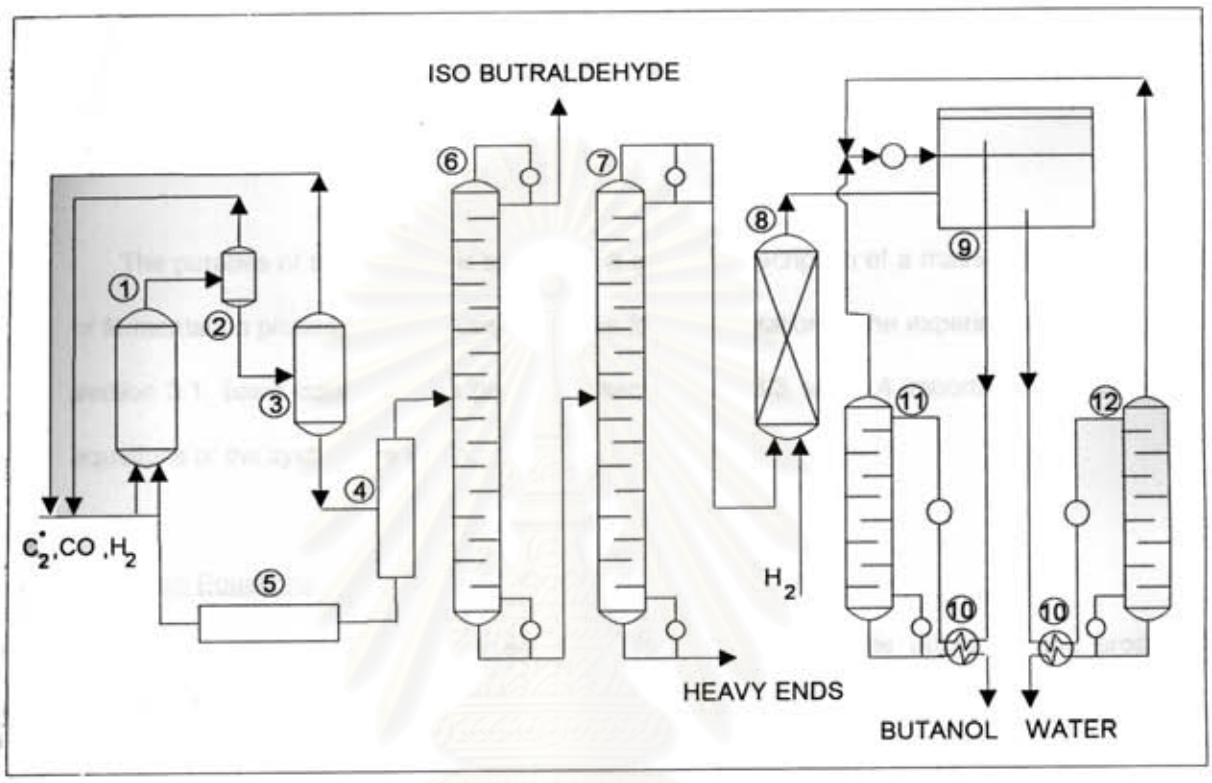

FIGURE 2.5 Process of n-butanol production by hydroformulation of propylene.*

1. oxo reactor
2. low pressure separator
3. high pressure separator
4. catalyst removal
5. catalyst storage
6. iso-butylaldehyde column
7. normal-butylaldehyde column
8. hydrogenation reactor
9. decanter
10. condenser
11. butanol column
12. aqueous column

* Data from Higher Oxo Alcohols.

CHAPTER III

THEORY

The purpose of this chapter is to explain a general description of a mathematical analysis of fermentation processes to be used as basis for interpretation of the experimental results. In section 3.1, basic equations are given. In section 3.2, 3.3 and 3.4 important mathematical equations of the system are given.

3.1 Basic Equations

The general material balance equations for growth, substrate utilization and product formation can be written as follows:

Growth:

Cell mass accumulation	=	Cell growth	+	Cells in	-	Cells out	-	Cell death

$$dX / dt = \mu X + F X_0 / V - F X / V - r X \qquad (1)$$

Substrate Utilization:

Substrate accumulation	=	Substrate consumed	+	Substrate feed	-	Substrate removal	-	Maintenance requirement	-	Product formation

$$-dS / dt = -\mu X / Y_{x/s} + F S_0 / V - F S / V - m X - \upsilon X / Y_{p/s} \qquad (2)$$

Product Formation:

product accumulation	=	Product synthesis	+	Product feed	-	Product removal	+	Product destruction

$$dP / dt = \upsilon X + F P_0 / V - F P / V - K P \qquad (3)$$

In equations (1), (2) and (3), X denotes the cell mass, μ the cell growth rate, F the feed flow rate , X_0 the initial cell mass, V the volume, r the death rate, S the substrate concentration, S_0 the initial substrate concentration, m the specific maintenance rate, υ the specific production rate, $Y_{x/s}$ the conversion yield of substrate to product, $Y_{p/s}$ the conversion

yield of substrate to product, P the product concentration, P_o the initial product concentration and K the product destruction rate, respectively. The above equations are applicable for both steady and unsteady state fermentation.

3.2 Batch Fermentation

For simplicity, it can be based on the following assumptions:

1. At the exponential growth phase,

$$\mu >> r \; ; \tag{4}$$

2. At the product formation phase,

$$\upsilon >> K \quad ; \tag{5}$$

3. At the substrate consumption phase

$$m X << \mu X / Y_{x/s} \; ; \quad \upsilon X / Y_{p/s} = 0 \tag{6}$$

$$\upsilon X / Y_{p/s} = 0$$

Since for the batch process, the feed rate F=0, equation (1), (2) and (3) can be rearranged with the help of assumptions (4), (5) and (6) to be in form

$$\mu = (1 / X) (dX / dt) \tag{7}$$

$$\mu X / Y_{x/s} = (1 / X) (dS / dt) = \upsilon_s \tag{8}$$

$$\upsilon = (1 / X) (dP / dt) \tag{9}$$

Where υ_s denotes the specific substrate consumption rate, the production yield from carbon source, $Y_{p/s}$, can be calculated from

$$Y_{p/s} = \upsilon / \upsilon_s \tag{10}$$

3.3 Single-stage Continuous Fermentation (Figure 3.1)

In addition to the assumptions (4), (5) and (6), it can be assumed that

4. Substrate is free from strain at initial condition, i.e.,

$$X_o = 0 \tag{11}$$

5. None of the product is fed to the first fermenter, i.e.,

$$P_o = 0 \tag{12}$$

With assumption (4)-(6) and (11)-(12), equation (1) can be simplified as shown ;

$$-F X / V + \mu X = dX / dt \quad (13)$$

For steady state, dX / dt = 0, equation (13) becomes

$$\mu = F / V \quad (14)$$

Let D denotes the dilution rate defined by the ratio between the flow rate of the substrate to the culture volume, i.e.,

$$D = F / V \quad (15)$$

At steady state, it can be seen that the specific growth rate is exactly equal to the dilution rate.

From equation (2), using assumption (4) - (6) and (11) - (12), there obtain at steady state ,i.e., dS / dt = 0 ,

$$D (S_0 - S) = \mu X / Y_{x/s} \quad (16)$$

Where D is defined in equation (15). Recalling from equation (15) that $\mu = D$, equation (16) becomes

$$X = Y_{x/s} (S_0 - S) \quad (17)$$

Similarly, from equation (3) there obtain at steady state

$$\upsilon = D P / X \quad (18)$$

3.4 Two-stage Continuous Fermentation (Figures 3.2,3.3)

The material balance on cell mass, limiting substrate and product formation for the first stage at steady state are identical to the single-stage chemostat and are given by equation (14),(16), and (18), respectively. Writing the general material balance equations for cell mass, substrate and product in the second stage yields

Cell accumulation = Cells in - Cells out + Cell growth

$$dX_2 / dt = F X_1 / V_2 - F X_2 / V_2 + \mu_2 X_2 \quad (19)$$

Substrate accumulation = Substrate in - Substrate out + Substrate consumed

$$dS_2 / dt = F S_1 / V_2 - F S_2 / V_2 + \mu_2 X_2 / Y_{x/s} \quad (20)$$

Product accumulation = Product in - Product out + Product formation

$$dP_2 / dt = F P_1 / V_2 - F P_2 / V_2 + \upsilon_2 X_2 \quad (21)$$

The steady state solution of equation (19), (20) ,and (21) for the second stage are compared with equations for the first stage in Table 3.1.

TABLE 3.1 Comparison of the steady state solutions for material balance on each stage of a two-stage chemostat.

Stage	Cell mass	Substrate	Product
1st	$\mu_1 = D$	$X_1 = Y_{x/s} (S_0 - S_1)$	$\upsilon_1 = D_1 P_1 / X_1$
2nd	$\mu_2 = D_2 (1 - X_1/X_2)$	$X_2 = (D_2/\mu_2) Y_{x/s} (S_1 - S_2)$	$\upsilon_2 = D_2 (P_2 - P_1) / X_2$

The dilution rates of the first stage and the second stage are equal to F/V_1 and F/V_2, respectively. From the steady state solutions for the second stage, the growth rate and the dilution rate are not equal. In addition, if no additional limiting nutrient is added to the second stage, the net growth in the second stage will be small. The value of this system is that cells may be grown in the first stage under constant controlled conditions and then maintained in the growth limiting environment for prolonged periods of time in the second stage.

Significant modification of this arrangement is the addition of feed stream to the second stage. This stream contain the limiting nutrient. Then material balances for cell mass, limiting nutrient and product formation in the second stage are given by equation (22), (23), and (24), respectively.

Cell accumulation = Cells from 1st stage + Cell from additional feed - Cells out + Cell growth

$$dX_2 / dt = F X_1 / V_2 + F' X' / V_2 - (F+F') X_2 / V_2 + \mu_2 X_2 \quad (22)$$

Where F' is the flow in the additional feed stream and X' is the cell mass in the additional feed stream. The dilution rate for the second stage is $D_2 = (F+F') / V_2$. Material balance of limiting nutrient is

Substrate = Substrate + Substrate - Substrate - Substrate
accumulation from 1st stage from additional feed removal consumed

$$dS_2 / dt = F S_1 / V_2 + F' S' / V_2 - (F+F') S_2 / V_2 + \mu_2 X_2 / Y_{x/s} \quad (23)$$

Where S' is the concentration of limiting nutrient in the additional feed. Similarly, the product formation balance is given by

Product = Product - Product - Product
accumulation from 1st stage removal formation

$$dP_2 / dt = F P_1 / V_2 - (F+F') P_2 / V_2 + \upsilon_2 X_2 \quad (24)$$

It is assumed that the additional feed to the second stage is sterilised (X' = 0). At steady state, equation (22), (23) , and (24) are rearranged.

$$\mu_2 = D_2 - (F / V_2)(X_1/X_2) \quad (25)$$

$$X_2 = (Y_{x/s} / \mu_2) (F S_1/ V_2 + F' S' / V_2 - D_2 S_2) \quad (26)$$

$$\upsilon_2 = D_2 P_2 / X_2 - (F / V_2)(P_1/X_2) \quad (27)$$

From these equations, it may be seen that the additional feed stream now permits further growth in the second stage. Furthermore, the dilution rate can be set at value greater than the maximal growth rate (μ_{max}) and the culture will not wash out because the first stage supplies fresh cells continuously.

3.5 Two-stage Continuous Coupled by Microfiltration (Figures 3.4, 3.5)

The two-stage coupled by microfiltration in order to increase the concentration of cells. A schematic for this system is shown in Figures 3.4,3.5. The nomenclature is the same as for the single-stage chemostat except for two additional parameter α, the recycle ratio and C, the concentration factor.

A material balance for cell mass is

Cell = Cells + Cell - Cells + Cell
accumulation from 1st stage in recycle stream out growth

$$dX_2 / dt = F X_1 / V_2 + \alpha F C X_2 / V_2 - (1 + \alpha) F X_2 / V_2 + \mu_2 X_2 \quad (28)$$

Death cell is assumed to be small. Solving equation (28) for steady state, dX / dt = 0, substituting $D_2 = F / V_2$, gives

$$\mu_2 = (1+ \alpha - \alpha C) D_2 - (X_1/X_2) D_2 \quad (29)$$

or $$\mu_2 = (B - (X_1/X_2)) D_2$$

Where B denotes the bleed ratio and equals $1+ \alpha - \alpha C$.

A material balance on the limiting nutrient over the same boundary yields

Substrate accumulation	=	Substrate from 1st stage	+	Substrate in additional feed	-	Substrate removal	-	Substrate consumed

$$dS_2 / dt = F S_1 / V_2 + \alpha F S_2 / V_2 - (1 + \alpha) F S_2 / V_2 + \mu_2 X_2 / Y_{x/s} \quad (30)$$

Solving equation (30) for steady state, dS / dt = 0, gives

$$X_2 = (D_2 Y_{x/s} / \mu_2) (S_1 - S_2) \quad (31)$$

Substituting equation (26) into (28) gives

$$X_2 = (Y_{x/s} (S_1 - S_2) + X_1) / B \quad (32)$$

A material balance on the product formation is identical to equation (27).

If additional limiting nutrient is added to the second stage, the material balances at the steady state are given

$$\mu_2 = (1+ \alpha) D_2 - (F / V_2) ((X_1/X_2) - \alpha C) \quad (33)$$

$$X_2 = (Y_{x/s} / \mu_2) ((F / V_2)(S_1 - S_2) + F' S'/ V_2 - (1 + \alpha) D_2 S_2) \quad (34)$$

$$\upsilon_2 = D_2 P_2 / X_2 - (F / V_2)(P_1/X_2) \quad (35)$$

Where $D_2 = (F+F') / V_2$ and F' denotes the additional feed.

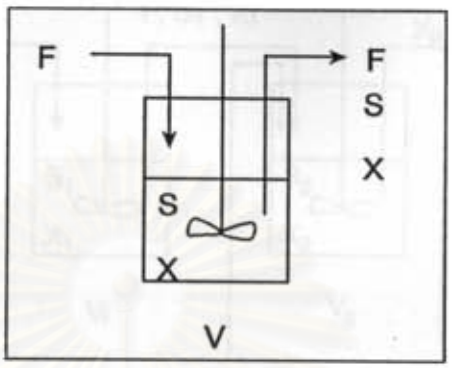

FIGURE 3.1 A single-stage continuous fermenter.

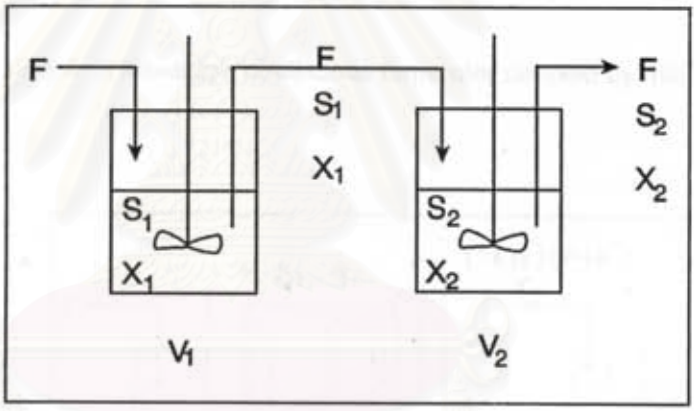

FIGURE 3.2 A two-stage continuous fermenter.

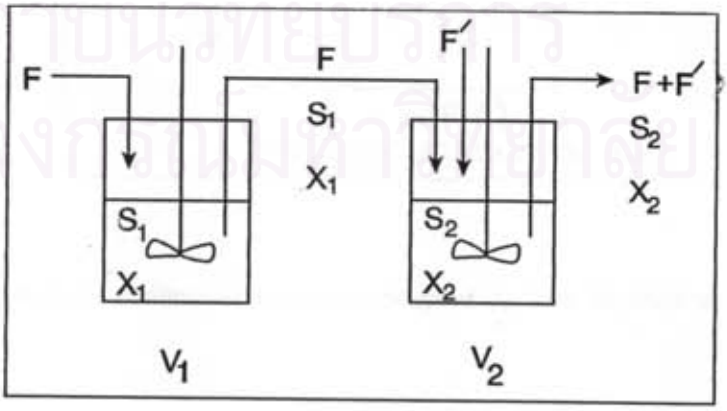

FIGURE 3.3 A two-stage continuous fermenter which applied an additional feed (F').

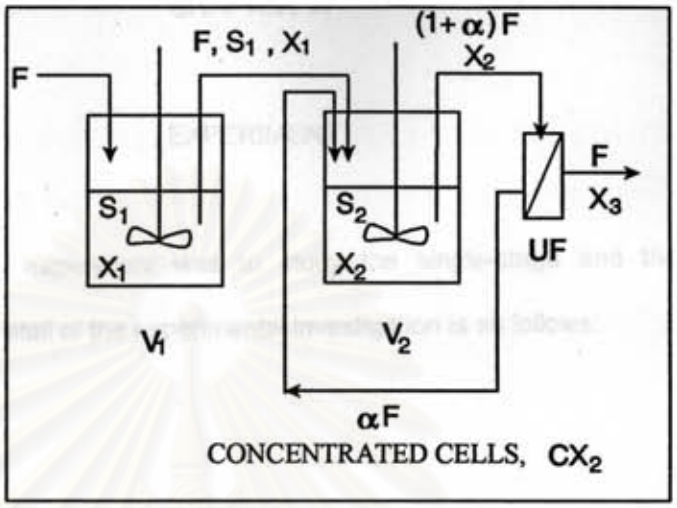

FIGURE 3.4 A two-stage continuous fermenter coupled by microfiltration.

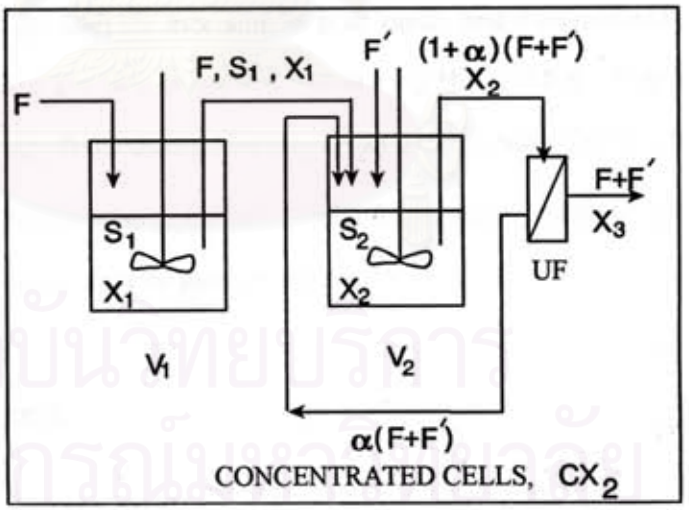

FIGURE 3.5 A two-stage continuous fermenter coupled by microfiltration which applied an additional feed (F').

CHAPTER IV

EXPERIMENT

The purpose of this experiment was to study the single-stage and the two-stage continuous system. The detail of the experimental investigation is as follows:

4.1 Strain

The strain used in this study was Clostridium acetobutylicum ATCC 824 stored as liquid samples from non-pH regulated cultures at 0°C in glass tubes.

4.2 Medium

The same medium was used for inoculum in both batch and continuous cultures. The synthetic medium contained per litre of distillate water : $K_2 HPO_4$ 0.5 g ; $KH_2 PO_4$ 0.5 g ; $MgSO_4 7H_2O$ 0.2 g ; $MnSO_4 7H_2O$ 0.01 g ; $FeSO_4 7H_2O$ 0.01 g ; NaCl 0.01 g ; Yeast extract 6.0 g ; D (+) Glucose anhydrous 50 g. For continuous fermentation, antifoam (vegetable oil) was added to the fermentation broth for foam inhibition.

4.3 Experimental Equipments

4.3.1 Fermenter

4.3.1.1 Batch System : A Eylla-M100 1 litre glass fermenter was used for the batch system. The pH of the experimental fermentation broth was controlled by a pH controller which controlled an alkali feed pump. The temperature of the system was controlled at 35°C by a temperature controller and the agitation speed was 200 rpm.

4.3.1.2 Single-stage Continuous System : A KMJ-2B 3 litres glass fermenter from Mituwa Rikagaku Kogyo Co.,Japan was used for the single-stage continuous system. The flow rate of broth was controlled by controlling the medium feed pump and overflow. The pH of

total fermentation broth was controlled by the pH controller which controlled the alkali feed pump. The temperature was maintained at 35°C by a temperature controller and the agitation speed was 200 rpm.

4.3.1.3 Two-stage Continuous System Coupling with and without Microfiltration : A 1 litre glass fermenter was used as the first stage and the KMJ-2B 3 litres glass fermenter as the second stage. Agitation speeds were 200 rpm, temperatures were 35°C and the pH of the total fermentation broth was controlled by two pH controllers which controlled alkali feed pumps. The volume of broth of the first stage was controlled with a level controller which controlled the medium feed pump. For the second stage, overflow at 3 litres was used. This experiment may applied the additional feed to the second stage by a medium additional feed pump in case of shortage of nutrient from the first stage fermenter.

4.3.2 The Cell Recycling System

The microfiltration was a ceramic filter (type 1M-1, Japan) (Figure 4.1) consisting of 19 carbon tubes with microfiltering ceramic coat inside. The tubes were 4 mm. of internal diameter, 85 cm. long and a 0.2030 m^2 of filtration area. The pore diameter was 0.2 μm (molecular weight cut off 100,000 approximately). A peristaltic pump was used for pumping the nutrient which was controlled by a level controller. The fermentation broth was circulated through the microfiltration module with a sanitary rotary pump (0.75 kW). The circulation velocity was 0.465 m/s. The permeate was continuously discharge at a fixed flow rate F and collected in a storage tank. The excess permeate was directly recycled to the fermenter.

