DEVELOPMENT OF COMPOSITE HOLLOW FIBER MEMBRANES FOR SEPARATION OF ACETONE-BUTANOL-ETHANOL (ABE) FROM FERMENTATION BROTH USING PERVAPORATION TECHNIQUE

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การพัฒนาท่อใยกลวงเชิงประกอบสำหรับใช้ในกระบวนการแยก อะซีโตน-บิวทานอล-เอทานอล จากน้ำหมักชีวภาพโดยใช้กระบวนการเพอร์แวปโพเรชั่น

นายวิรัตน์ อินทวี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for a Master's Degree.

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วิรัตน์ อินทวี : การพัฒนาท่อใยกลวงเชิงประกอบสำหรับใช้ในกระบวนการแยกอะซีโตน-บิวทานอล-เอทานอล จากน้ำหมักชีวภาพโดยใช้ระบบเพอร์แวปโพเรชั่น (DEVELOPMENT OF COMPOSITE HOLLOW FIBER MEMBRANES FOR SEPARAION OF ACETONE-BUTANOL-ETHANOL (ABE) FROM FERMENTATION BROTH USING PERVAPORATION TECHNIQUE), อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.อภิชาติ บุญทาวัน, 95 หน้า.

ไบโอบิวทานอลได้รับการพิจารณาว่าเป็นพลังงานทดแทนที่มีศักยภาพคล้ายกับน้ำมันเบนซิน แต่อย่างไรก็ตาม การเกิดสารยับยั้งผลผลิต ผลผลิตที่ต่ำ และต้นทุนในการแยกผลิตผลที่สูงยังเป็นปัญหาหลักในกระบวนการหมัก อะซีโตน-บิวทานอล-เอทานอล (ABE) ดังนั้น เยื่อเลือกผ่านเชิงประกอบโพลีไดเมทิลไซโลเซน (PDMS) เยื่อเลือกผ่านแบบท่อใยกลวงเชิงประกอบยางธรรมชาติ (NR) และยางคาร์บอกซิเลตสตีรีนบิวทาไดอีน (XSBR) จึงถูกนำมาศึกษาในการแยกบิวทานอลโดยใช้ระบบเพอร์แวปโพเรชั่น สารละลายบิวทานอลได้ถูกเตรียมขึ้นเพื่อศึกษาอิทธิพลของความเข้มข้นที่ร้อยละ 1.25 – 10 โดยปริมาตร และอุณหภูมิการแยกที่ 35 – 80 °C พบว่าค่าบิวทานอลฟลักซ์และความเข้มข้นของบิวทานอลที่แยกได้ของเยื่อเลือกผ่านทั้งสามชนิดเพิ่มขึ้นเมื่อเพิ่มความเข้มข้นของบิวทานอลในสารละลาย ขณะที่ค่าการคัดเลือกบิวทานอลสวนทางกันกับปัจจัยนี้ การเพิ่มอุณหภูมิในการทดลองยังส่งผลทำให้เกิดการเพิ่มขึ้นของค่า ฟลักซ์และการคัดเลือกบิวทานอลด้วย ทั้งนี้เกิดขึ้นเฉพาะในเยื่อเลือกผ่าน PDMS และ NR แต่ในเยื่อเลือกผ่าน XSBR พบค่าการคัดเลือกบิวทานอลที่ลดลงเมื่ออุณหภูมิในการแยกเพิ่มขึ้น ภายใต้สภาวะการทดลองเดียวกันเยื่อเลือกผ่าน PDMS แสดงค่าฟลักซ์และการเป็นเยื่อเลือกผ่านบิวทานอลมากกว่าเยื่อเลือกผ่านอีกสองชนิด อย่างไรก็ตามค่าการคัดเลือกบิวทานอลที่พบในเยื่อเลือกผ่าน XSBR และ NR สูงกว่าในเยื่อเลือกผ่าน PDMS แต่ไม่สามารถทำงานได้อย่างมีประสิทธิภาพภายใต้อุณหภูมิต่ำ (35 °C) ดังนั้น เยื่อเลือกผ่าน PDMS จึงถูกเลือกใช้ในการผลิต ABE โดยใช้ระบบเพอร์แวปโพเรชั่นที่เป็นการแยกแบบทันทีที่เกิดผลผลิต (ISPR) ซึ่งจากผลการทดลองพบว่าค่าความเข้มข้นของ ABE และผลิตผลที่ได้ (17.94 กรัม/ลิตร และ 0.37 กรัม/กรัม, ตามลำดับ) ซึ่งมีค่าสูงกว่าที่พบในการผลิตแบบกะทั่วไป (14.38 กรัม/ลิตร และ 0.32 กรัม/กรัม, ตามลำดับ) และนอกจากนี้ค่าผลผลิตต่อเวลาที่ได้ยังมากกว่า 1.5 เท่า เมื่อเทียบกับระบบการผลิตแบบทั่วไป