4.4 Experimental Procedure

4.4.1 Study of Single-stage Continuous Fermentation

The aim of this study was to find two optimum conditions. One allowed a high solvent productivity to compare with other systems and the another allowed a high cell productivity to used for the best condition of the first stage. The parameter under studied was the dilution rate. Other parameters such as temperature, pH, agitator speed, glucose

concentration were fixed at the optimum point in a batch acetone-butanol fermentation process. Photograph and schematic diagram of this operation were shown in Figures 4.2 and 4.3, respectively. The procedure of this study was described as follows.

4.4.1.1 Preparation of the Clostridium acetobutylicum ATCC 824 Cultures : A 10 ml of Clostridium acetobutylicum ATCC 824 stored as liquid at 0°C in a glass tube was aseptically transferred to 100 ml of the sterilised medium in a 500 ml flask. The medium was kept anaerobic by bubbling pure nitrogen for 15 minutes, and was then kept at 35°C for an 18 hours growth. Culture tubes and inoculum flask were shown in Figures 4.4 and 4.5 , respectively.

4.4.1.2 Batch Operation in the Single-stage System : The 100 ml of the prepared culture (from 4.4.1.1) was aseptically transferred to 3 litres of the sterilised medium in the fermenter. The agitation speed was maintained at about 200 rpm and the temperature was controlled at 35°C. The pH of the medium was controlled to be not less than 4.8 by automatic addition of 4 N NaOH. The medium was kept anaerobic by bubbling of pure nitrogen.

After the residue substrate concentration reached about 25 g/l or until the cell concentration was constant) , 50 g/l glucose of the sterilised medium was added by medium feed pump. Every 4 or 6 hours of the operation, 5 ml of broth was aseptically taken from the fermenter for determination of products, cell and glucose concentrations.

4.4.1.3 Continuous Operation in the Single-stage System : The continuous operation was started after the residual substrate concentration reached about 25 g/l or until the cell concentration was constant. After that, 50 g/l glucose of sterilised medium was fed into the fermenter by a multichannel flow tube pump and the fermentation broth removed by overflow with a fixed flow rate (F) to the storage tank. The pH of the medium was controlled to be not less than 4.8 by automatic of 4 N NaOH. The temperature was maintained at 35°C.

The dilution rate (D) was at first controlled to be at 0.03 hr^{-1} until the residual substrate or cell concentrations were constant at least 3 times of retention time. Then the dilution rate (D) was changed to 0.05 hr^{-1}, 0.07 hr^{-1}, 0.1 hr^{-1},0.12 hr^{-1} , 0.14 hr^{-1}, 0.15 hr^{-1}, 0.17 hr^{-1}, 0.18 hr^{-1}, and 0.23 hr^{-1}, respectively. Then a repeated run at 0.05 hr^{-1} was made to compare the productivity to the first run. The data from experiments were used for optimization of this system. Every 4 or 6 hours of the operation, 5 ml of the fermentation broth was aseptically taken from the fermenter for determination of cell, products and glucose concentrations.

FIGURE 4.1 Ceramic filter (type 1M-1).

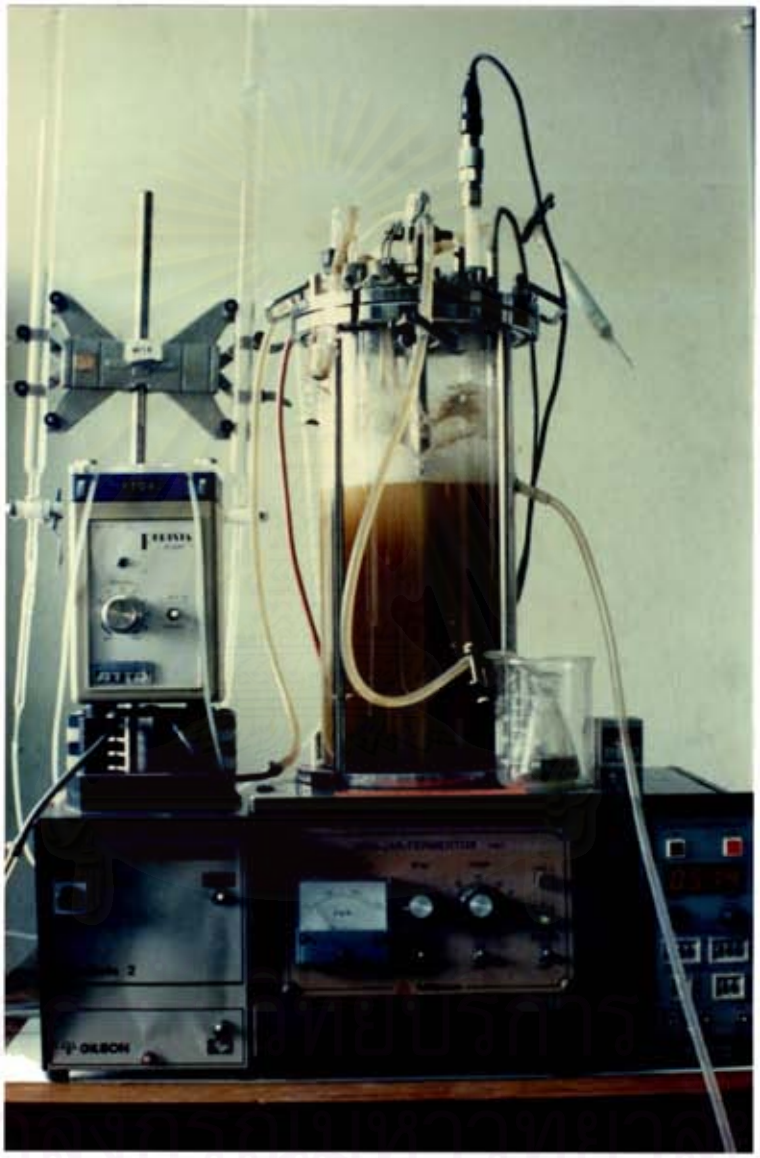

FIGURE 4.2 The single-stage continuous fermentation.

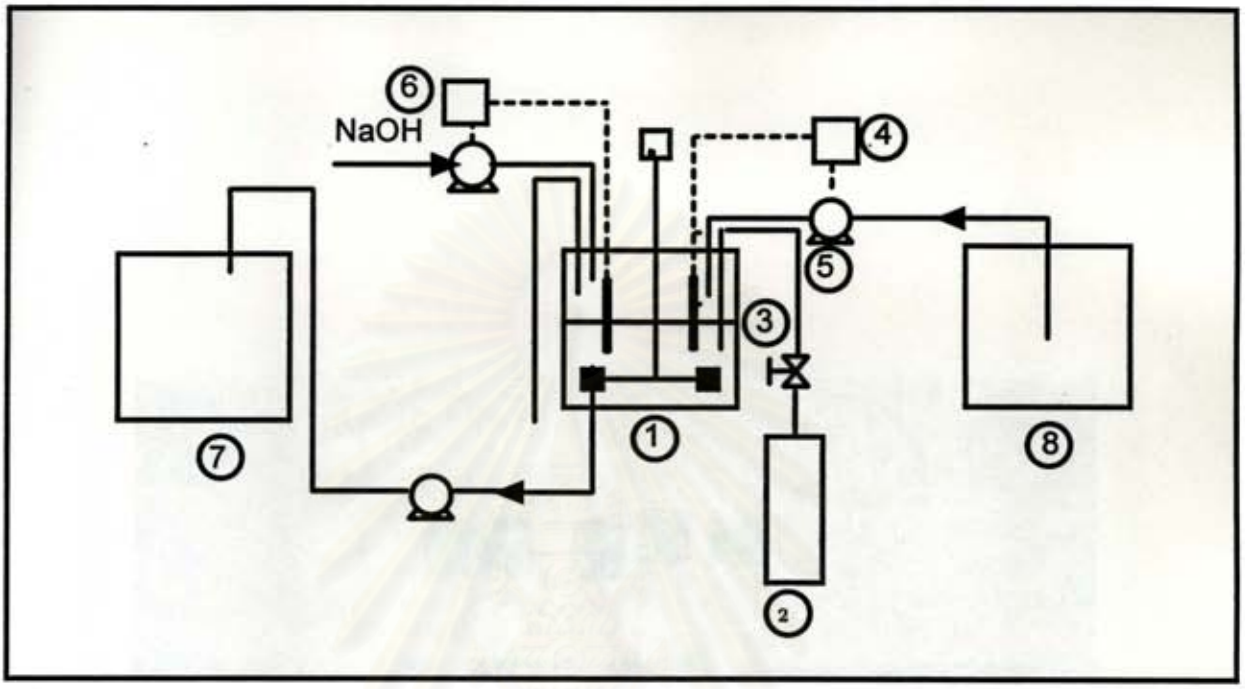

FIGURE 4.3 Schematic diagram of the single-stage continuous fermentation.

1. fermenter
2. N_2 storage tank
3. level controller
4. level controller
5. feed pump
6. alkali feed pump
7. storage tank
8. feed tank

FIGURE 4.4 Culture tubes.

FIGURE 4.5 An inoculum flask.

4.4.2 Study of Two-stage Continuous Fermentation

4.4.2.1 Batch Operation in the Two-stage Continuous System : The 100 ml of the prepared culture (from 4.4.1.1) was aseptically transferred to 1 litre of the sterilised medium in the 1-L first stage fermenter which was connected with the 3-L second stage fermenter. Both agitation speeds were maintained at about 200 rpm and temperatures were also controlled at 35°C. The pH of this system was controlled to be not less than 4.8 by automatic addition of 4 N NaOH and kept anaerobic by bubbling of pure nitrogen.

After the residual substrate concentration of the first stage reached about 25 g/l or until the cell concentration was constant, 50 g/l glucose of the sterilised medium was added into the first stage by medium feed pump. Every 4 or 6 hours of the operation, 5 ml of broth was aseptically taken from the fermenter for determination of cell, products and glucose concentrations.

4.4.2.2 Continuous Operation in the Two-stage System : The continuous operation was started after the residual substrate concentration reached about 25 g/l until the cell concentration was constant. After that, 50 g/l glucose of the sterilised medium was fed into the first stage fermenter and the residual glucose was removed from the first stage fermenter to the second stage fermenter by a multichannel flow tube pump. The volume of the first stage was maintained at 1 litre by equaling the feed flow rate and the outflow rate, while the fermentation broth was continuously overflow at a fixed flow rate (F) from the second stage fermenter to storage tank. In case of shortage of glucose in the second stage, a 500 g/l glucose of additional feed was fed into the second stage by a peristaltic pump. The overall dilution rate (D=F/V) was defined by the continuous outflow rate of the fermentation broth (at 4-L of the total fermentation broth volume). The pH of the medium was controlled to be not less than 4.8 by automatic addition of 4 N NaOH. The temperature was kept at 35°C. Photograph and schematic diagram of the two-stage continuous fermentation were shown in Figures 4.6 and 4.7, respectively.

The dilution rate of the first stage ($D_1 = F/V_1$) was at first kept at 0.15 hr^{-1} until residual substrate concentration of the first and the second stage were constant at least 3 times of the retention time. Then the dilution rate (D_1) was changed to 0.17 hr^{-1}, 0.12 hr^{-1}, and 0.10 hr^{-1}, respectively. If the residual substrate concentration in the second stage reached about 5 g/l , the 500 g/l glucose was fed until the residual substrate concentration reached more than 5 g/l.

In this study, run A (Table 4.1) was operated with a fixed dilution rate of 0.15 hr^{-1} in the first stage and 500 g/l glucose of additional feed was added to the second stage fermenter with a fixed flow rate of 0.03 l/hr. Run B, C and D were the same as run A in the first stage and added the 500 g/l glucose of additional feed into the second stage with a fixed flow rate of 0.02 l/hr, 0.01 l/hr, and no additional feed, respectively. Run E was operated with a fixed dilution rate of 0.17 hr^{-1} in the first stage and no addition to the second stage. Run F was operated with a fixed dilution rate of 0.12 hr^{-1} in the first stage and no addition to the second stage until the shortage of glucose occurred, then for run G, 500 g/l glucose was fed with a fixed flow rate of 0.0045 l/hr. Run H was operated with a fixed dilution rate of 0.10 hr^{-1} in the first stage and 500 g/l glucose was added to the second stage with a fixed flow rate of 0.0045 l/hr. Every 4 or 6 hours of the operation, 5 ml of broth of each stage were aseptically taken from both fermenters for determination of cell ,products and glucose concentrations. Table 4.1 was the operation parameters in run A - H.

4.4.2.3 A Two-stage Continuous System coupling with Cell recycling : The working volume of 1 litre of the first stage fermenter was connected with a total working volume of 2.7 litres of the second stage fermenter coupled by the microfiltration. Preparation of the ATCC 824 culture and batch operation for this system were the same as (4.4.1.1) and (4.4.1.2), respectively. The continuous operation was also started after the residual substrate concentration reached about 25 g/l or until the cell concentration was constant. After that, a sterilised medium was fed into the first stage fermenter and removed to the second stage fermenter by a multichannel flow tube pump. A volume of the first stage fermenter was

maintained at 1 litre by allowing the feed flow rate was equal to the outflow rate. The permeate was continuously discharged at a fixed flow rate (F) to the storage tank by peristaltic pump controlled with a level controller of the second stage. Sometimes the additional feed was used in the second stage to avoid the shortage of glucose. The dilution rate (D=F/V) was defined by the flow rate of permeate (at 3.7 litres of total fermentation broth volume). The pH of the medium was controlled to be not less than 4.8 and the temperature of the system was kept constant at 35°C. Photograph and schematic of the two-stage continuous coupled by microfiltration were shown in Figures 4.8 and 4.9 , respectively.

The dilution rate of the first stage (D_1) was maintained at 0.17 hr^{-1} and that of the second stage (D_2) was at first kept constant at 0.063 hr^{-1} for run I until the residual substrate concentration reached about 5 g/l. Then the 500 g/l glucose was added to the second stage with a fixed flow rate of 0.0045 l/hr for run J and increased to 0.0157 l/hr for run K. Run L and run M were operated with 50 g/l glucose added to the second stage with a fixed flow rate of 0.75 l/hr and 1.323 l/hr, respectively, while the first stage was also maintained at 0.17 hr^{-1}. Every 4 or 6 hours of the operation, 5 ml of broth was aseptically taken from the first stage fermenter for determination of cell, products and glucose concentrations. For the second stage fermenter, 5 ml of broth was aseptically taken for determination of the cell concentration and 5 ml of the permeate was taken for determination of products and glucose concentrations. Table 4.2 was the operation parameter in run I - M.

4.4.2.4 A Batch System : The 100 ml of prepared culture (from 4.4.1.1) was aseptically transferred to the 1 litre of sterilised medium which contained 50 g/l glucose. The agitation speed was 200 rpm and the temperature was controlled at 35°C. The pH of the medium was maintained to be not less than 4.8 by automatic addition of 4 N NaOH. The incubation time was 60-70 hours. Photograph and schematic of this operation were shown in Figures 4.10 and 4.11, respectively.

4.4.3 Analysis

4.4.3.1 Determination of Cell Concentration

Biomass concentration was determined by two independent method :

1. Optical density measurement at 625 nm with a spectrophotometer : The samples were diluted in order to be in the linear range of the instrument (Figure 4.12).

2. Dry weight : A 5 ml sample of the fermentation broth was centrifuged at 4,000 rpm for 10 minutes. The cell pellet was resuspended and washed twice with distilled water and then dried for 48 hours, at 90°C, and weighed.

4.4.3.2 Determination of Product Concentrations

Acetone, n-butanol, ethanol, acetic acid and butyric acid were determined by gas chromatography using a Shimadzu Model G_c 7 A_G equipped with a flame ionization detector. Separation took place in a 2 m x 0.125 in stainless steel column packed with Porapak Q 80-100 mesh at 210°C and nitrogen was used as carrier gas. The injector temperature was 280°C and the detector temperature was 300°C. The analysis of the chromatographic data was with a Chromatopac CR14 recorder injector. Flow rate of carrier gas was 50 ml/min, retention time of butanol, acetone, ethanol, acetic acid and butyric acid are 4.09, 1.77, 1.31, 3.10, and 9.96 minutes, respectively.

4.4.3.3 Determination of Glucose Concentration

Glucose was determined by a YSI Model 27 glucose analyzer (Figure 4.13).

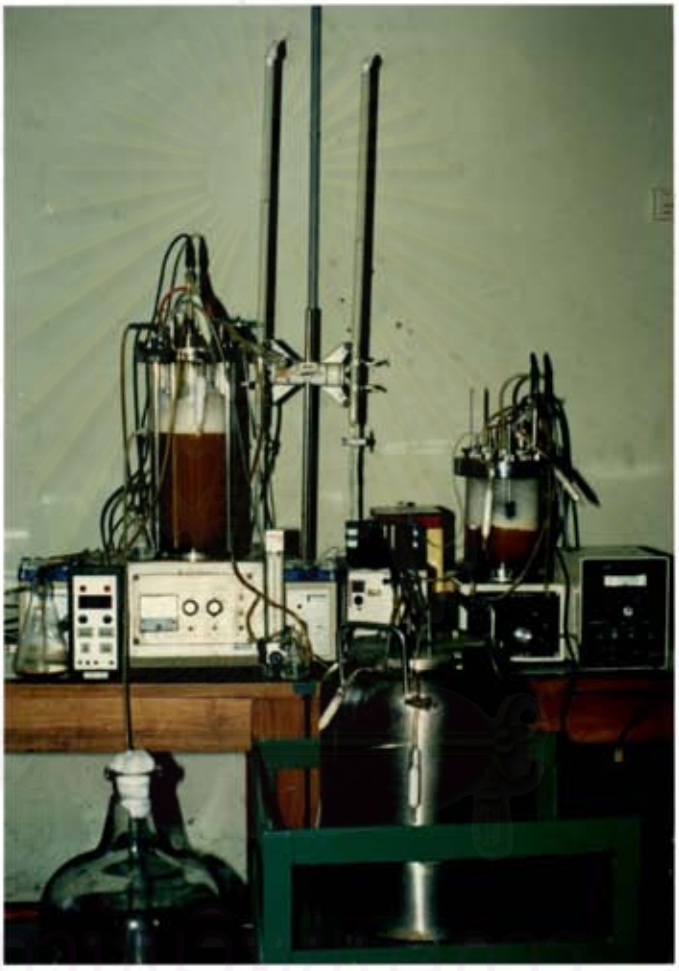

FIGURE 4.6 The two-stage continuous fermentation.

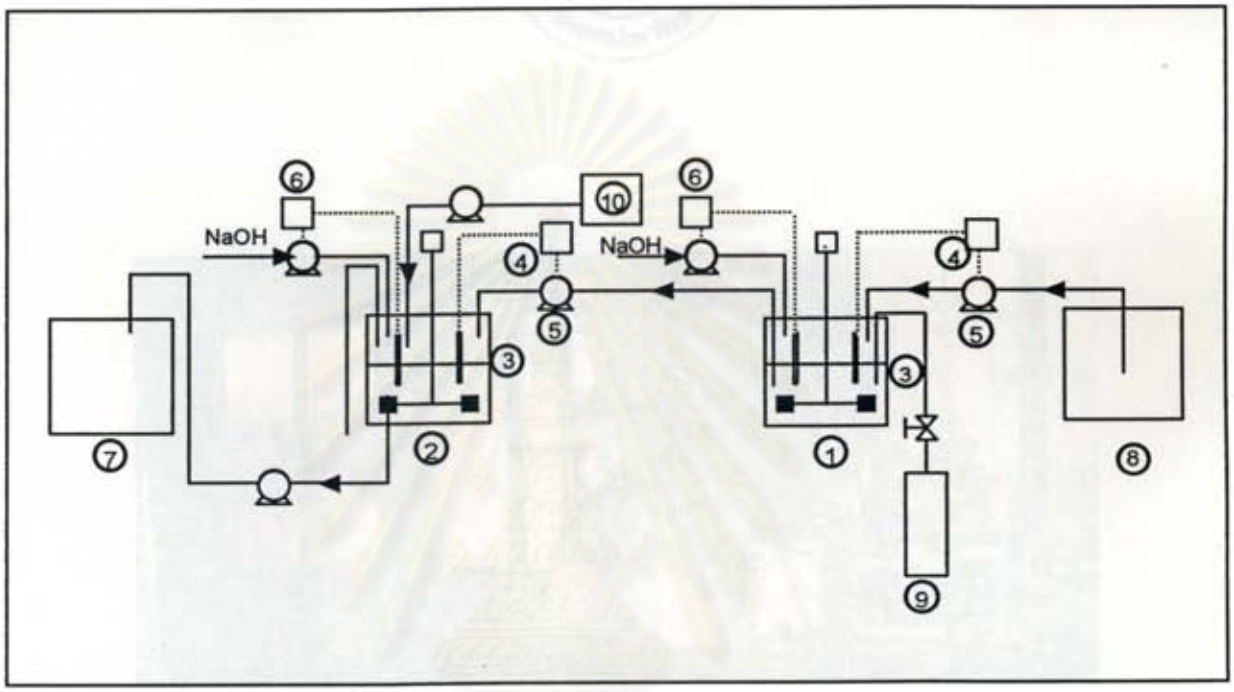

FIGURE 4.7 Schematic diagram of the two-stage continuous fermentation.