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนักศึกษา_____

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WIRAT INTHAVEE : DEVELOPMENT OF COMPOSITE HOLLOW FIBER MEMBRANES FOR SEPARATION OF ACETONE-BUTANOL-ETHANOL (ABE) FROM FERMENTATION BROTH USING PERVAPORATION TECHNIQUE, THESIS ADVISOR : ASST. PROF. APICHAT BOONTAWAN, Ph.D. 95 PP.

HOLLOW FIBER MEMBRANE/PERVAPORATION/ACETONE-BUTANOL-ETHANOL FERMENTATION/*IN SITU* PRODUCT REMOVAL

Biobutanol has been considered as a potential alternative fuel with sufficiently similar characteristics to gasoline. However, product inhibitions, low productivities, and high recovery costs are the consequent limitations of acetone-butanol-ethanol (ABE) fermentation. A Polydimethyl siloxane (PDMS) composite membrane, Natural rubber (NR) composite hollow fiber membrane, and Carboxylated Styrene-Butadiene Rubber (XSBR) composite hollow fiber membrane were used to investigate the membrane performances by pervaporation technique. A *n*-butanol/water binary solution was prepared to study the effect of feed butanol concentration at a varying concentration of 1.25 – 10 % v/v. The effect of operating temperature was also investigated with the increasing of the feed temperature in range of 35 – 80 °C. The results showed that the butanol flux and permeate butanol concentration of all membranes used in this experiment increased with the increasing of the feed butanol concentration, while the corresponding butanol selectivity showed the reverse tendency. An increase in operating temperature resulted in increasing the permeation flux and butanol selectivity of the PDMS and NR composite membranes. However, in

the case of XSBR composite hollow fiber membrane, the butanol selectivity at higher operating temperature was shown to decrease. Under the same experimental condition, the PDMS composite membrane offered significantly better results in terms of permeation flux and butanol permeance. However, NR and XSBR composite hollow fiber membrane showed higher performance in terms of butanol selectivity, but they did not work efficiently with low temperature (35 °C). The PDMS composite membrane was, therefore, chosen to perform the *in situ* product removal (ISPR) equipped with batch ABE production by using *Clostridium acetobutylicum* TISTR 1462. The experimental results revealed that the total solvent concentration and production yield were higher (17.94 g/L and 0.37 g/g, respectively) than that of typical batch fermentation (14.38 g/L and 0.32 g/g, respectively). Compared to batch fermentation, this system achieved 1.5 times more productivity.

School of Biotechnology

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Wirat Inthavee

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LIST OF ABBREVIATIONS

A	=	Membrane area (m^2)
ABE	=	Acetone-butanol-ethanol
BR	=	Polybutadiene
cP	=	Centipoise
°C	=	Degree Celsius
DRC	=	Dry rubber content
EPDM	=	Ethylene propylene diene monomer
et al.	=	Et alia (and other)
g	=	Gram
h	=	hour
IR	=	Synthetic polyisoprene
ISPR	=	*In situ* product removal
J_i	=	Permeation flux ($g\ h^{-1}\ m^{-2}$)
Kg	=	Kilogram
L	=	Liter
l	=	Membrane thickness (µm)
M_w	=	Molecular weight
NBR	=	Nitrile rubber (copolymer of polybutadiene and acrylonitrile)
NR	=	Natural rubber
p	=	Pressure (bar)
PDMS	=	Polydimethyl siloxane

LIST OF ABBREVIATIONS (Continued)

P_i	=	Membrane permeability
p^{sat}	=	Saturated vapor pressure (bar)
PVDF	=	Polyvinylidene fluoride
Q	=	Permeance (kg/h m^2kPa)
RO	=	Reverse osmosis
SBR	=	Styrene-butadiene rubber
SEM	=	Scanning electron microscope
T	=	Temperature (°C)
t	=	Time (h)
TSC	=	Total solid content
W	=	Weight (g)
x	=	Mass fraction in the feed side
XSBR	=	Carboxylated styrene-butadiene rubber
y	=	Mass fraction in the permeate side
ΔP	=	Partial pressure gradient (bar)

CHAPTER I

INTRODUCTION

1.1 Significance of the problem

At present, the petroleum exploration has met a lot of problems, such as increasing petroleum price which has been opposed to rapid decrease of whole petroleum stock in the world. In addition, the fuel crisis is an important problem in Thailand which will be possibly extended in the near future. Moreover, other growing concerns are the greenhouse gas emission, and global warming. Therefore, these reasons induce to the necessity of researches for the alternative fuels.

In the past two decades, there were many researches related to the renewable fuels which can either be completely replaced or blended with the petroleum fuel without requiring specially adopted engines in vehicles (Ranjan and Moholkar, 2009). The most popular alternative fuel is ethanol, which has been recommended as a great alcohol fuel. However, there are some disadvantages of this alcohol fuel such as limitation of low energy content (or heat of combustion) and it causes problems with corrosion including phase separation in the gasoline mixture. Another alternative alcohol fuel that has emerged in recent past is biobutanol, which overcomes most of above constraints. Biobutanol has currently attracted considerable attention as an alternative biofuel to the petroleum-derived fuel (Ha *et al*., 2008) due to several advantages including high energy content, low water absorption, and easy application to the existing gasoline infrastructure.

Biobutanol can be produced through the process of acetone-butanol-ethanol (ABE) fermentation of various substrates by using solvent producing strains of *Clostridium spp*. However, ABE fermentation processes still have limited productivity. A common reason for this is the presence of the products that can cause alcohol inhibitory or toxic effects (making poor use of the enzyme) or promote unfavourable equilibria (giving low conversions) (Lye and Woodley, 1999). In each case, the desired product needs to be removed as soon as it is formed in order to overcome these constraints and hence increase the productivity and yield of the biocatalytic process. The usual concentration of total solvents in the fermentation broth is 18–33 g/L (using starch or glucose) of which butanol is only about 13–18 g/L (Ezeji *et al*., 2004). Such a low product concentration adversely affects the economics of recovery of these solvents from dilute fermentation broth by distillation, making the process unable to compete with the petroleum-based products.

Recently, a variety of butanol recovery techniques have been developed to reduce the cost of butanol production. Pervaporation is one of downstream processing which appears to be particularly promising. Furthermore, a combination of production process and downstream technology (integrated processing) offers a great potential in micro-biotechnology. These operation steps can be influenced positively the time and cost intensive downstream processes. Another importance, high permeation flux and high selectivity are the essential requirements for a successful product separation process by pervaporation. In order to meet these requirements, a hydrophobic polymeric membrane is played an important role on high specific recovery and should also have an ultra thin layer, while this layer must maintain its integrity and mechanical stability under operation. Composite hollow membrane has attracted great

attentions because of its many advantages contrast to the traditional extraction process (Liu *et al.* 2003). In the fabrication of a composite hollow membrane, a microporous tubular support with good mechanical strength is coated with a thin layer of selective hydrophobic polymer to perform the separation. Generally, the defect free top layer should be as thin as possible while the support membrane should possess a high porosity with reasonably small pore size.

In many researches for ABE production, it was found that organic solvents had very strong action on biotransformation cells and membranes. Here, the work was aimed to produce the ABE with *in situ* product-removal (ISPR), integration of production and separation process with development of composite membrane using pervaporation technique. The ISPR processing in which a potentially inhibitory product is continuously removed from the fermentation broth as it is produced has important advantages in improving yield and conversion relative to conventional processes.

1.2 Research objectives

1.2.1 To construct the hollow fibre membrane (including spinning and coating steps) which will be serve as specific function for butanol separation by using dry-wet phase inversion method.

1.2.2 To compare the membrane performances between commercial flat-sheet and hollow fiber membrane spun in the laboratory by pervaporation technique using model solution (acetone-butanol-ethanol/water) in terms of permeation flux, selectivity, and permeance.

1.2.3 To compare the yield and productivity of ABE fermentation in batch, and batch with *in situ* product removal (ISPR) using composite membrane by pervaporation system.

1.3 Research hypothesis

1.3.1 Product (acetone-butanol-ethanol) inhibition and dilute product streams are main constrain of biobutanol production that results in limited productivity and yield of ABE fermentation process.