1. 1st fermenter
2. 2nd fernenter
3. level controller
4. level controller
5. feed pump
6. alkali feed pump
7. storage tank
8. feed tank
9. N_2 storage tank
10. additional feed tank

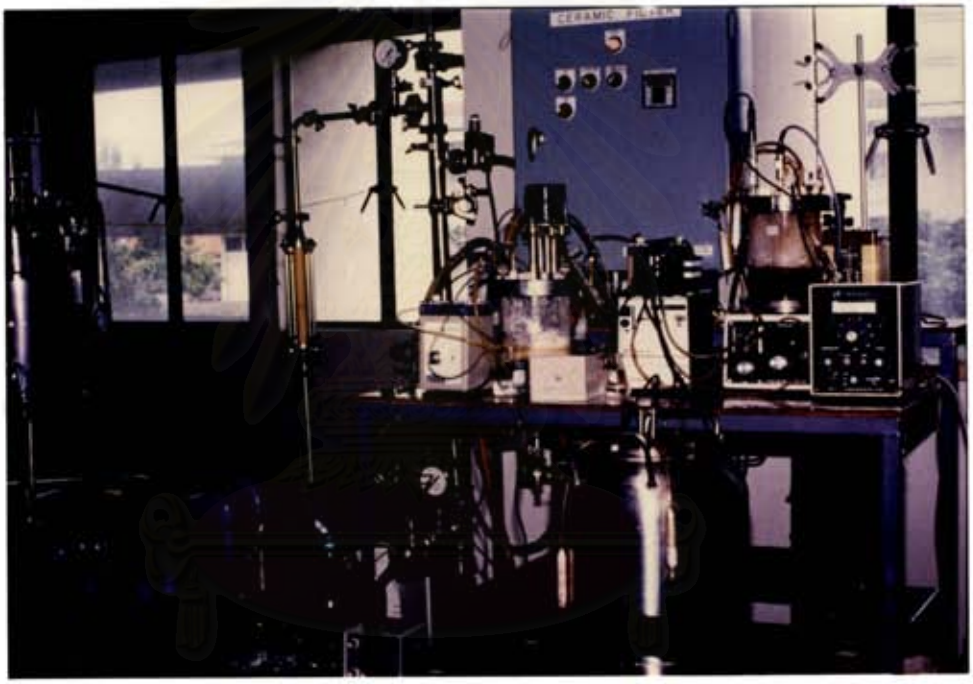

FIGURE 4.8 The two-stage continuous fermentation coupled by microfiltration.

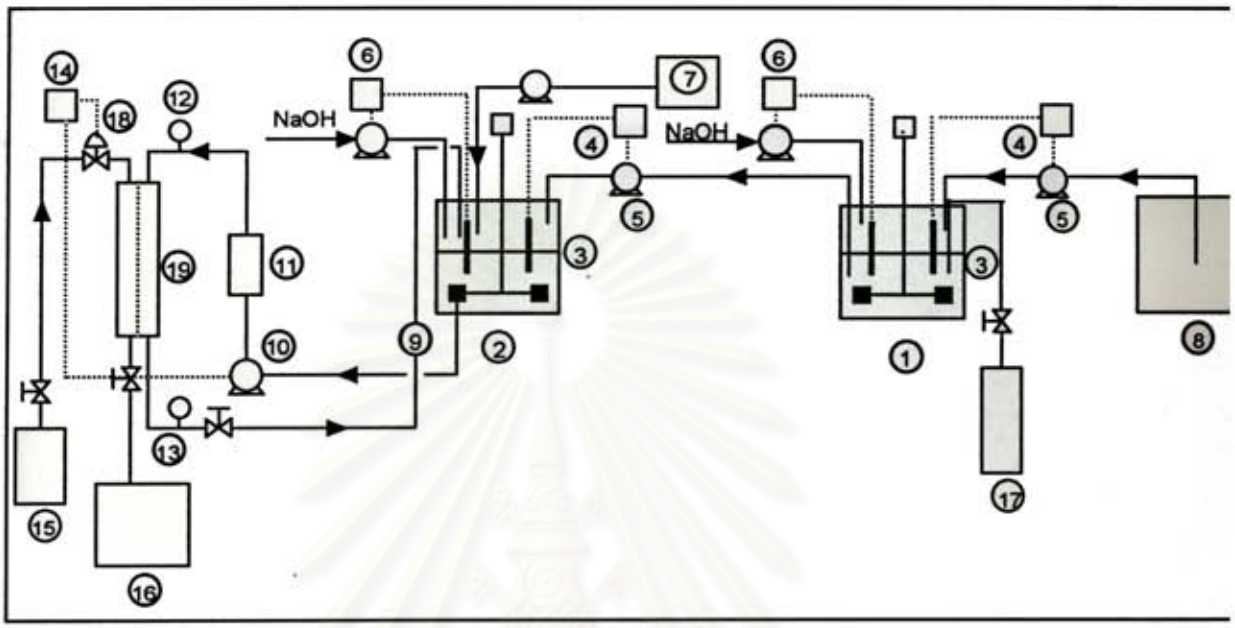

FIGURE 4.9 Schematic diagram of the two-stage continuous fermentation coupled by microfiltration.

1. 1st fermenter
2. 2nd fermenter
3. level controller
4. level controller
5. feed pump
6. alkali feed pump
7. additional feed tank
8. feed tank
9. pH controller
10. recirculate pump
11. flow meter
12. inlet pressure gauge
13. outlet pressure gauge
14. control panel
15. N_2 storage tank
16. storage tank
17. N_2 storage tank
18. control valve
19. recycling unit

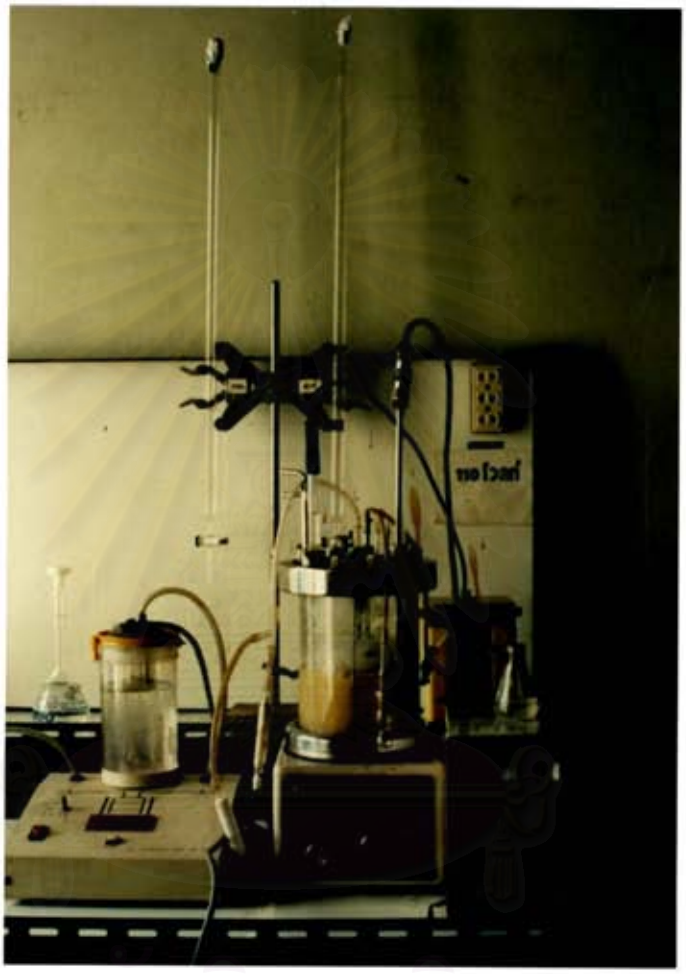

FIGURE 4.10 The batch fermentation.

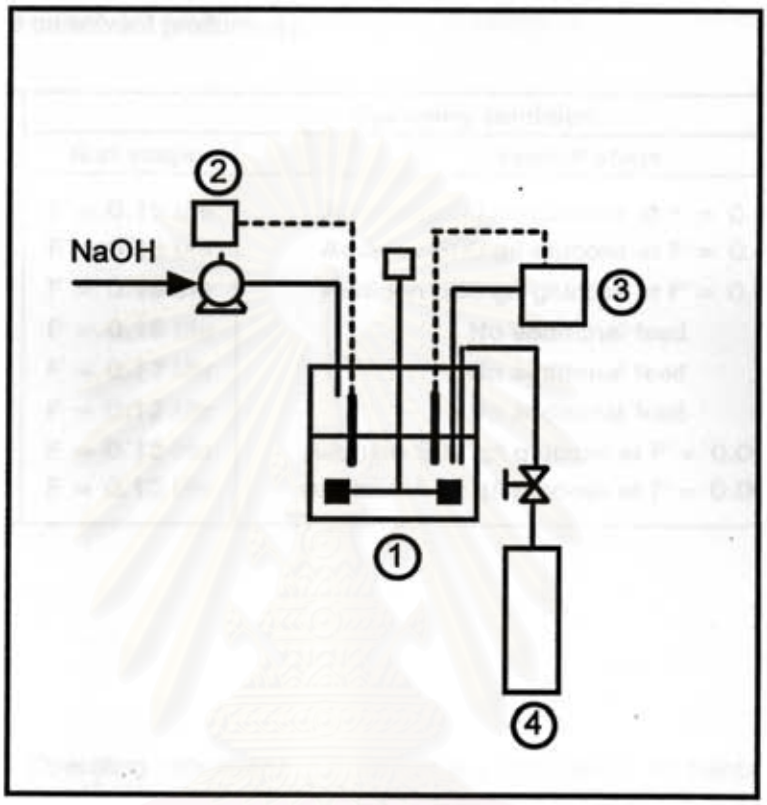

FIGURE 4.11 Schematic diagram of the batch fermentation.

1. fermenter
2. alkali feed pump
3. temperature controller
4. N_2 storage tank

TABLE 4.1 Operating conditions of two-stage continuous fermentation for studying the effect of dilution rate on solvent productivity.

Run	Operating condition	
	first stage	second stage
A	F = 0.15 l/hr	Addition 500 g/l glucose at F' = 0.03 l/hr
B	F = 0.15 l/hr	Addition 500 g/l glucose at F' = 0.02 l/hr
C	F = 0.15 l/hr	Addition 500 g/l glucose at F' = 0.01 l/hr
D	F = 0.15 l/hr	No additonal feed
E	F = 0.17 l/hr	No additonal feed
F	F = 0.12 l/hr	No additonal feed
G	F = 0.12 l/hr	Addition 500 g/l glucose at F' = 0.0045 l/hr
H	F = 0.10 l/hr	Addition 500 g/l glucose at F' = 0.0045 l/hr

TABLE 4.2 Operating conditions of two-stage continuous fermentation coupled by microfiltration for studying the effect of dilution rate on solvent productivity.

Run	Operating condition	
	first stage	second stage
I	F = 0.17 l/hr	No additonal feed
J	F = 0.17 l/hr	Addition 500 g/l glucose at F' = 0.0045 l/hr
K	F = 0.17 l/hr	Addition 500 g/l glucose at F' = 0.0157 l/hr
L	F = 0.17 l/hr	Addition 50 g/l glucose at F' = 0.55 l/hr
M	F = 0.17 l/hr	Addition 50 g/l glucose at F' = 1.323 l/hr

CHAPTER V

RESULTS AND DISCUSSIONS

5.1 The Study of Single-stage Continuous Fermentation

The experimental results of this study are shown in Figures 5.1-5.5 and Table 5.1. After the batch operation, the cell mass reached 1.72 g/l with the glucose consumption and residual glucose of 2.05 g/l-hr and 25 g/l , respectively. Then the first continuous fermentation was started at a fixed dilution rate of 0.05 hr^{-1}.

After changing from batch operation to continuous operation, the cell mass and glucose consumption began to increase after 20 hours. The operation was allowed to reach steady state and was maintained for 60 hours with the glucose consumption rate of 2.12 g/l-hr, glucose concentration of 7.5 g/l and cell mass of 1.96 g/l. The solvent concentration was 10.7 g/l and butanol was 6.37 g/l. Then the dilution rate was changed from 0.05 hr^{-1} to 0.07 hr^{-1}.

After 92 hours of the operation at 0.07 hr^{-1} dilution rate, the cell mass reached 2.10 g/l and the residual glucose was 10 g/l with 8.03 g/l solvent concentration and 5.14 g/l butanol concentration. The glucose consumption increased to 2.66 g/l-hr. The dilution rate was then changed to 0.10 hr^{-1}.

This operation at 0.10 hr^{-1} was maintained for 72 hours. The glucose concentration in the system was 11 g/l and the glucose consumption was 3.92 g/l-hr. Solvent and butanol concentration reached at 8.84 g/l and 5.08 g/l, respectively, and the cell mass increased to 2.34 g/l. After that, the dilution rate was changed to 0.12 hr^{-1}.

With the fixed dilution rate of 0.12 hr^{-1}, the cell mass was 2.23 g/l and the residual glucose was 12 g/l with 10.50 g/l solvent concentration and 5.55 g/l butanol concentration. The glucose consumption rate increased to 5.63 g/l-hr. The operation was maintained for 72 hours and then changed to a fixed dilution rate of 0.14 hr^{-1}.

The operation at 0.14 hr^{-1} dilution rate was maintained for 70 hours. The cell mass was 2.08 g/l with 9.25 g/l solvent concentration and 5.0 g/l butanol concentration. The glucose consumption was 4.62 g/l-hr and the residual glucose concentration in the fermenter was 17.5 g/l. Then the dilution rate was increased to 0.15 hr^{-1}.

About 60 hours of the operation at 0.15 hr^{-1} dilution rate, the residual glucose concentration in the fermenter reached to 18.5 g/l with 8.82 g/l solvent concentration and 4.44 g/l butanol concentration. The glucose consumption increased to 5.44 g/l-hr and the cell mass was 2.14 g/l. After that, the dilution rate was increased to 0.17 hr^{-1}.

At the dilution rate 0.17 hr^{-1}, the cell mass reached 2.17 g/l with 20.5 g/l residual glucose concentration. Solvent and butanol concentration were 8.96 g/l and 4.47 g/l, respectively, and the glucose consumption rate was 4.97 g/l-hr. After 64 hours of this operation, the dilution rate was changed to 0.18 hr^{-1}.

The operation of 0.18 hr^{-1} dilution rate was maintained for 78 hours and the cell mass was constant at approximately 2.12 g/l with 23 g/l residual glucose concentration and 4.86 g/l-hr glucose consumption. Solvent and butanol concentration were 8.44 g/l and 4.37 g/l, respectively. After that, the dilution rate was increased to 0.23 hr^{-1}.

About 40 hours of 0.23 hr^{-1}dilution rate, the cell mass decreased to 1.24 g/l and the residual glucose concentration increased to 31 g/l with the glucose consumption rate of 4.1 g/l-hr. This is because the high dilution rate led to culture washed out from the fermenter. Solvent and butanol concentration reduced to 4.42 g/l and 2.26 g/l, respectively. Finally, a run at the fixed dilution rate of 0.05 hr^{-1} was repeated for comparison with the first 0.05 hr^{-1} dilution rate.

For the second 0.05 hr^{-1} dilution rate, the average cell mass increased to 1.96 g/l with 7 g/l residual glucose concentration and 2.26 g/l-hr glucose consumption. The solvent concentration was 10.99 g/l and butanol was 6.5 g/l. This second operation was almost identical to the first one. After 106 hours of this operation, the dilution rate was decreased to 0.03 hr^{-1}.

At 0.03 hr^{-1} dilution rate, the average cell mass was 1.95 g/l with 3 g/l residual glucose concentration and 1.43 g/l-hr glucose consumption. The solvent concentration was 14.27 g/l and butanol was 7.47 g/l. The operation was maintained for 72 hours and then was stopped because cell would loose activity in the very low glucose concentration (< 5 g/l) if it is allowed to continue for a long time.

Figures 5.1, 5.2 showed the behavior of microorganism during operation of the single-stage, i.e., specific growth rate (μ), specific production rate (υ) and specific acid production rate (υ_{acid}). Table 5.1 showed the productivity and concentration of total solvent and butanol of this experiment. It could be seen that the maximum solvent productivity and cell productivity were achieved from the operation when the dilution rate was 0.17 hr^{-1} with 1.52 g/l-hr solvent productivity, 0.76 g/l-hr butanol productivity and 0.37 g/l-hr cell productivity. Therefore the optimum condition for the first stage of two-stage continuous fermentation was taken to be the dilution rate of 0.17 hr^{-1}.

In this experiment, low solvent productivity, low glucose consumption rate but high butanol concentration were achieved while the dilution rate was rather low. This could be explained by the inhibitory effect of butanol on glucose uptake. Moriera et al. (1985) and Bowles et al. (1985) reported that at high concentration (> 7 g/l approximately), butanol could inhibit the rate of glucose uptake into cells for growth and solvent production. Thus, the glucose consumption rate was limited by butanol concentration. For increasing dilution rate, solvent productivity, growth rate and glucose consumption rate were higher because the butanol concentration decreased due to glucose and cell removal. Also, the glucose consumption rate and growth rate were limited by the dilution rate. At a high dilution rate, solvent productivity, growth rate and glucose consumption rate decreased because the culture was washed out from the fermenter.

The comparison of the first run with 0.05 hr^{-1} dilution rate with the second is shown in the same Table 5.1. The second result was almost identical to the first and it indicated that the fermenter still had a lot of active cells so there was no need to repeat other experiments in order to check cell activity.

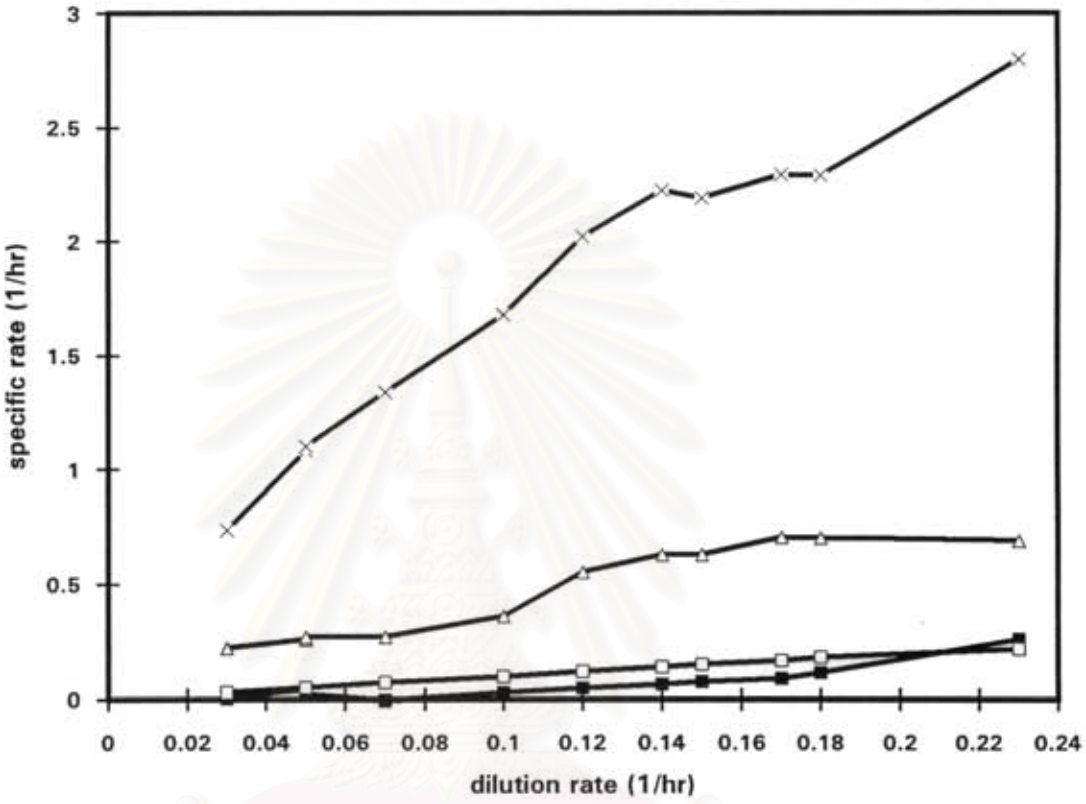

FIGURE 5.1 Influence of the dilution rate on specific substrate consumption rate(υ_s), specific solvent production rate (υ), specific acid production rate (υ_{acid}) and specific growth rate (μ) at pH 4.8, temperature of 35 °C and steady state condition of a single-stage continuous fermentation of Cl.acetobutylicum.

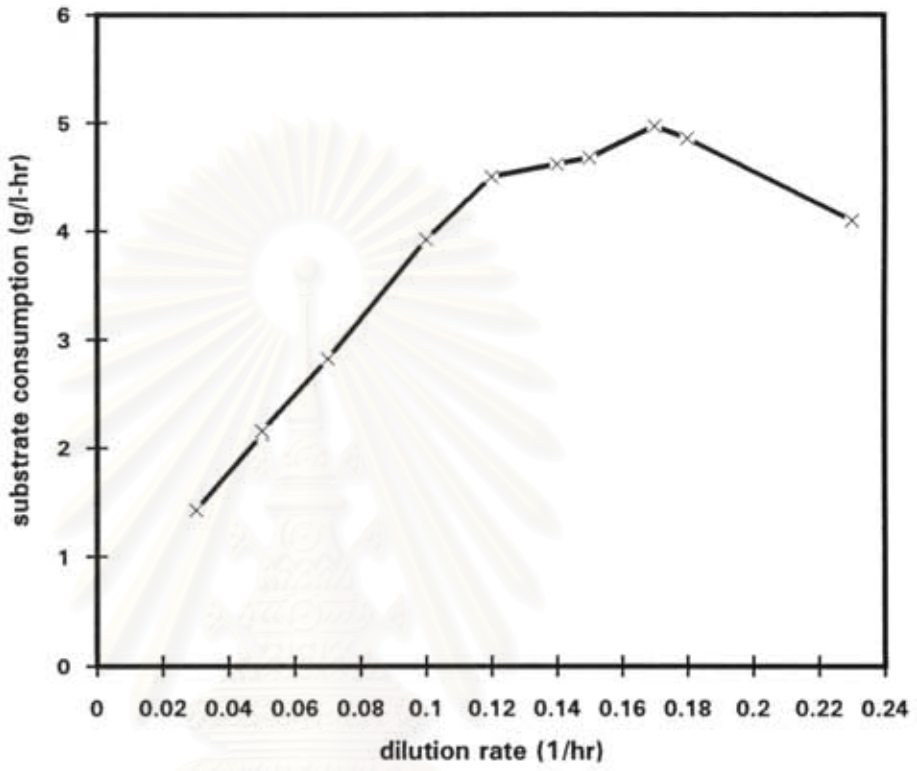

FIGURE 5.2 Influence of the dilution rate on the substrate consumption rate (r_s) at pH 4.8, temperature of 35 °C and steady state condition of a single-stage continuous fermentation of Cl.acetobutylicum.

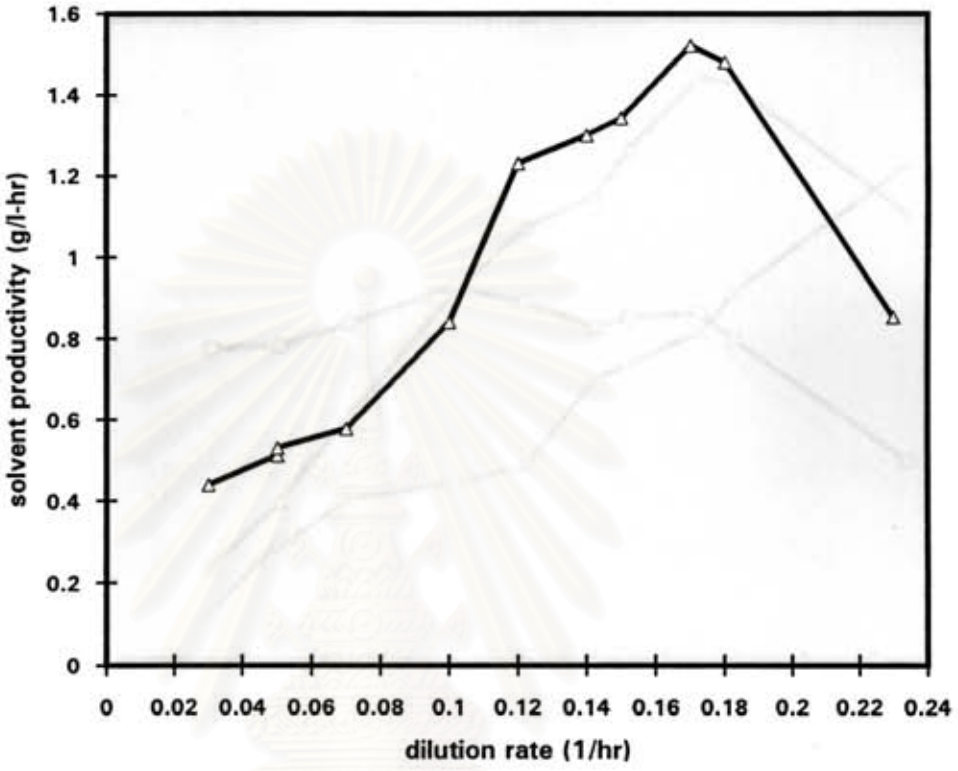

FIGURE 5.3 Influence of the dilution rate on the solvent productivity (r_p) at pH 4.8, temperature of 35 °C and steady state condition of a single-stage continuous fermentation of Cl.acetobutylicum.

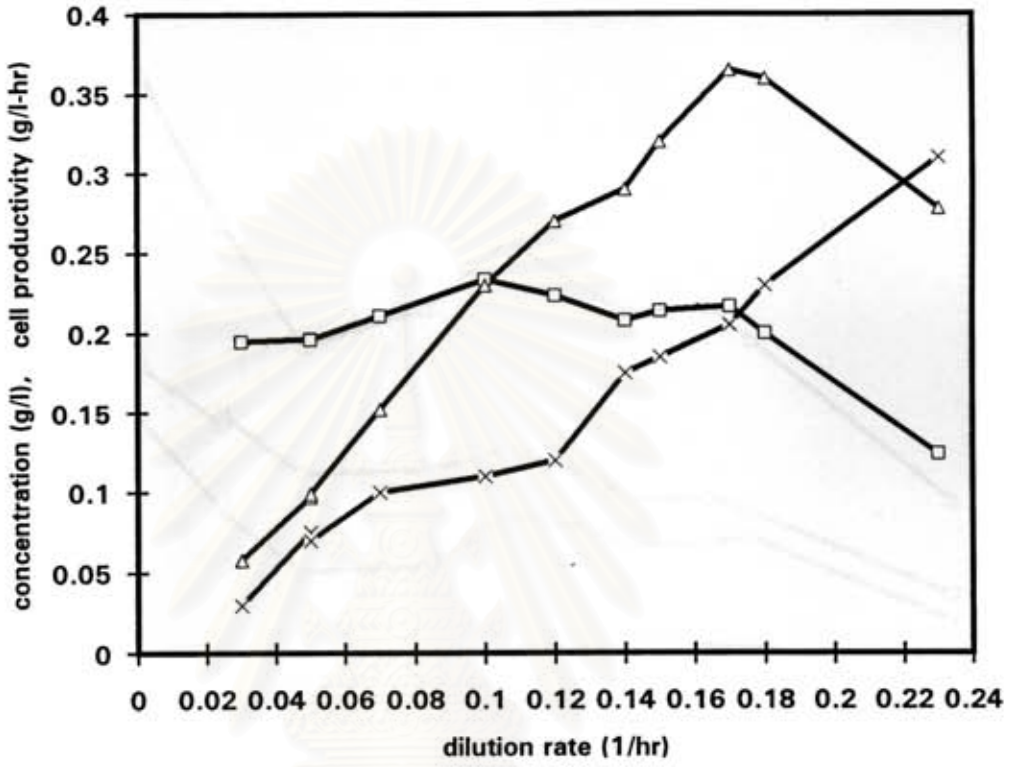

FIGURE 5.4 Influence of the dilution rate on cell concentration, glucose concentration and cell productivity (r_x) at pH 4.8, temperature of 35 °C and steady state condition of a single-stage continuous fermentation of Cl.acetobutylicum.

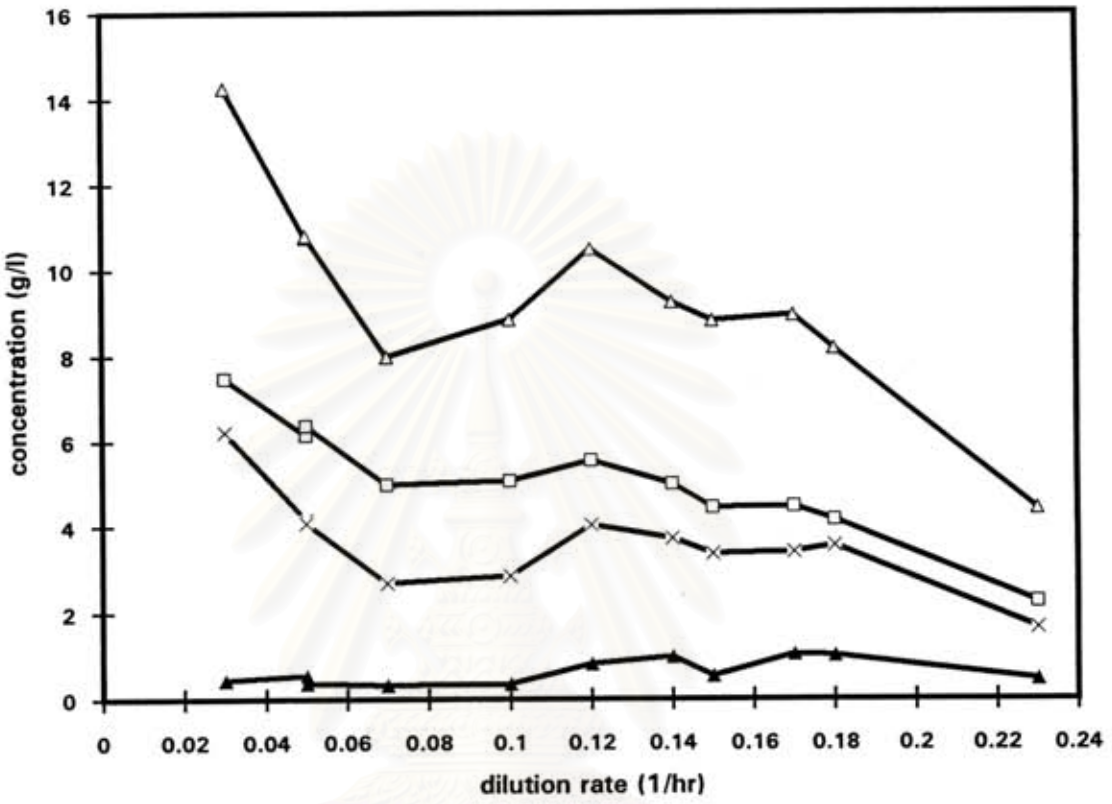

FIGURE 5.5 Influence of the dilution rate on the butanol, acetone, ethanol, and total solvent at pH 4.8, temperature of 35 ºC and steady state condition of a single-stage continuous fermentation of Cl.acetobutylicum.

TABLE 5.1 The results of single-stage continuous fermentation of Cl.acetobutylicum.

System	Dilution rate (1/hr)	Solvent concentration (g/l)	Butanol concentration (g/l)	Solvent productivity (g/l-hr)	Butanol productivity (g/l-hr)
Single-stage	0.03	14.27	7.47	0.44	0.22
	0.05	10.77	6.15	0.51	0.31
Repeated	0.05	10.81	6.37	0.53	0.32
	0.07	7.98	4.98	0.58	0.35
	0.10	8.84	5.07	0.84	0.51
	0.12	10.50	5.55	1.23	0.67
	0.14	9.25	5.00	1.30	0.70
	0.15	8.82	4.44	1.34	0.67
	0.17	**8.96**	**4.47**	**1.52**	**0.76**
	0.18	8.17	4.15	1.48	0.75
	0.23	4.42	2.26	1.01	0.52

5.2 The Study of The Two-stage Continuous Fermentation (Runs A-H)

The results of this experiment are shown in Figures 5.6-5.13 and Table 5.2. After 10 hours of the batch operation, the cell mass reached 1.50 g/l and the residual glucose was 25 g/l. Then first continuous fermentation was started at a fixed dilution rate of 0.15 hr^{-1} while 500 g/l glucose concentration was added at a fixed flow rate of 0.03 l/hr or equivalent dilution rate of 0.06 hr^{-1} to the second stage fermenter.

After 20 hours of the continuous operation, the volume of the second stage fermenter became constant at 3 litres. In the first stage fermenter with the dilution rate of 0.15 hr^{-1}, the average cell mass was 2.16 g/l and the residual glucose was 18.25 g/l. The glucose consumption was 4.82 g/l-hr with 9.11 g/l solvent concentration and 4.53 g/l butanol concentration approximately. In the second stage fermenter, cell mass and residual glucose were 1.71 g/l and 65 g/l, respectively. The glucose consumption was 3.49 g/l-hr with 12.44 g/l solvent concentration and 6.23 g/l butanol concentration. This operation (Run A) was maintained for 144 hours and then the flow rate of the 500 g/l glucose additional feed was decreased from 0.03 l/hr to 0.02 l/hr while the dilution rate of the first stage was kept constant at 0.15 hr^{-1}

At the 0.02 l/hr flow rate of additional feed which is equivalent to the dilution rate of 0.057 hr^{-1} (Run B), the cell mass reached at 2.12 g/l and the residual glucose decreased to 25 g/l with the glucose consumption rate of 5.43 g/l-hr. Solvent and butanol concentration were 12.58 g/l and 6.53 g/l, respectively, in the second stage. After 102 hours of this operation, the additional feed flow rate was decreased to 0.01 l/hr or a 0.053 hr^{-1} dilution rate (Run C).

In the second stage, the 0.053 hr^{-1} dilution rate was maintained for 132 hours. The cell mass was 2.07 g/l with the glucose consumption rate at 3.06 g/l-hr and residual glucose was 16 g/l. Solvent and butanol concentration were 13.25 g/l and 7.12 g/l, respectively. Then the additional feed was stopped.

For this run without additional feed (Run D), we obtained 2.23 g/l cell mass and 3.5 g/l residual glucose. Solvent and butanol concentration were 15.29 g/l and 8.44 g/l, respectively,

with the glucose consumption of 1.65 g/l-hr. After 96 hours operation, the dilution rate of the first stage fermenter was increased to 0.17 hr^{-1}.

In the first stage fermenter at the fixed dilution rate of 0.17 $hr^{-1}$and with no additional feed into the second stage (Run E), the cell mass and residual glucose were 2.17 g/l and 21 g/l, respectively, with the glucose consumption of 5.01 g/l-hr. The solvent concentration was 8.89 g/l and the butanol concentration was 4.0 g/l. In the second stage fermenter with the 0.057 hr^{-1} dilution rate, the cell mass reached 2.12 g/l, the residual glucose was 9 g/l and the glucose consumption was 1.38 g/l-hr. Solvent and butanol concentration were 15.04 g/l and 8.53 g/l, respectively. After 96 hours, the dilution rate was decreased from the 0.17 hr^{-1} in the first stage fermenter to 0.12 hr^{-1}(Run F).

At the 0.12 hr^{-1}dilution rate, with no additional feed to the second stage fermenter, the cell mass was 2.38 g/l and the residual glucose was 13 g/l. The glucose consumption was 4.41 g/l-hr with 11.15 g/l solvent concentration and 6.05 g/l butanol concentration in the first stage fermenter. In the second stage fermenter with a 0.04 hr^{-1} dilution rate, the cell mass remained constant at 2.18 g/l and the residual glucose decreased to 2.0 g/l with 1.68 g/l-hr glucose consumption. Solvent and butanol concentration were 16.07 g/l and 9.0 g/l, respectively. After 24 hours, the next run with additional feed of 500 g/l glucose with a fixed flow rate of 0.0045 l/hr into the second stage fermenter was started.

For this run with additional glucose at a fixed flow rate of 0.0045 l/hr or a 0.0415 hr^{-1} dilution rate (Run G) into the second stage fermenter, the cell mass was 2.36 g/l and the residual glucose increased to 13.5 g/l with the glucose consumption rate of 1.79 g/l-hr. Solvent and butanol concentration were 18.13 g/l and 10.58 g/l, respectively, in the second stage fermenter. After 84 hours of this operation, the dilution rate of the first stage fermenter was changed from 0.12 hr^{-1} to 0.10 hr^{-1} with the same additional glucose at the flow rate of 0.0045 l/hr or a 0.035 hr^{-1} dilution rate into the second stage fermenter.

In the first stage fermenter for this run, the cell mass and residual glucose were 1.86 g/l and 10 g/l, respectively, with the glucose consumption of 4.03 g/l-hr. The solvent concentration was 10.07 g/l and the butanol concentration was 5.04 g/l. In the second stage

fermenter with the 0.035 hr^{-1}dilution rate, the cell mass was 1.86 g/l with 14.0 g/l residual glucose and the glucose consumption rate of 2.13 g/l-hr. Solvent and butanol concentration were 16.51 g/l and 9.77 g/l, respectively. This operation was maintained for 88 hours (Run H) and then the experiment was stopped.

Figures 5.6, 5.8 and 5.9 showed the behavior of microorganism during operation in the form specific growth rate (μ), specific production rate (υ) and specific acid production rate (υ_{acid}), respectively. Table 5.2 showed the productivity and concentration of total solvent and butanol in this experiment.

It can be seen that the overall maximum productivity was achieved when the dilution rate was 0.17 hr^{-1} and with no additional feed into the second stage fermenter (overall dilution rate was 0.0425 hr^{-1} or Run E) resulting in 15.04 g/l solvent concentration and 8.53 g/l butanol concentration. According to the experiment, the influence of the dilution rate on the behavior of microorganism in the first stage was almost the same as that in the single-stage continuous fermentation. In the second stage fermenter, specific growth rate, glucose consumption and specific solvent productivity were clearly lower than the first stage continuous while solvent and butanol concentration were higher because of the inhibitory effect of butanol on glucose uptake. Cells were mainly produced in the first stage where a small cell growth was maintained in the second stage.

From Runs A, B and C, which 500 g/l glucose was added, the low productivity might be the result of the very excessive residual glucose with very high feeding glucose concentration. Another factor that might reduce solvent productivity was the effect of burned glucose. In many fermentation processes, the amount of burned glucose could reduced the activity of microorganism to produce solvent. The burned glucose occurred when high glucose concentration in the broth was kept at high temperature for a long time. Comparing Run B with Run E which operated at the same dilution rate of 0.057 hr^{-1} by feeding and not feeding excessive glucose, respectively, it can be seen that the effect of butanol on glucose uptake was stronger than the effect of burned glucose.

Comparing the second stage with the first stage, the acid formation was very low while solvent formation was higher because acid was changed almost completely to acetone and butanol. The overall solvent productivity of this fermentation was 0.63 g/l-hr at 0.0425 hr^{-1} overall dilution rate which was slightly higher than the solvent productivity of single-stage continuous culture under nearly the same overall dilution rate (i.e., 0.54 g/l-hr at 0.05 hr^{-1} dilution rate for the single-stage continuous). When comparing the two-stage (Run E) with the single-stage (at 0.17 hr^{-1} dilution rate), it can be shown that the solvent productivity of the single-stage was 1.4 times higher than the two-stage but the solvent concentration of the two-stage was 1.7 times which was more satisfied for separation economically. However, the two-stage fermentation could also be achieved to increase the longevity of the continuous fermentation at high solvent concentration, as a result of the continuous feeding of fresh cells to the second stage. Additional gains in productivity might still be expected by coupling the second stage with a microfiltration device so as to reach high cell concentration by cell recycling (Godin et al., 1988).

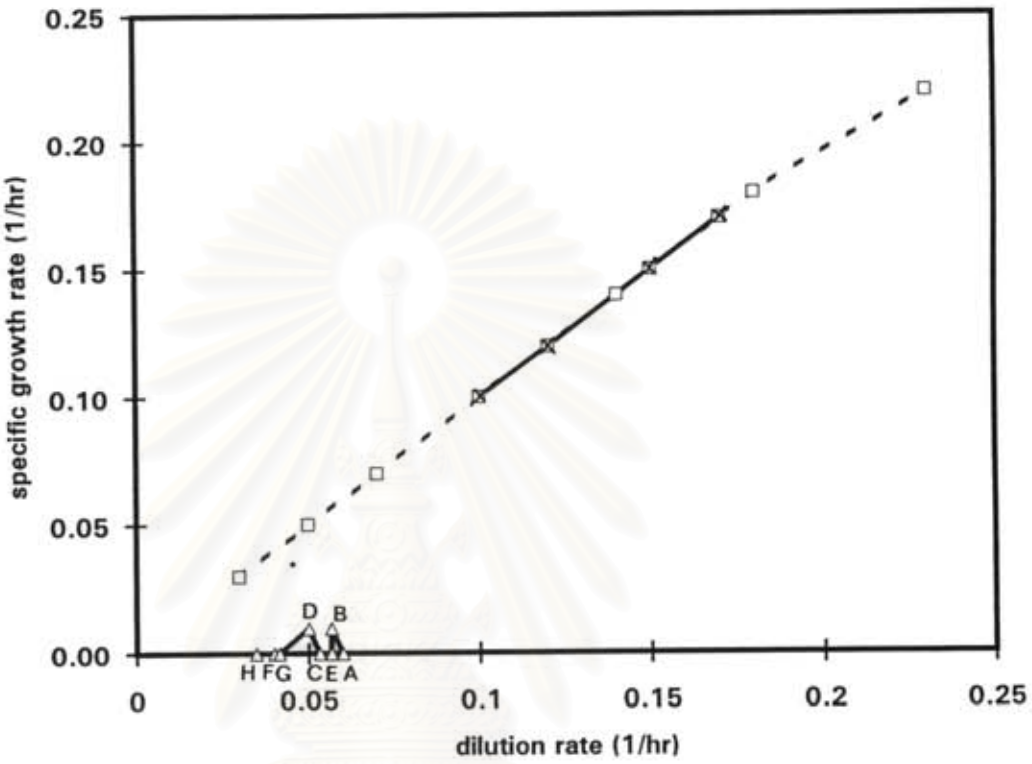

FIGURE 5.6 Influence of the dilution rate on specific growth rates (μ) at pH 4.8, temperature of 35 °C and steady state condition of a two-stage continuous compared with a single-stage continuous fermentation of Cl.acetobutylicum.

* A-H denoted the operating condition (see Table 4.1).

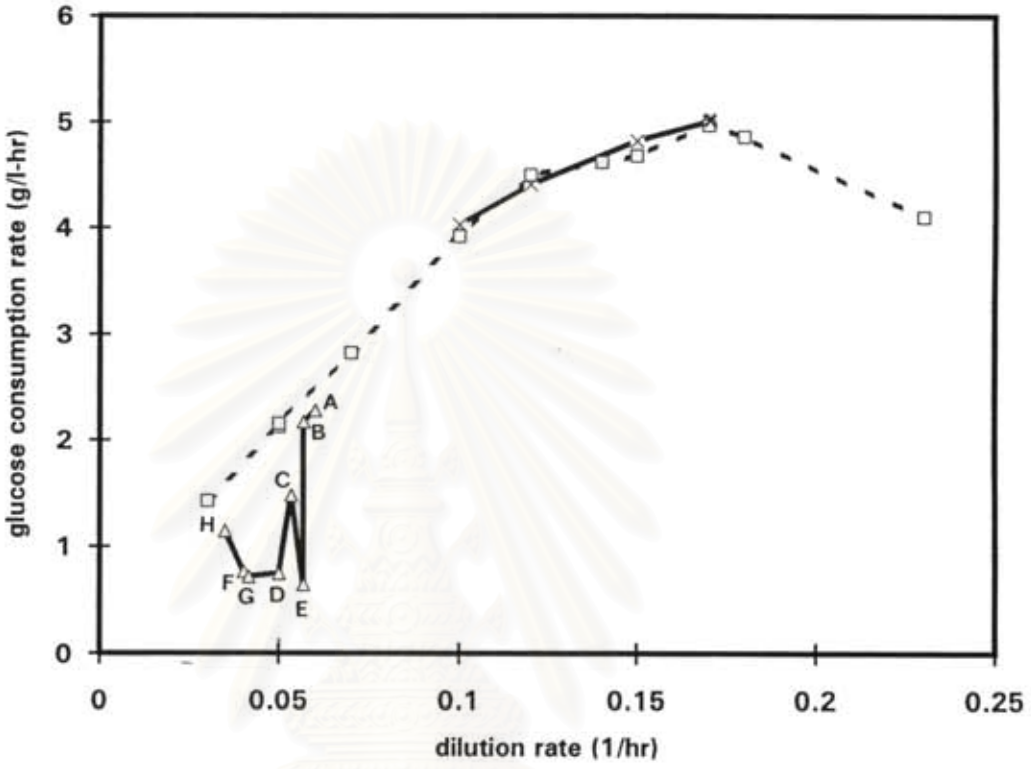

FIGURE 5.7 Influence of the dilution rate on glucose consumption rates (r_s) at pH 4.8, temperature of 35 °C and steady state condition of a two-stage continuous compared with a single-stage continuous fermentation of Cl.acetobutylicum.

* A-H denoted the operating condition (see Table 4.1).

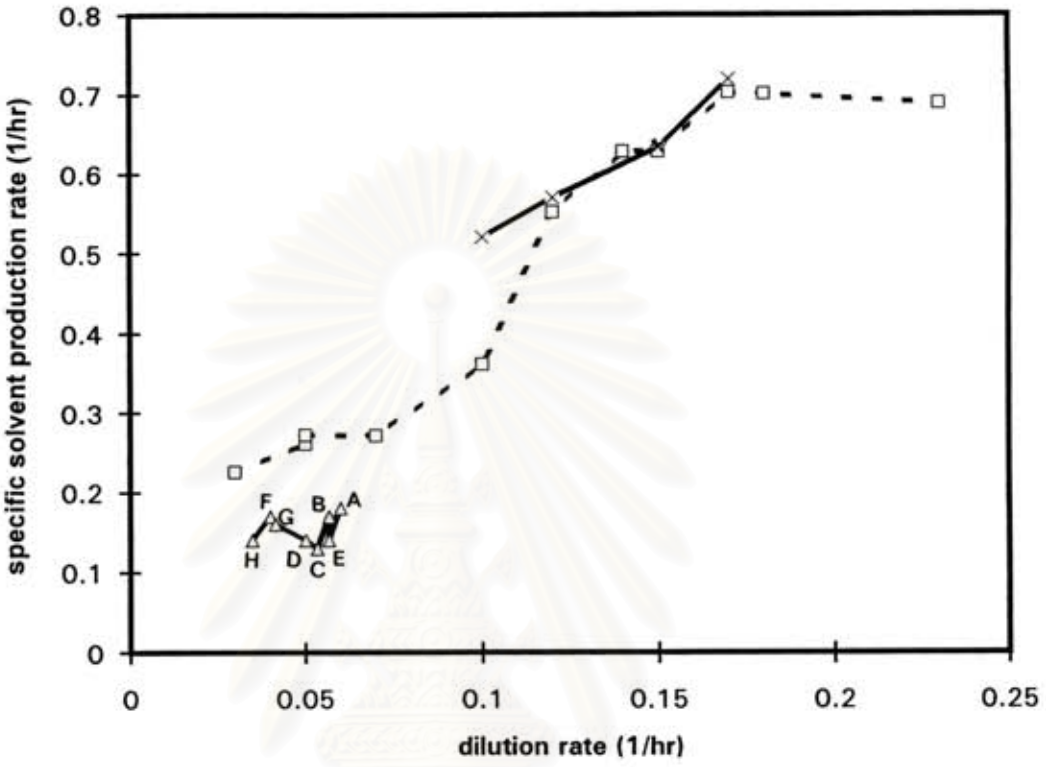

FIGURE 5.8 Influence of the dilution rate on specific solvent productivities (υ) at pH 4.8, temperature of 35 °C and steady state condition of a two-stage continuous compared with a single-stage continuous fermentation of Cl.acetobutylicum.

* A-H denoted the operating condition (see Table 4.1).

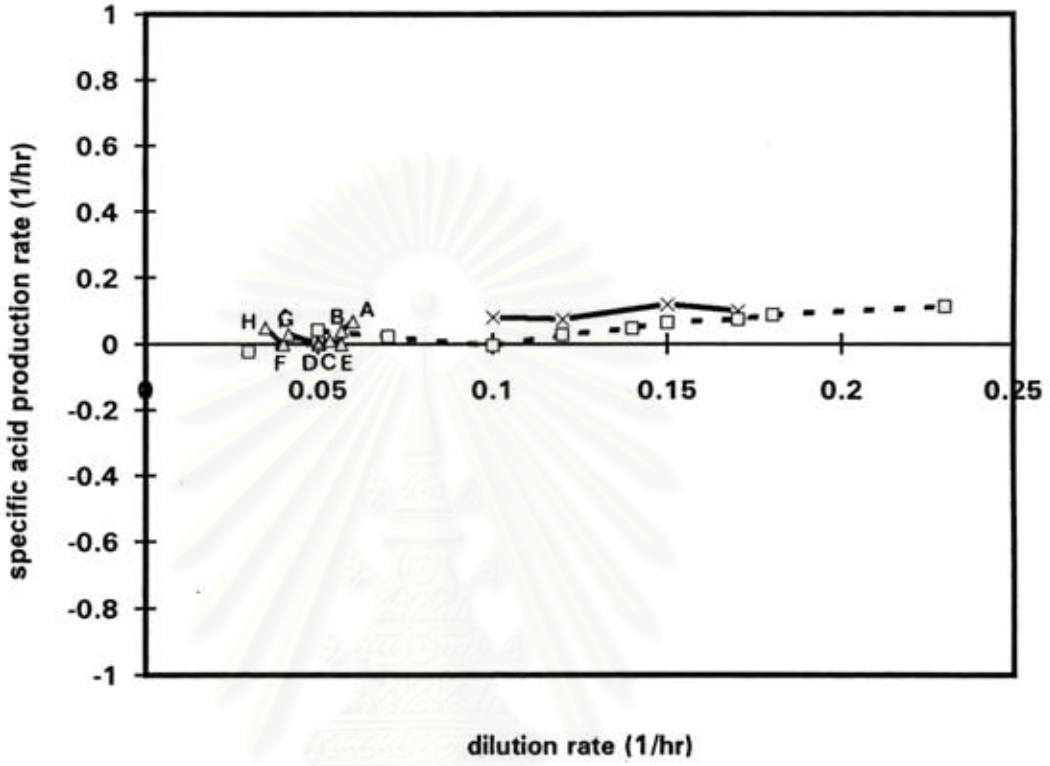

FIGURE 5.9 Influence of the dilution rate on specific acid productivities (υ_{acid}) at pH 4.8, temperature of 35 ºC and steady state condition of a two-stage continuous compared with a single-stage continuous fermentation of Cl.acetobutylicum.

* A-H denoted the operating condition (see Table 4.1).

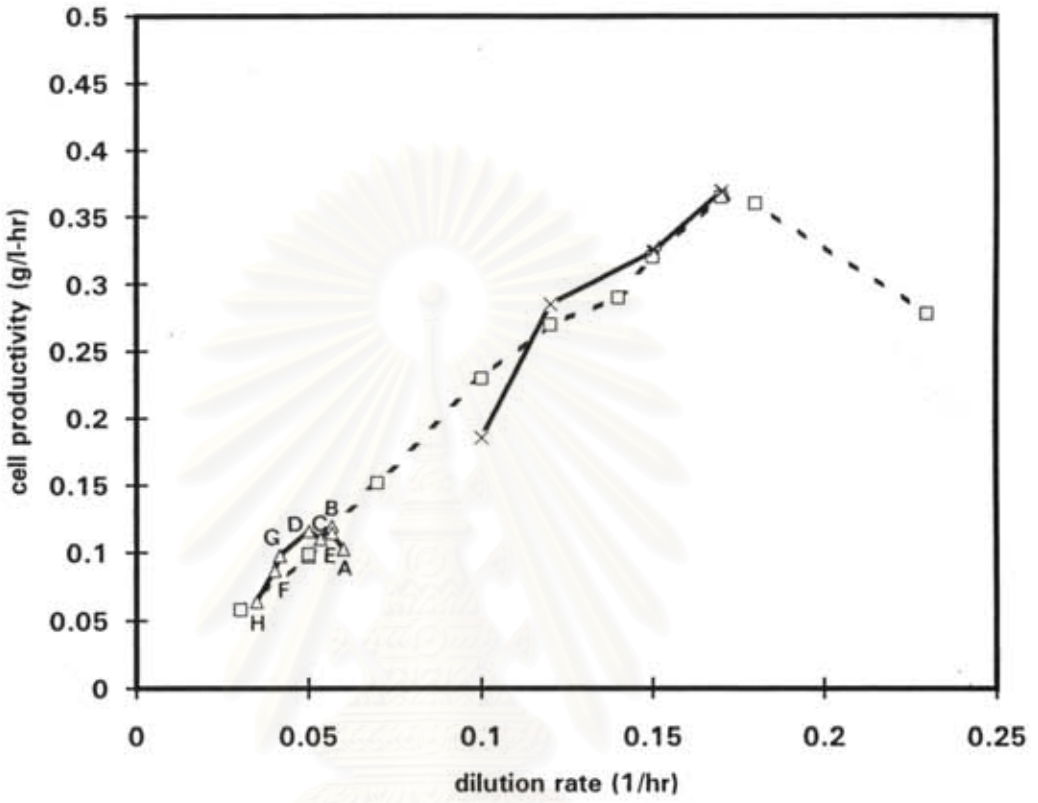

FIGURE 5.10 Influence of the dilution rate on cell productivities (r_x) at pH 4.8, temperature of 35 °C and steady state condition of a two-stage continuous compared with a single-stage continuous fermentation of Cl.acetobutylicum.

* A-H denoted the operating condition (see Table 4.1).

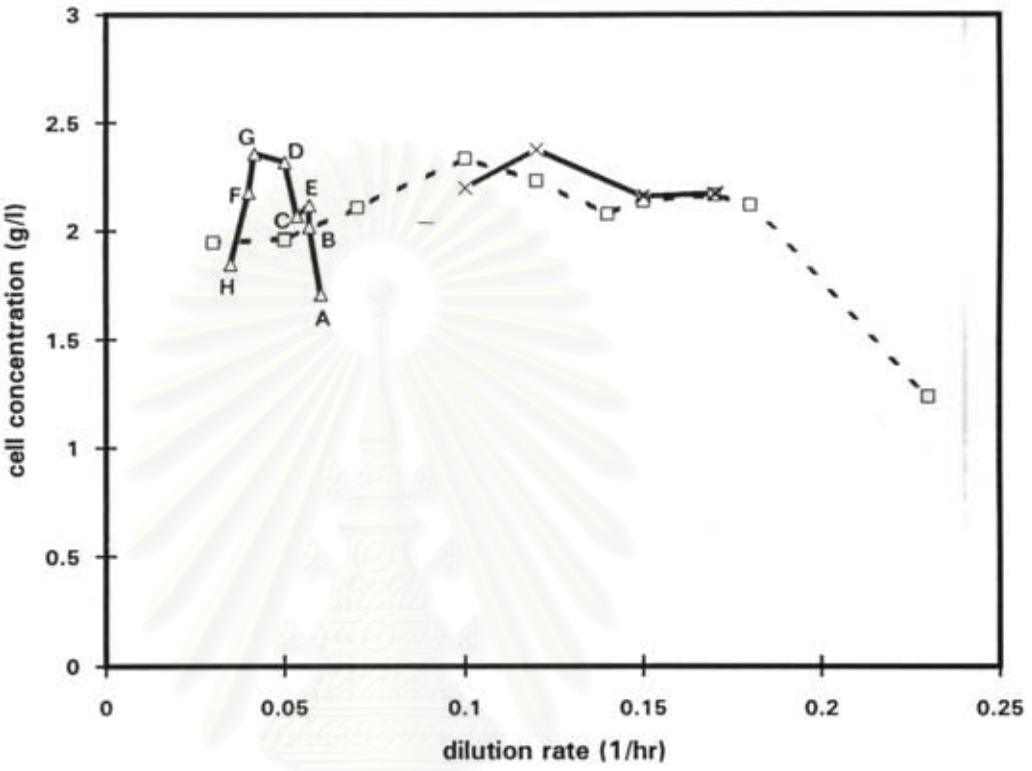

FIGURE 5.11 Influence of the dilution rate on cell concentrations at pH 4.8, temperature of 35 °C and steady state condition of a two-stage continuous compared with a single-stage continuous fermentation of Cl.acetobutylicum.

* A-H denoted the operating condition (see Table 4.1).

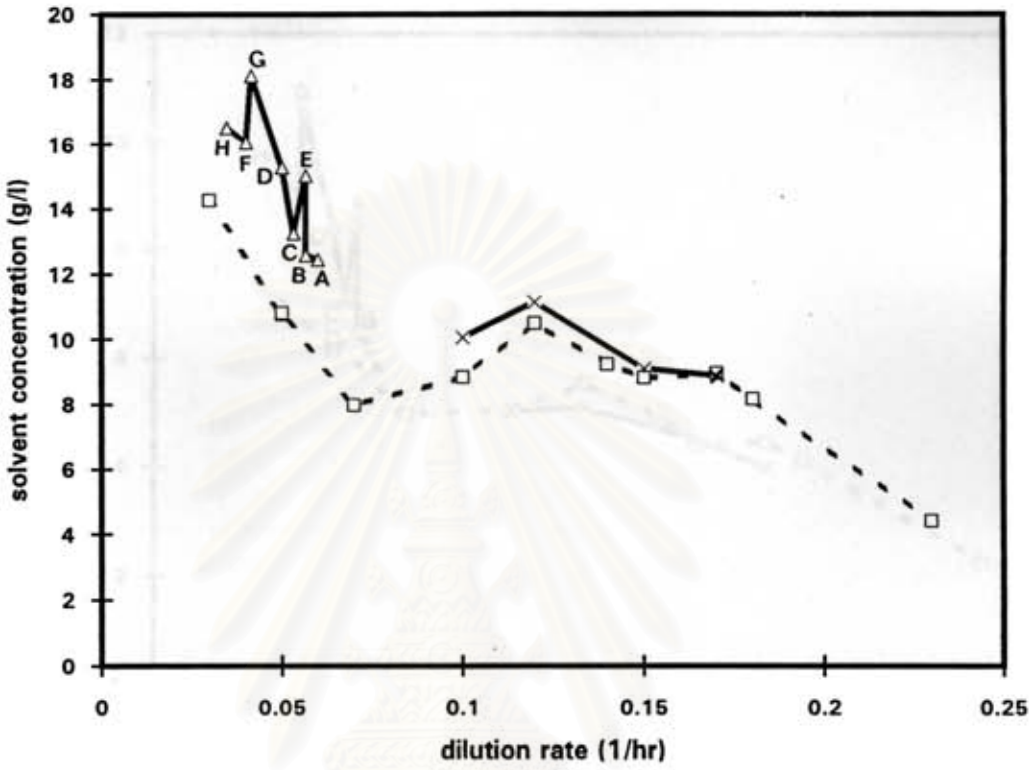

FIGURE 5.12 Influence of the dilution rate on solvent concentrations at pH 4.8, temperature of 35 °C and steady state condition of a two-stage continuous compared with a single-stage continuous fermentation of Cl.acetobutylicum.

* A-H denoted the operating condition (see Table 4.1).

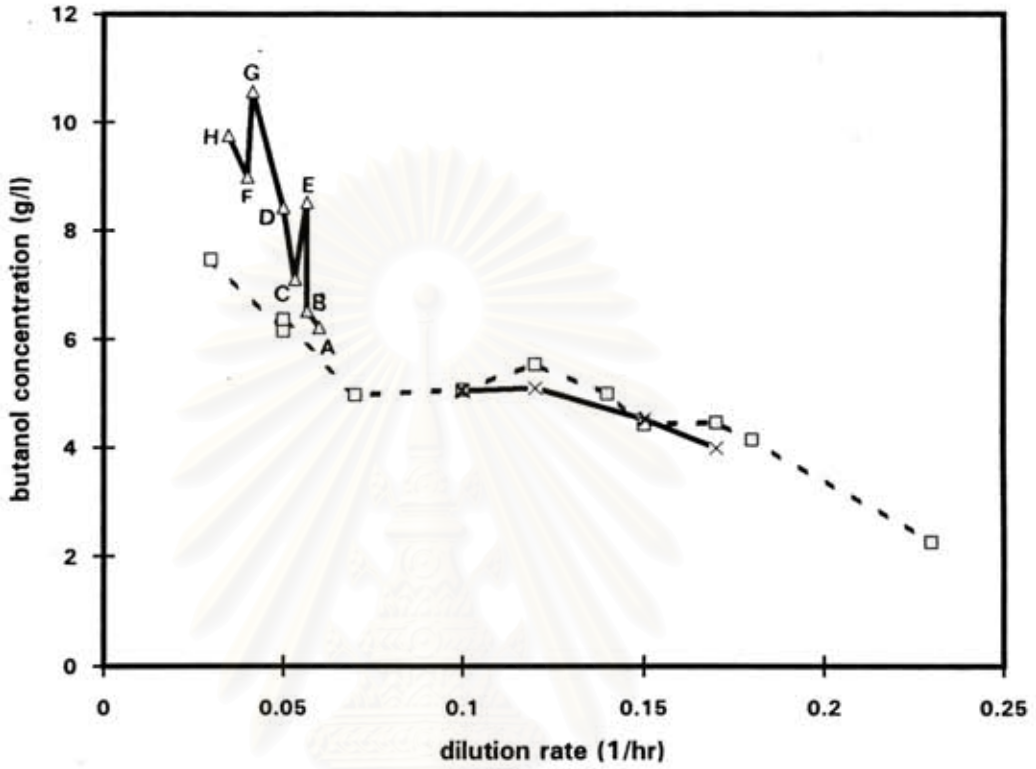

FIGURE 5.13 Influence of the dilution rate on butanol concentrations at pH 4.8, temperature of 35 °C and steady state condition of a two-stage continuous compared with a single-stage continuous fermentation of Cl.acetobutylicum.

* A-H denoted the operating condition (see Table 4.1).

TABLE 5.2 The results of two-stage continuous fermentation of Cl.acetobutylicum.

Operating condition	Overall dilution rate (1/hr)	Dilution rate of		Solvent concentration (g/l)	Butanol concentration (g/l)	Solvent productivity (g/l-hr)	Butanol productivity (g/l-hr)
		first stage	second stage				
Run A	0.045	0.15	0.06	12.45	6.23	0.56	0.28
Run B	0.0425	0.15	0.057	12.61	6.53	0.54	0.28
Run C	0.04	0.15	0.053	13.30	7.12	0.53	0.28
Run D	0.0375	0.15	0.05	15.24	8.44	0.57	0.32
Run E	**0.0425**	**0.17**	**0.057**	**14.94**	**8.53**	**0.63**	**0.36**
Run F	0.03	0.12	0.04	16.07	9.00	0.48	0.27
Run G	0.031	0.12	0.042	18.21	10.58	0.56	0.33
Run H	0.026	0.10	0.035	16.45	9.77	0.43	0.25

5.3 The Study of Two-stage Fermentation Couple by Microfiltration (Runs I-M)

The result of this experiment are shown in Figures 5.14-5.20 and Table 5.3. After the batch operation, the cell mass reached 1.83 g/l and the residual glucose was 25 g/l with glucose consumption rate of 1.50 g/l-hr, respectively. Then the first continuous fermentation was started at a fixed flow rate of 0.17 l/hr and 0.17 hr^{-1}dilution rate (Run I).

After changing from batch operation to continuous operation, the cell mass and glucose consumption began to increased rapidly. After 63 hours, the second stage fermentation clearly showed shortage of glucose with 3 g/l residual glucose concentration, 19.05 g/l cell mass and the consumption rate 0.762 g/l-hr. Solvent and butanol concentration were 13.93 g/l and 7.83 g/l, respectively. Then 500 g/l glucose was added to the second stage fermenter at a fixed flow rate of 0.0045 l/hr (Run J). In this case, cell mass and residual glucose in the first stage fermenter fluctuated between 1.70-1.84 g/l and 20-22 g/l, respectively, with 7.18-8.13 g/l solvent concentration, 3.68-3.89 g/l butanol concentration and 4.66-5.02 g/l-hr glucose consumption rate.

With the addition of 500 g/l glucose at the flow rate of 0.0045 l/hr (Run J), the dilution rate for the second stage was 0.0646 hr^{-1}. After 30 hours, the shortage of glucose occurred in the second stage fermenter with 38.42 g/l cell mass, 5 g/l residual glucose concentration and 1.20 g/l-hr glucose consumption rate. The solvent concentration was 16.26 g/l and the butanol concentration was 8.66 g/l. Then the flow rate of 500 g/l glucose was increased to 0.0157 l/hr (Run K).

After 42 hours of increasing the flow rate of 500 g/l glucose to 0.0157 l/hr which is equivalent to 0.069 hr^{-1}dilution rate of the second stage, the cell mass reached at 45.49 g/l with 16.78 g/l solvent concentration and 9.90 g/l butanol concentration. The glucose consumption rate was 2.89 g/l-hr in the second stage and the residual glucose was 19 g/l.

After that, the residual glucose concentration increased to 39 g/l because cell lysis and loss of glucose consuming activity occurred. Therefore, a part of cells were removed from the second stage fermenter and additional feed of 50 g/l glucose was introduced at a fixed flow rate of 0.55 l/hr or a 0.267 hr^{-1}dilution rate.

In the second stage fermenter with additional feed of 50 g/l glucose at the 0.267 hr^{-1} dilution rate (Run L), the cell mass increased to 71.03 g/l with 12.57 g/l solvent concentration. The glucose consumption rate was rather constant at 8.75 g/l-hr and the residual glucose show sign of shortage at 9 g/l after 124 hours. Then the flow rate of 50 g/l glucose was increased to 1.323 l/hr (Run M).

At the fixed flow rate of 1.323 l/hr or a 0.55 hr^{-1} dilution rate of the second stage, the cell mass remained constant at 80.06 g/l. The residual glucose was 14 g/l and the average total solvent and butanol concentration were 14.01 g/l and 7.09 g/l, respectively. The glucose consumption reached at 17.79 g/l-hr. After 118 hours of operation, the high cell concentration reduced permeate flux of the recycling unit lower than the point which the 0.55 hr^{-1} dilution rate could be maintained. So the experiment was stopped.

Figures 5.14 and 5.16 showed the behavior of microorganism during operation in terms of specific growth rate (μ) and specific production rate (υ). Table 5.3 showed the productivity and concentration of total solvent and butanol as functions of the dilution rate. Glucose consumption, cell concentration and residual glucose concentration were demonstrated in Figures 5.14, 5.17 and 5.18, respectively. From Table 5.3, the solvent productivity rose with increasing dilution rate and attained a maximum value of 5.65 g/l-hr at 0.40 hr^{-1} overall dilution rate (Run M) with 14 g/l solvent and 7.09 g/l butanol concentration.

In this experiment, the first stage was kept constant at the fixed dilution rate of 0.17 hr^{-1} and the second stage was coupled by the microfiltration in order to increase the cell concentration in the fermenter. As could be seen from Table A7, in the second stage, growth rate (r_x), consumption rate (r_s) and solvent productivity (r_p) increased with the dilution rate because of the increase in cell concentration and the decrease in butanol concentration. At the same time, the ability of microorganism to grow, i.e., the specific growth rate (μ), the product formation, i.e., the specific solvent production rate (υ), and the substrate utilization, i.e., the specific glucose consumption (υ_s), were all rather small. This could be explained by the effect of butanol on glucose uptake. Acids were almost entirely changed to acetone and butanol, so that there was less acid-accumulation in this system.

Comparing this operation with the two-stage fermentation, the solvent productivity from the two-stage continuous coupled by microfiltration was higher than the two-stage continuous because of the increase in cell concentration by cell recycling. However, the solvent productivity of this experiment was limited by cell concentration. High cell concentration reduced permeate flux of cell recycling unit due to blockage in microfilter tubes. This problem could be solved by operating at optimum cell concentration in order to obtain the maximum solvent productivity for a long time by using turbidostatically controller.

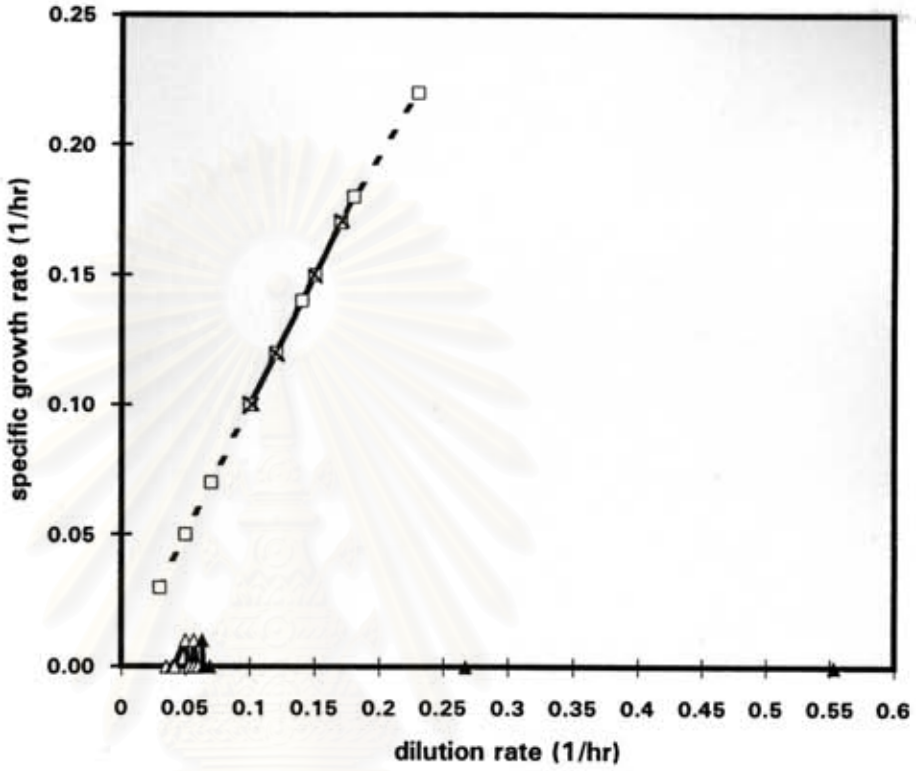

FIGURE 5.14 Influence of the dilution rate on specific growth rates (μ) at pH 4.8, temperature of 35 °C and steady state condition of a two-stage continuous coupled by microfiltration compared with single-stage and two-stage continuous of Cl.acetobutylicum.

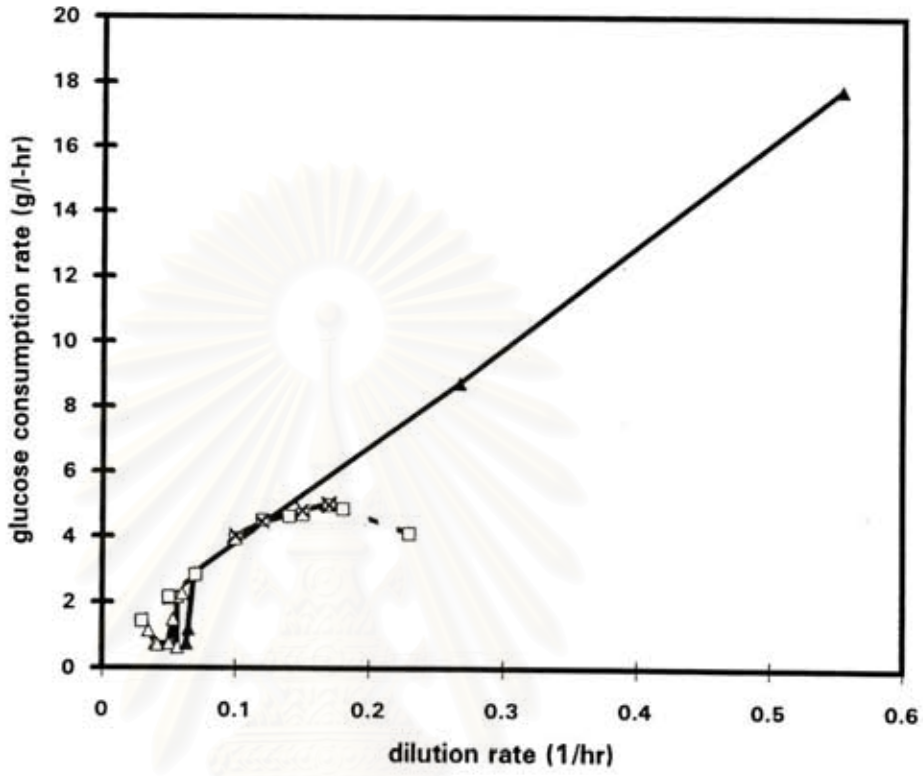

FIGURE 5.15 Influence of the dilution rate on glucose consumption rates (r_s) at pH 4.8, temperature of 35 °C and steady state condition of a two-stage continuous coupled by microfiltration compared with single-stage and two-stage continuous of Cl.acetobutylicum.

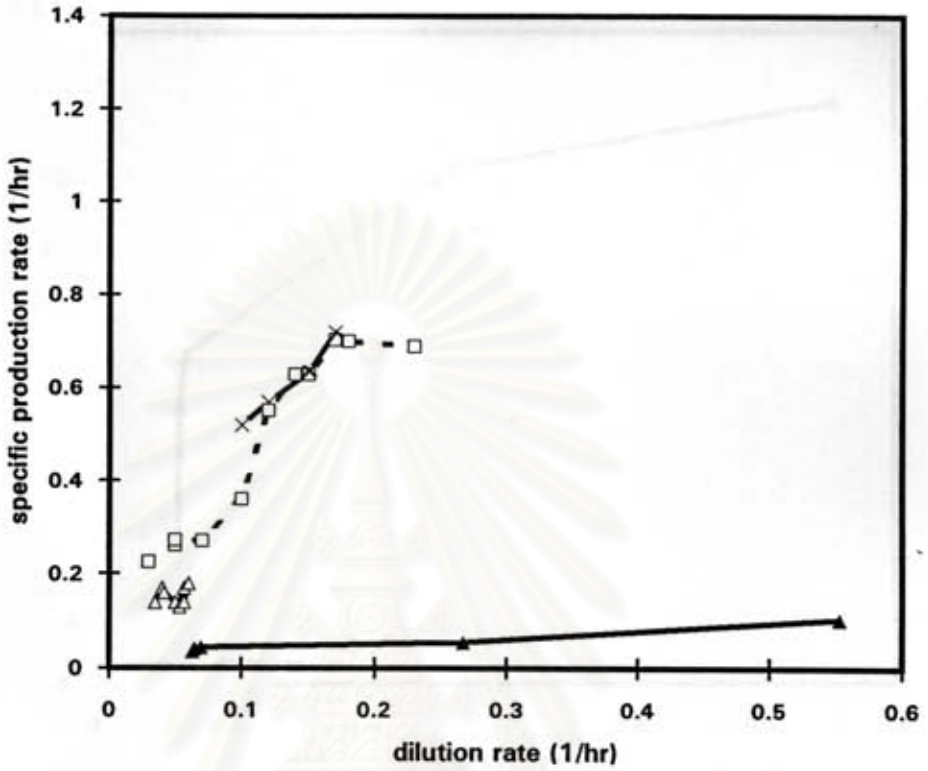

FIGURE 5.16 Influence of the dilution rate on specific solvent productivities (υ) at pH 4.8, temperature of 35 ºC and steady state condition of a two-stage continuous coupled by microfiltration compared with single-stage and two-stage continuous of Cl.acetobutylicum.

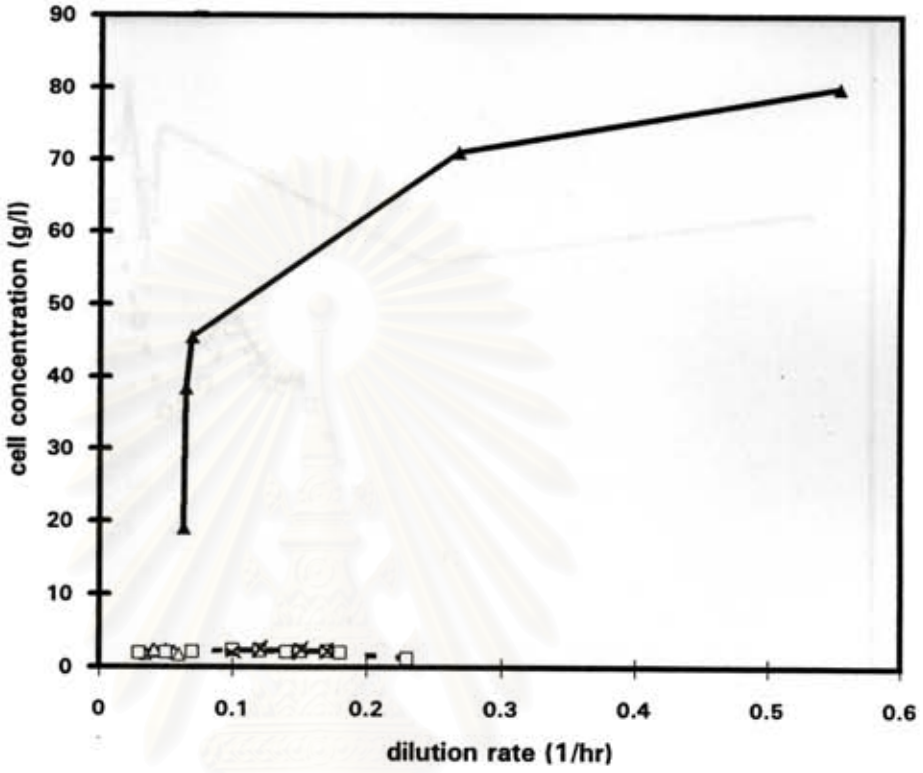

FIGURE 5.17 Influence of the dilution rate on cell concentrations at pH 4.8, temperature of 35 °C and steady state condition of a two-stage continuous coupled by microfiltration compared with single-stage and two-stage continuous of Cl.acetobutylicum.

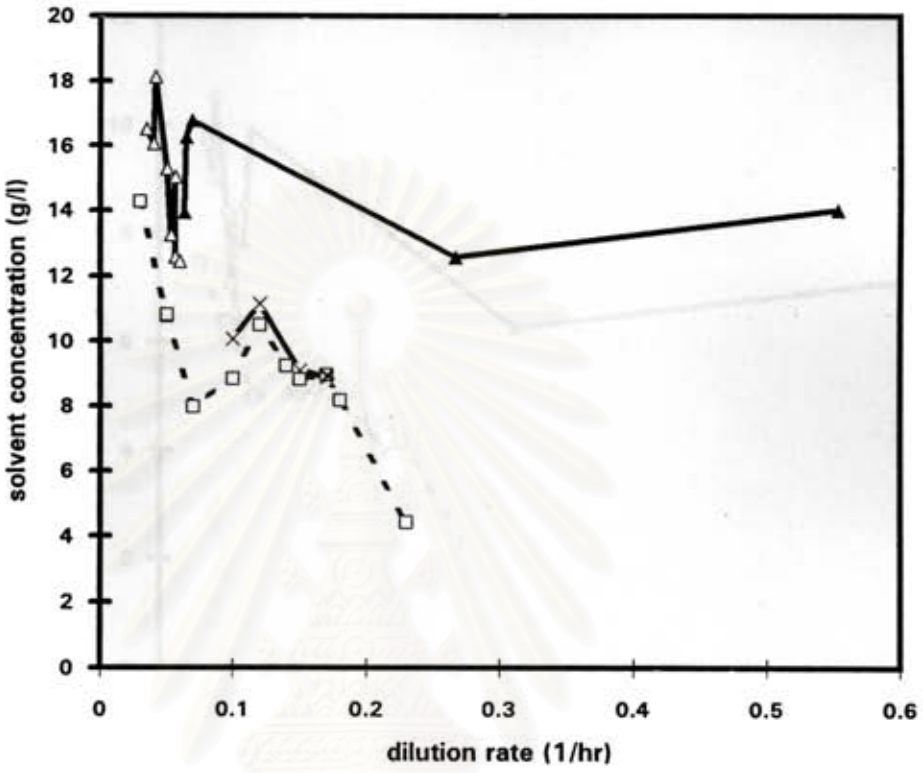

FIGURE 5.18 Influence of the dilution rate on solvent concentrations at pH 4.8, temperature of 35 °C and steady state condition of a two-stage continuous coupled by microfiltration compared with single-stage and two-stage continuous of Cl.acetobutylicum.

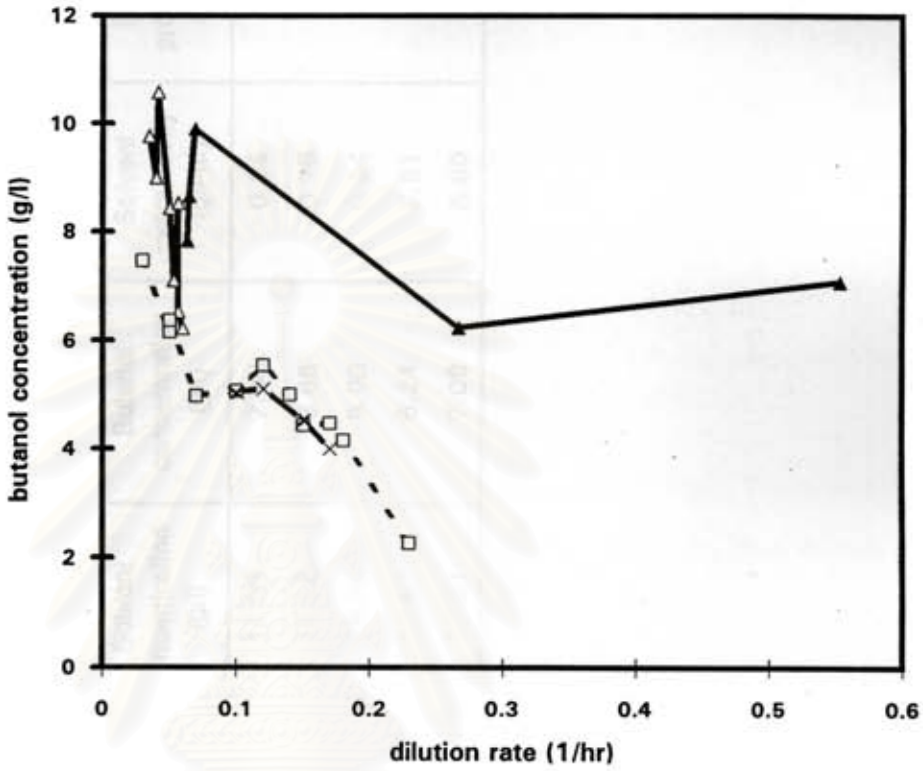

FIGURE 5.19 Influence of the dilution rate on butanol concentrations at pH 4.8, temperature of 35 ºC and steady state condition of a two-stage continuous coupled by microfiltration compared with single-stage and two-stage continuous of Cl.acetobutylicum.

TABLE 5.3 The results of two-stage continuous fermentation coupled by microfiltration of Cl.acetobutylicum.

Operating condition	Overall dilution rate (1/hr)	Dilution rate of		Solvent concentration (g/l)	Butanol concentration (g/l)	Solvent productivity (g/l-hr)	Butanol productivity (g/l-hr)
		first stage	second stage				
Run I	0.046	0.17	0.063	13.93	7.83	0.64	0.36
Run J	0.047	0.17	0.065	16.26	8.66	0.76	0.41
Run K	0.05	0.17	0.069	16.78	9.90	0.84	0.50
Run L	0.20	0.17	0.27	12.57	6.24	2.51	1.25
Run M	**0.40**	**0.17**	**0.55**	**14.01**	**7.09**	**5.60**	**2.84**

5.4 Optimization of Two-stage Continuous Fermentation

The comparison of total solvent productivity was reported in Table 5.4. From the results, the maximum solvent productivity of two-stage continuous fermentation was achieved at 0.0425 hr^{-1} overall dilution rate (Run E). The solvent concentration was 14.94 g/l which consisted of 8.53 g/l of butanol, 5.12 g/l of acetone, and 1.29 g/l of ethanol. There was less acid accumulation in this system and the cell mass concentration was 2.12 g/l. Therefore, the operation at a 0.17 hr^{-1} dilution rate of the first stage and a 0.057 hr^{-1}dilution rate of the second stage (at the 0.0425 hr^{-1} overall dilution rate) was considered the optimum condition for this two-stage continuous fermentation.

When the second stage fermenter was coupled by a microfiltration, the maximum solvent productivity at the 0.40 hr^{-1} overall dilution rate with 80 g/l cell mass (Run M) was 8 times higher than two-stage continuous without microfiltration.

TABLE 5.4 Comparison of the results of acetone-butanol fermentation from single-stage continuous, two-stage continuous, and two-stage continuous coupled by microfiltration.

Operating condition	Overall dilution rate (1/hr)	Solvent concentration (g/l)	Butanol concentration (g/l)	Solvent productivity (g/l-hr)	Butanol productivity (g/l-hr)
Single-stage	0.03	14.27	7.47	0.44	0.22
	0.05	10.77-10.81	6.15-6.37	0.51-0.53	0.31-0.32
	0.07	7.98	4.98	0.58	0.35
	0.10	8.84	5.07	0.84	0.51
	0.12	10.50	5.55	1.23	0.67
	0.14	9.25	5.00	1.30	0.70
	0.15	8.82	4.44	1.34	0.67
	0.17	**8.96**	**4.47**	**1.52**	**0.76**
	0.18	8.17	4.15	1.48	0.75
	0.23	4.42	2.26	1.01	0.52
Two-stage					
Run A	0.045	12.45	6.23	0.56	0.28
Run B	0.0425	12.61	6.53	0.54	0.28
Run C	0.04	13.30	7.12	0.53	0.28
Run D	0.0375	15.24	8.44	0.57	0.32
Run E	**0.0425**	**14.94**	**8.53**	**0.63**	**0.36**
Run F	0.03	16.07	9.00	0.48	0.27
Run G	0.031	18.21	10.58	0.56	0.33
Run H	0.026	16.45	9.77	0.43	0.25
Two-stage coupled by MF					
Run I	0.046	13.93	7.83	0.64	0.36
Run J	0.047	16.26	8.66	0.76	0.41
Run K	0.05	16.78	9.90	0.84	0.50
Run L	0.20	12.57	6.24	2.51	1.25
Run M	**0.40**	**14.01**	**7.09**	**5.60**	**2.84**

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5.5 Comparison of Two-stage Continuous Fermentation with Other Processes

5.5.1 The Study of Batch Fermentation

An experiment of batch fermentation with initial glucose concentration at 50 g/l was operated to compare with the two-stage fermentation. The result were shown in Figures 5.21-5.29. Figures 5.21 and 5.23 showed the behavior of microorganism during operation of the batch fermentation in the form of specific production rate (υ) and specific acid production rate (υ_{acid}) and specific growth rate (μ). The maximum solvent concentration occurred after 40 hours of fermentation with 17.42 g/l solvent, 8.56 g/l butanol and 5.98 g/l cell mass. The productivity of this batch fermentation was 0.21 g/l-hr while the glucose consumption rate was 0.62 g/l-hr with 0.30 production yield. In the comparison with the maximum productivity of two-stage fermentation (Table 5.5), the solvent productivity of two-stage continuous fermentation and two-stage continuous coupled by microfiltration were 3 times (Run E) and 27 times (Run M) higher than batch fermentation, respectively.

5.5.2 The Study of Single-stage Couple with Microfiltration

A comparison of the results obtained from the two-stage continuous (Run M) and the data obtained from single-stage continuous coupled by microfiltration utilizing the same apparatus (Muenduen Phisalphonge, 1989) was shown in Table 5.6. It could be seen that the second stage, which duplicated the system of single-stage continuous coupled by microfiltration, showed improvement in the solvent concentration by using the first stage fermenter as a supplier for continuous feeding of fresh cell to the system. Also, the solvent productivity of the two-stage was 7% less than the single-stage continuous coupled by microfiltration but obtainable solvent concentration was 21.2 % higher. However, the advantages of two-stage system was the longevity of operation due to the continuous feeding of fresh cells from the first stage and the solvent at high concentration which was more suitable for separation.

5.5.3 Other Processes

Comparing of the results obtain from the two-stage continuous fermentation at the overall dilution rate of 0.0425 hr^{-1} (Run E) with two-stage continuous coupled by microfiltration at the first stage dilution rate of 0.17 hr^{-1} and the second stage dilution rate of 0.55 hr^{-1} (0.40 hr^{-1} overall dilution rate, Run M) and with the data obtained from the literatures, the solvent productivity was higher than that of most published data (Table 5.7).

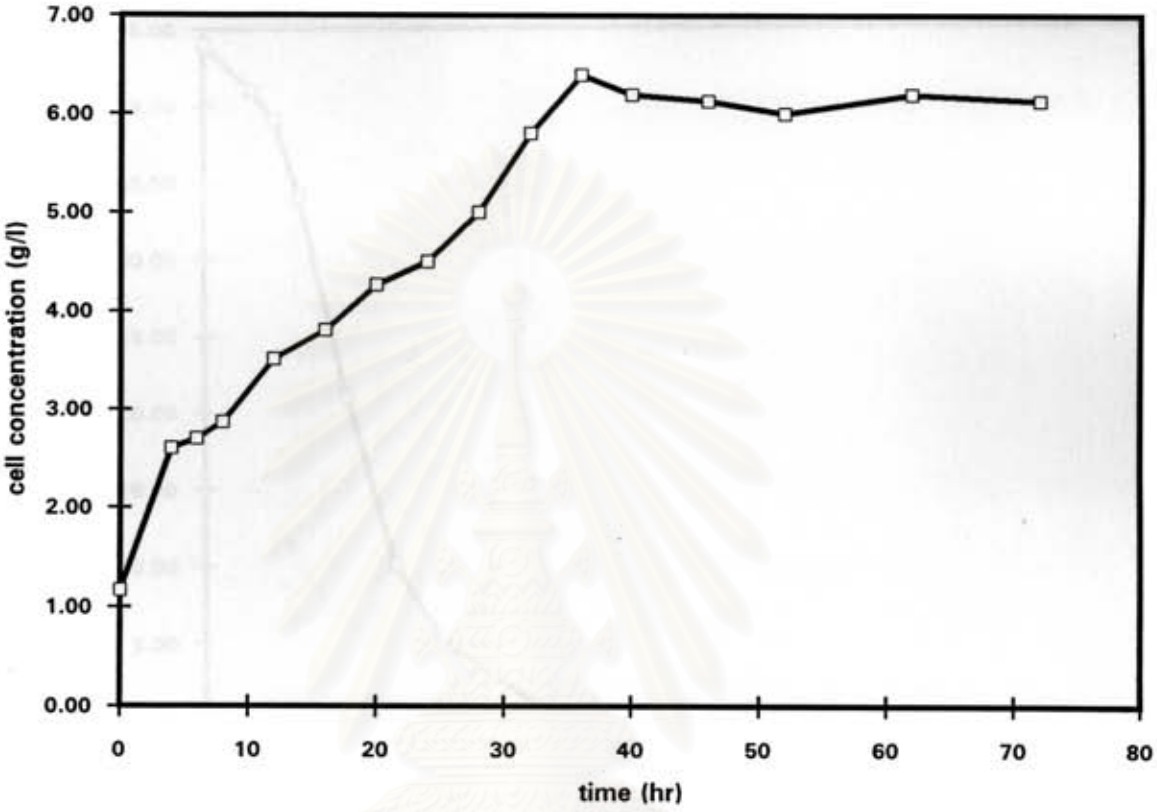

FIGURE 5.20 Time variation of cell concentration during a batch fermentation of Cl.acetobutylicum at pH 4.8 and temperature of 35 °C.

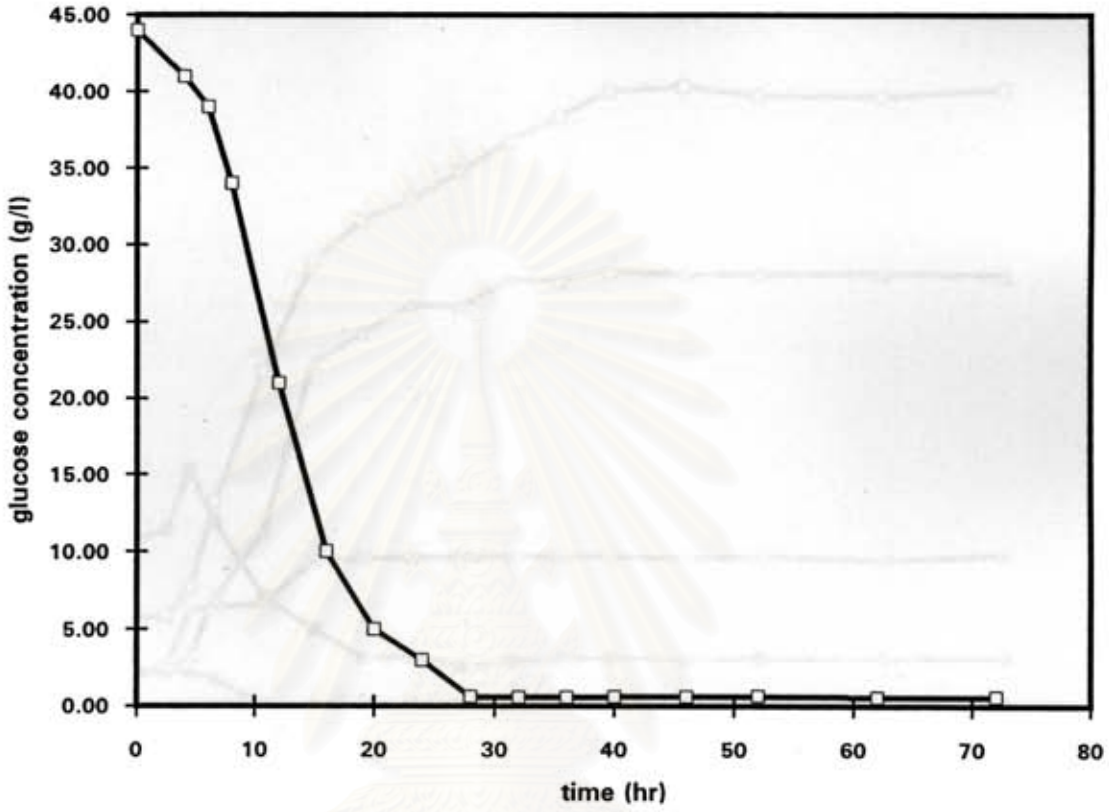

FIGURE 5.21 Time variation of glucose concentration during a batch fermentation of Cl.acetobutylicum at pH 4.8 and temperature of 35 ºC.

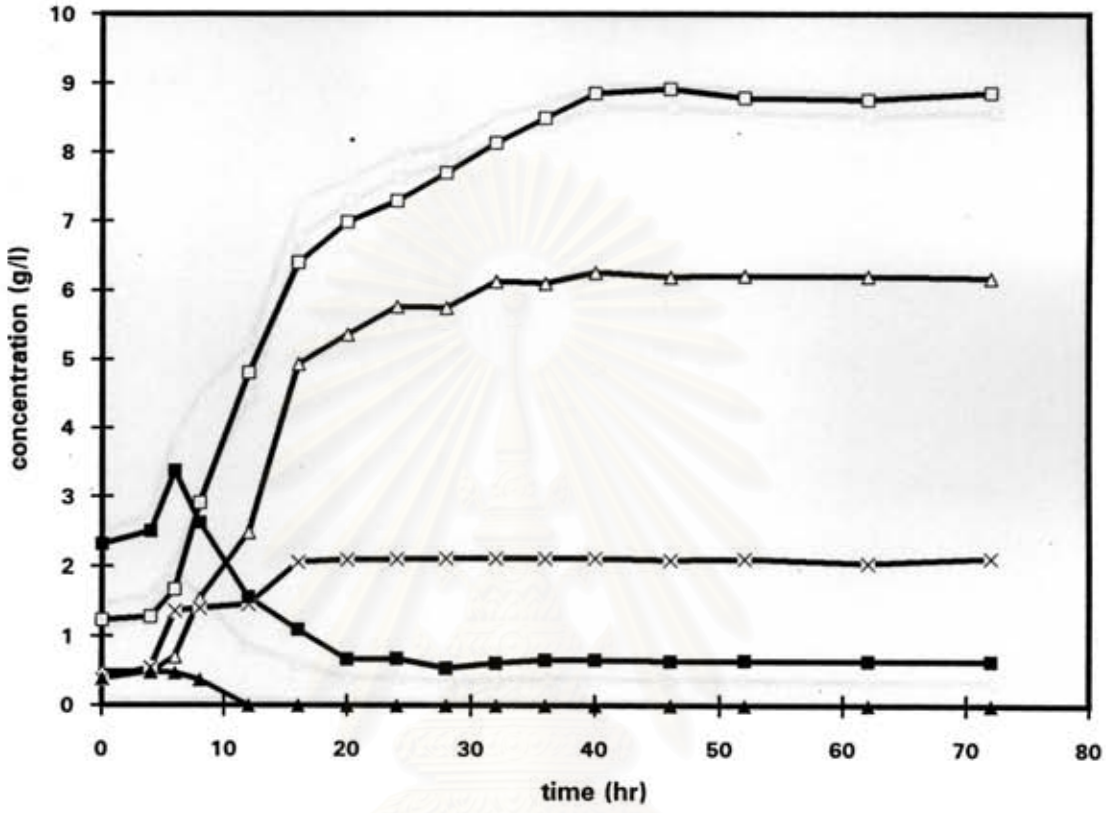

FIGURE 5.22 Time variation of butanol, acetone, ethanol, acetic acid and butyric acid concentration during a batch fermentation of Cl.acetobutylicum at pH 4.8 and temperature of 35 °C.

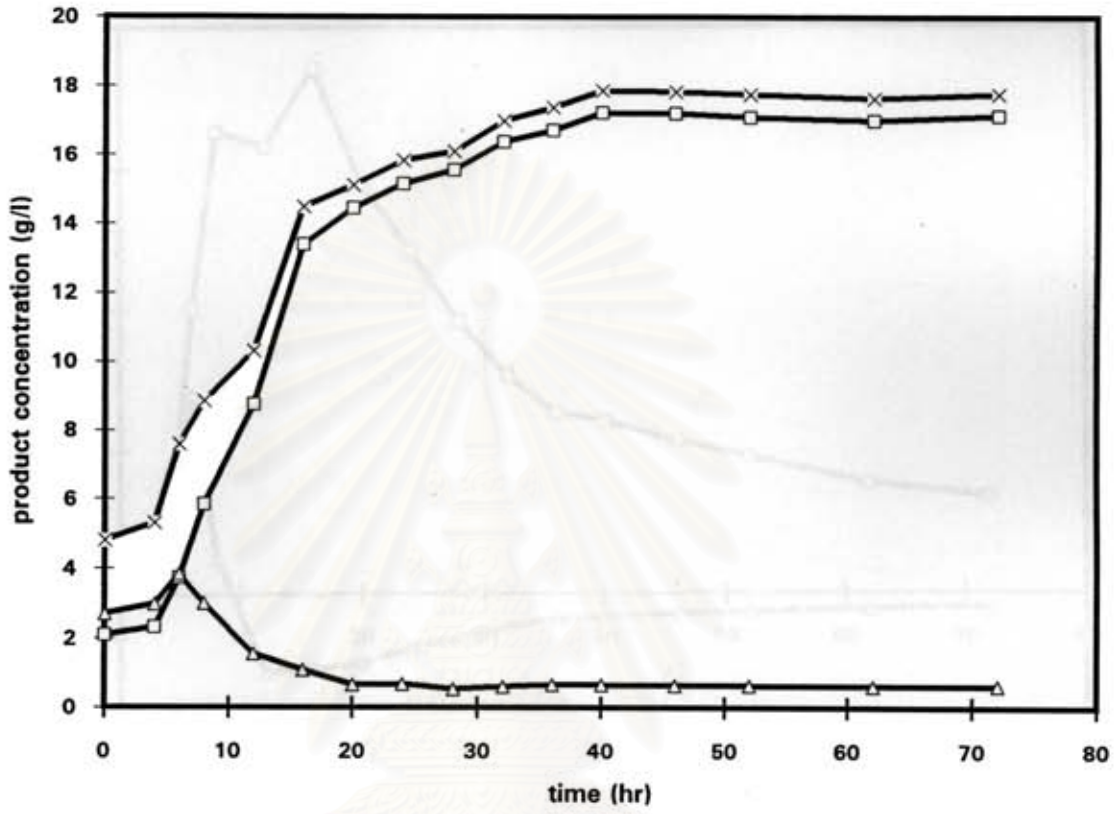

FIGURE 5.23 Time variation of solvent, acid and total product concentration during a batch fermentation of Cl.acetobutylicum at pH 4.8 and temperature of 35 ºC.

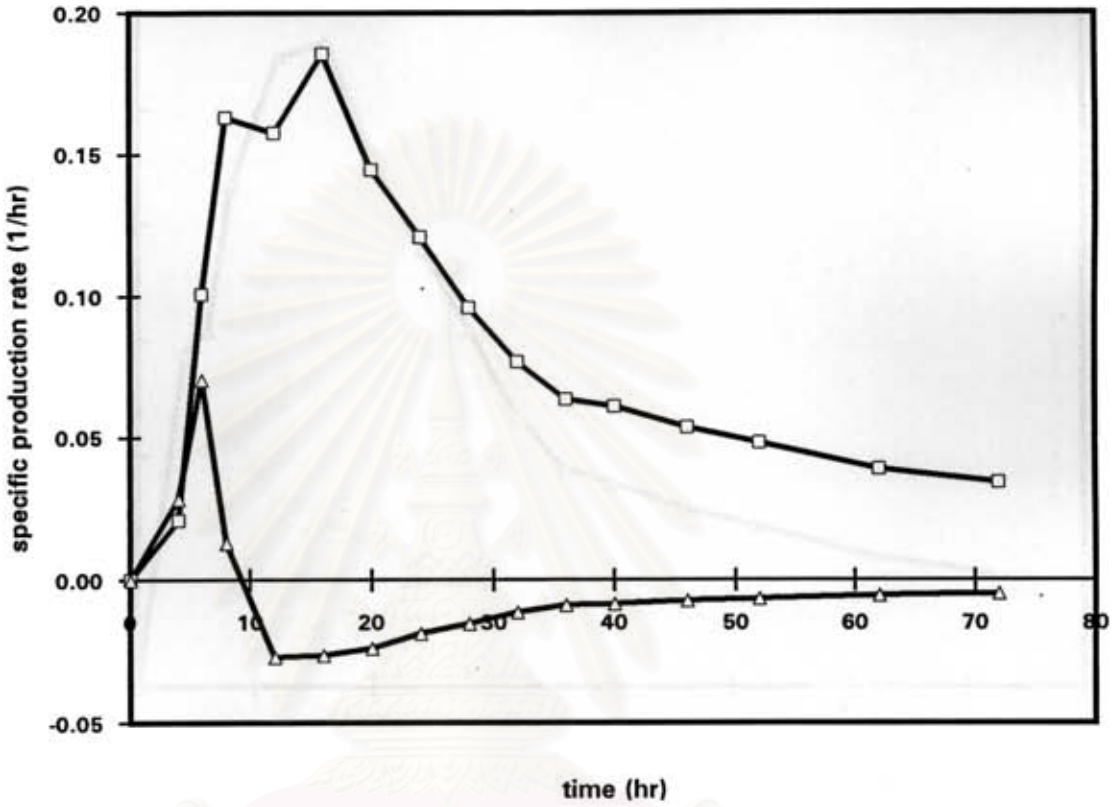

FIGURE 5.24 Time variation of specific production rate (υ) and specific acid production rate (υ_{acid}) during a batch fermentation of Cl.acetobutylicum at pH 4.8 and temperature of 35 °C.

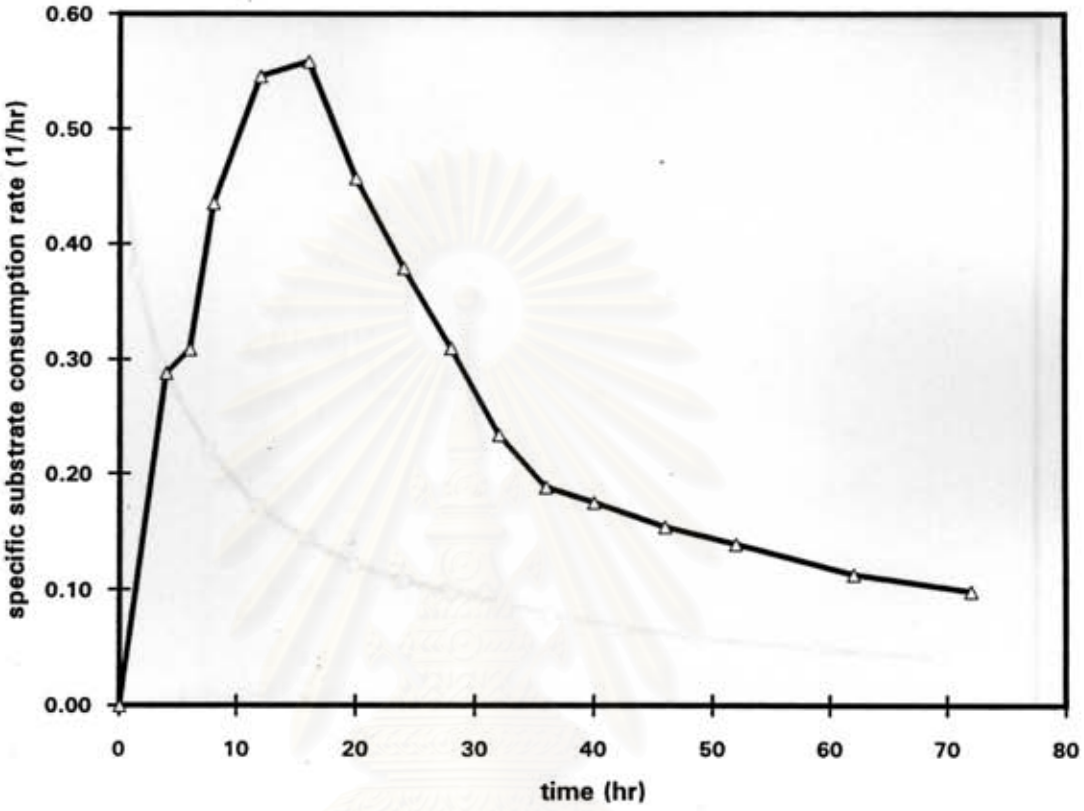

FIGURE 5.25 Time variation of specific substrate consumption rate (υ_s) during a batch fermentation of Cl.acetobutylicum at pH 4.8 and temperature of 35 °C.

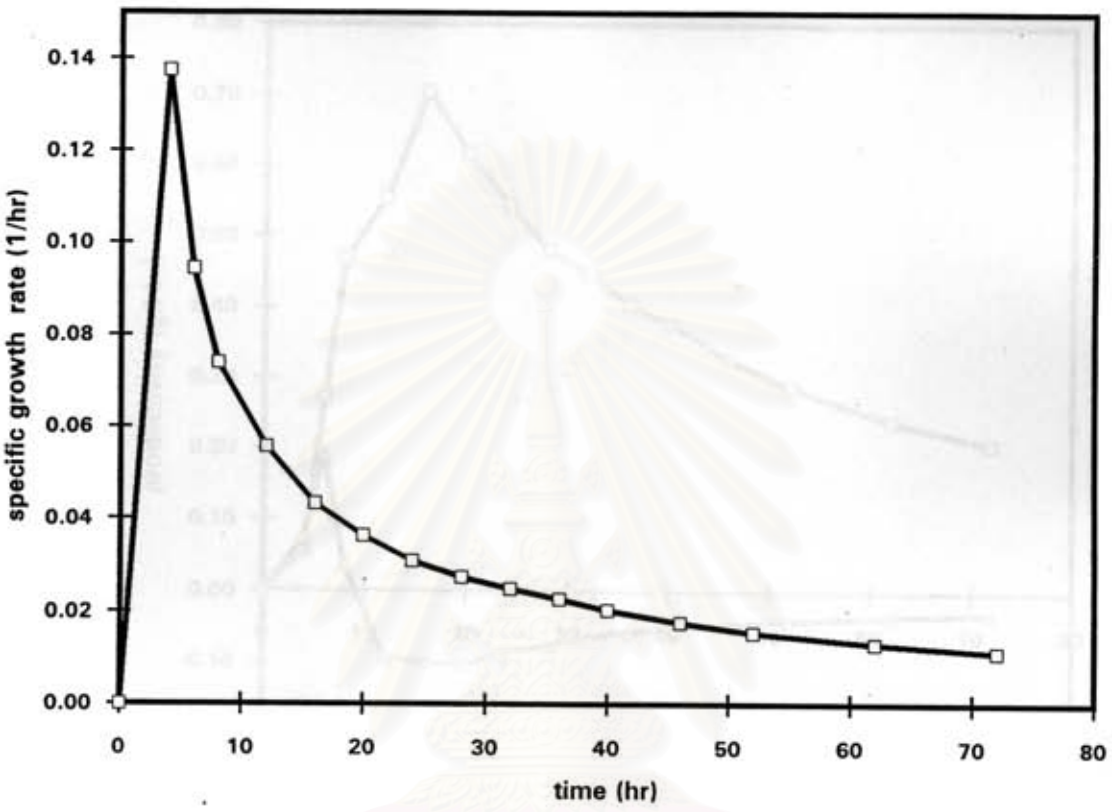

FIGURE 5.26 Time variation of specific growth rate (μ) during a batch fermentation of Cl.acetobutylicum at pH 4.8 and temperature of 35 ºC.

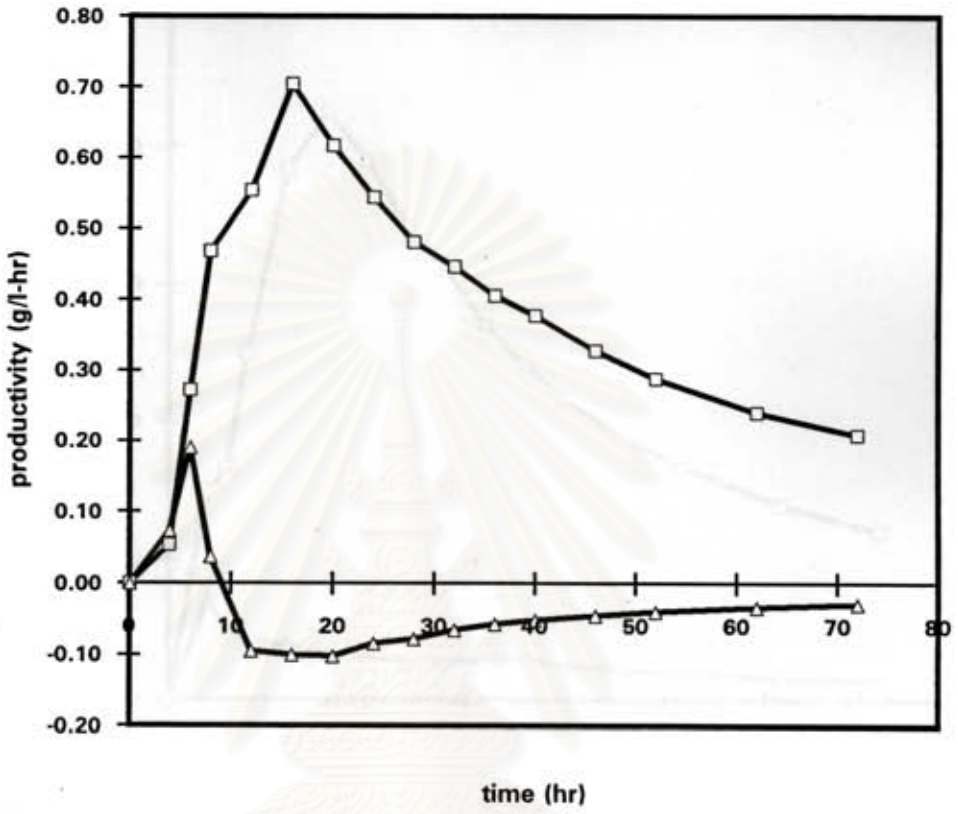

FIGURE 5.27 Time variation of solvent productivity (r_p) and acid productivity (r_{acid}) during a batch fermentation of Cl.acetobutylicum at pH 4.8 and temperature of 35 °C.

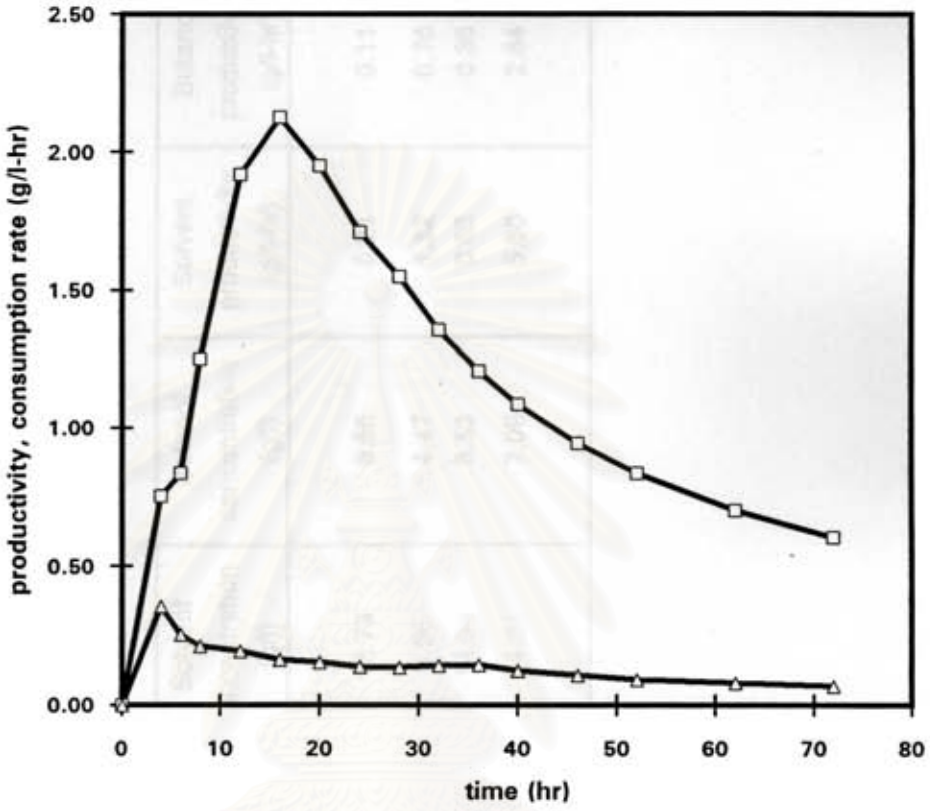

FIGURE 5.28 Time variation of consumption rate (r_s) and cell productivity (r_x) during a batch fermentation of Cl.acetobutylicum at pH 4.8 and temperature of 35 °C.

TABLE 5.5 Comparison of the results of acetone-butanol fermentation from two-stage continuous, two-stage continuous coupled by microfiltration and batch fermentation.

System	Overall dilution rate (1/hr)	Dilution rate of		Solvent concentration (g/l)	Butanol concentration (g/l)	Solvent productivity (g/l-hr)	Butanol productivity (g/l-hr)
		first stage	second stage				
Batch	-	-	-	16.79	8.56	0.21	0.11
Single-stage	0.17	0.17	-	8.96	4.47	1.52	0.76
Run E	0.0425	0.17	0.057	14.94	8.53	0.63	0.36
Run M	0.40	0.17	0.55	14.01	7.09	5.60	2.84

TABLE 5.6 Comparison of the results obtained from the two-stage of run M with the datas obtained from single-stage continuous coupled by microfiltration.

System	Dilution rate (1/hr)	Concentration (g/l)		Productivity (g/l-hr)		Cell concentration (g/l)
		Solvent	Butanol	Solvent	Butanol	
Single-stage coupled by MF	0.55	11.03	6.26	6.06	3.44	80.00
Two-stage coupled by MF [Run M]	0.40 [D2 = 0.55]	14.01	7.09	5.60	2.84	80.06

TABLE 5.7 Comparison of the results obtained from this experiment with datas of Cl. acetobutylicum obtained from the literatures.

	Ref. 25	Bahl et al. [1982]	Monot et al. [1983]	Afschar et al. [1985]	Pierrot et al. [1986]	Schlote et al. [1986]	Forberg et al. [1983]	Godin et al. [1988]	Fick et al. [1986]	Present work	
										Run E	Run M
Maximum solvent productivity [g/l-hr]	< 0.6	0.54	0.4	4.5	6.5	4.1	0.7	0.5	4.02	0.63	5.60
Solvent concentration [g/l]	18	18	12	7	13	10.3	1	20.00	3.94	14.94	14.01
Substrate	Molasses	Synthetic P-limited	Synthetic	Synthetic 0.5g/l yeast extract	Synthetic	Synthetic	Complex 10g/l yeast extract	Synthetic	Complex 6g/l yeast extract	Synthetic	Synthetic
Fermenter	Industrial batch	Two-stage	Single-stage	Two-stage Cell recycle	Single-stage Cell recycle	Single-stage Cell recycle	Single-stage immobilized cells	Two-stage	Two-stage immobilized cells	Two-stage	Two-stage Cell recycle

CHAPTER VI

CONCLUSIONS AND RECOMMENDATIONS

It was clear that solvent productivity can be improved by using a two-stage continuous fermentation. With this system, the solvent productivity was 3 times of the batch fermentation and approximately 1.2-1.4 times of the single-stage continuous. From the experiment, the maximum solvent productivity was 0.63 g/l-hr with butanol productivity of 0.36 g/l-hr. The cell mass was 2.12 g/l and the total solvent concentration was 14.94 g/l which consisted of 8.53 g/l of butanol, 5.12 g/l of acetone and 1.29 g/l of ethanol.

The second stage of the two-stage continuous system produced solvent at high concentration while the first stage produced the active cell. The second stage could be considered as a single-stage system which was connected with an active cell feeding unit, i.e., the first stage. The two-stage continuous system could also be expected to increase the stability of the continuous process as a result of continuous feeding of active cells.

Replacing the second stage with one coupled by microfiltration, the cell mass increaved to a high concentration of 80 g/l which was 40 times of the system without microfiltration. The solvent productivity reached a maximum value of 5.60 g/l-hr with butanol productivity of 2.84 g/l-hr. The total solvent concentration was 14 g/l which was the combination of 7.09 g/l butanol, 5.42 g/l-of acetone and 1.50 g/l of ethanol. The technique using a cell recycling could overcome the problem of growth rate limitation due to the dilution rate. Moreover, the operation with high cell concentration might allow higher productivity (8 times higher than two-stage without coupling by microfiltration) at optimum parameters.

However, this microfiltration system which made of ceramic could not be operated for a long period of time due to a lack of equipment for cell bleeding. Then the cell mass increased to a very high concentration rapidly until the equipment became inoperable caused

by the blockage of cell mass at high density in the microfilter tubes. This problem might be solved by incorporating a turbidostatically controller to control cell concentration in the system. This would allow the fermentation to be operated at optimum cell concentration which is expected to yield a higher solvent productivity for a long period of time. Therefore, it might be studied continuously to determine the optimum cell concentration for operation of two-stage coupled by microfiltration at the maximum solvent productivity by using cell concentration controller.

Based on this experiment, two techniques for improvement solvent productivity, i.e., two-stage continuous system and cell recycling unit, were used and yielded higher solvent productivity. It clearly demonstrated the possibility of further improvement of acetone-butanol fermentation and other fermentation processes.

รายการอ้างอิง

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APPENDIX

EXPERIMENTAL DATA

TABLE A1 The results of acetone-butanol fermentation from single-stage continuous process.

Operating condition	Solvent concentration (g/l)			Total solvent (g/l)	Acid concentration (g/l)		Cell concentration (g/l)
	Butanol	Acetone	Ethanol		Butyric acid	Acetic acid	
Batch (3 L)	2.23	1.65	0.44	4.32	1.73	0.41	1.72
Continuous (D=0.03 hr-1)	7.47	6.22	0.55	14.24	0.36	0.00	1.95
Continuous (D=0.05hr-1) repeated	6.15	4.17	0.38	10.70	1.73	0.00	1.96
Continuous (D=0.05 hr-1)	6.37	4.09	0.33	10.79	0.90	0.00	1.96
Continuous (D=0.07 hr-1)	4.98	2.68	0.36	8.02	0.00	0.00	2.11
Continuous (D=0.10 hr-1)	5.07	2.86	0.82	8.75	0.00	0.00	2.34
Continuous (D=0.12 hr-1)	5.55	4.04	0.98	10.56	0.92	0.00	2.23
Continuous (D=0.14 hr-1)	5.00	3.72	0.53	9.25	1.00	0.00	2.08
Continuous (D=0.15 hr-1)	4.44	3.36	1.04	8.83	1.01	0.00	2.14
Continuous (D=0.17 hr-1)	4.47	3.41	1.02	8.90	1.17	0.00	2.17
Continuous (D=0.18 hr-1)	4.15	3.57	0.47	8.18	1.37	0.00	2.00
Continuous (D=0.23 hr-1)	2.26	1.66	0.15	4.07	1.30	0.00	1.24

TABLE A2 The results of acetone-butanol fermentation from two-stage continuous process.

Operating condition	Solvent concentration (g/l)			Total solvent (g/l)	Acid concentration (g/l)		Cell concentration (g/l)
	Butanol	Acetone	Ethanol		Butyric acid	Acetic acid	
Batch (1 L)	2.71	2.80	0.55	6.06	0.31	1.54	1.67
First stage							
Continuous (D=0.1hr-1)	5.04	4.18	0.94	10.15	0.30	0.90	2.20
Continuous (D=0.12hr-1)	5.10	4.09	1.03	10.22	0.10	0.90	2.38
Continuous (D=0.15hr-1)	4.20-4.70	3.24-3.31	1.23-1.37	8.67-9.38	0.23-0.28	0.90-1.48	2.00-2.34
Continuous (D=0.17hr-1)	4.00	3.57	1.35	8.92	0.45	0.00	2.17
Second stage							
D=0.10 hr-1 added 0.0045 l/hr	9.77	5.27	1.41	16.45	0.65	2.20	1.85
D=0.12 hr-1 no additional feed	9.00	5.77	1.30	16.07	0.35	0.95	2.18
D=0.12 hr-1 added 0.0045 l/hr	10.58	6.20	1.42	18.21	0.25	1.10	2.36
D=0.15 hr-1 no additional feed	8.44	5.43	1.37	15.24	0.22	0.90	2.32
D=0.15 hr-1 added 0.01 l/hr	7.12	4.69	1.49	13.30	0.15	1.50	2.07
D=0.17 hr-1 no additional feed	8.53	5.12	1.29	14.94	0.41	0.95	2.12
D=0.15 hr-1 added 0.02 l/hr	6.53	4.59	1.49	12.61	0.30	2.10	2.02
D=0.15 hr-1 added 0.03 l/hr	6.23	4.71	1.51	12.45	0.30	2.50	1.71

TABLE A5 Glucose consumption rate (r_s) , solvent productivity (r_p) , cell productivity (r_x) and production yield ($Y_{p/s}$) from single-stage continuous process.

Operating condition	rs (g/l-hr)	rp (g/l-hr)	rx (g/l-hr)	Yp/s
Batch (3 L)	2.05	0.29	0.15	0.17
Continuous (D = 0.03 hr-1)	1.43	0.44	0.06	0.21
Continuous (D = 0.05hr-1) repeated	2.12	0.51	0.10	0.24
Continuous (D = 0.05 hr-1)	2.16	0.53	0.10	0.25
Continuous (D = 0.07 hr-1)	2.82	0.58	0.15	0.20
Continuous (D = 0.10 hr-1)	3.92	0.84	0.23	0.21
Continuous (D = 0.12 hr-1)	4.50	1.23	0.27	0.22
Continuous (D = 0.14 hr-1)	4.62	1.30	0.29	0.28
Continuous (D = 0.15 hr-1)	5.44	1.34	0.32	0.25
Continuous (D = 0.17 hr-1)	4.97	1.52	0.37	0.31
Continuous (D = 0.18 hr-1)	4.86	1.48	0.36	0.31
Continuous (D = 0.23 hr-1)	4.10	0.85	0.28	0.21

TABLE A6 Glucose consumption rate (r_s) , solvent productivity (r_p) , cell productivity (r_x) and production yield ($Y_{p/s}$) from two-stage continuous process.

Operating condition	rs (g/l-hr)	rp (g/l-hr)	rx (g/l-hr)	Yp/s
Batch (1 L)	1.50	0.36	0.08	0.21
First stage				
Continuous (D=0.1 hr-1)	4.03	1.02	0.22	0.25
Continuous (D=0.12 hr-1)	4.41	1.23	0.29	0.28
Continuous (D=0.15 hr-1)	4.82	1.35	0.33	0.28
Continuous (D=0.17 hr-1)	5.01	1.52	0.37	0.30
Second stage				
D=0.10 hr-1 added 0.0045 l/hr	1.15	0.06	0.06	0.21
D=0.12 hr-1 no additional feed	0.77	0.09	0.09	0.25
D=0.12 hr-1 added 0.0045 l/hr	0.72	0.10	0.10	0.22
D=0.15 hr-1 no additional feed	0.75	0.12	0.12	0.23
D=0.15 hr-1 added 0.01 l/hr	1.48	0.11	0.11	0.19
D=0.17 hr-1 no additional feed	0.65	0.12	0.12	0.22
D=0.15 hr-1 added 0.02 l/hr	2.17	0.11	0.11	0.16
D=0.15 hr-1 added 0.03 l/hr	2.28	0.10	0.10	0.15

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TABLE A7 Glucose consumption rate (r_s) , solvent productivity (r_p) , cell productivity (r_x) and production yield ($Y_{p/s}$) from two-stage continuous coupled by microfiltration.

Operating condition	rs (g/l-hr)	rp (g/l-hr)	rx (g/l-hr)	Yp/s
Batch (1 L)	1.50	0.36	0.08	0.21
First stage (D=0.17hr-1)	4.66-5.02	1.21-1.37	0.28-0.31	0.21-0.28
second stage				
D=0.063 hr-1 no additional feed	0.76	0.609	1.90	0.34
D=0.0646 hr-1 added 500 g/l of glucose	1.20	1.584	0.00	0.32
D=0.069 hr-1 added 500 g/l of glucose	2.89	1.689	0.00	0.21
D=0.267 hr-1 added 50 g/l of glucose	8.75	3.838	1.42	0.20
D=0.55 hr-1 added 50 g/l of glucose	17.79	8.264	0.00	0.21

TABLE A8 Glucose consumption rate (r_s) , solvent productivity (r_p) , cell productivity (r_x) and production yield ($Y_{p/s}$) from batch process.

Operating condition	rs (g/l-hr)	rp (g/l-hr)	rx (g/l-hr)	Yp/s
Batch (1 L)	0.62	0.21	0.07	0.30

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