1.3.2 In order to produce bio-butanol, the traditional recovery process is still suffered from a high operation cost.

1.3.3 Production of biobutanol should include strategies for reducing or eliminating butanol toxicity to the culture and for manipulating the culture to achieve better product specificity and yield.

1.3.3 Advances in integrated fermentation and *in situ* product removal (ISPR) processes have been expected that it can result in a dramatic reduction of process streams, reduced butanol toxicity to the fermenting microorganisms, improved substrate utilization, and overall improved bioreactor performance.

1.4 Scope and limitation of the study

This research studied the separation and production of acetone butanol and ethanol (ABE) by using pervaporation process. For research experiment, a hollow fiber membrane instrument was constructed and was used for fabrication of composite hollow fiber membrane. In addition, this work was collaborated with Prince of Songkla University (PSU) for constructing the instrument and coating the

hollow fiber membrane. Furthermore, the membranes were used for separating ABE from solution model (synthetic ABE solution) in order to evaluate the membrane performances, prior to applying to ABE fermentation broth separation.

1.5 Expected results

1.5.1 Successful production and separation of butanol from fermentation broth by using *in situ* product removal with pervaporation technique.

1.5.2 High performance hollow fiber membrane will be obtained by using the own fabricating membrane instrument.

1.5.3 To publish the experimental results in scientific journal.

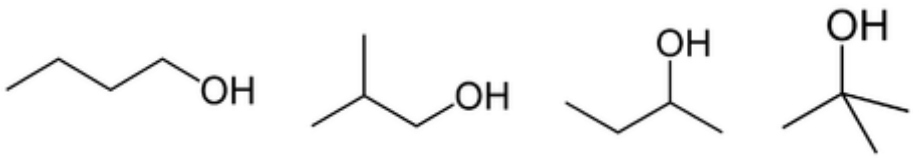
CHAPTER II

LITERATURE REVIEW

2.1 Butanol

Butanol is typically produced from petroleum sources, but that has not always been the case, and sometimes called biobutanol when produced biologically. It, also known as a butyl alcohol, can refer to any of the four isomeric alcohols of formula C_4H_9OH: *n*-Butanol, isobutanol, *sec*-butanol, and *tert*-butanol as shown in Figure 2.1. At room temperature, butanol is a flammable colorless liquid, and has a melting point of -89.5 °C and a boiling point of 117.2 °C as shown in Table 2.1. It is one of the groups of fuel alcohols, which have significant solubility in water. Other chemicals in the alcohol family include methanol (1-carbon), ethanol (2-carbon), and propanol (3-carbon). Butanol is used widely as an ingredient in perfumes and as a solvent for the extraction of essential oils (Mellan, 1950). Butanol is also used as an extractant in the manufacture of antibiotics, hormones, and vitamins; a solvent for paints, coatings, natural resins, gums, synthetic resins, dyes, alkaloids, and camphor (Doolittle, 1954; Mellan, 1950). Other industrial uses include the manufacture of pharmaceuticals, polymers, pyroxylin plastics, and herbicide esters (Monick, 1968).

Butanol is produced chemically using either the Oxo process starting from propylene (with H_2 and CO over a rhodium catalyst) or the Aldol process starting from acetaldehyde (Ezeji *et al*., 2007). Bio-fermentation is an attractive process for producing feedstock chemicals from renewable biomass. The production of butanol by



n-butanol isobutanol *sec*-butanol *tert*-butanol

Figure 2.1 Four isomeric alcohol

ABE fermentation used to be one of the largest bioprocesses until the 1950s, but later it was nearly disappeared in the 1960s because it could not compete on a cost basis with the less expensive petroleum-based chemical synthesis (Qureshi and Maddox, 1995). During the first half of the 20th century, the production of butanol from biological sources was a commercial reality. According to the National Renewable Energy Laboratory (NREL), biobutanol had previously been produced through a fermentation process known as ABE fermentation because it produced acetone, butanol, and ethanol in roughly 3:6:1 ratio. *Clostridium* strains were the fermenting organisms to create the chemicals from molasses-type feedstocks. In recent years, interest in bio-based butanol has been revived primarily due to concerns with petroleum fuel depletion, and microbial production of butanol is considered to be a potential source of liquid fuels. There is a relatively wide range of substrates suitable for ABE fermentation, but the process suffers from severe product inhibition, which is one of the primary reasons that the traditional batch process of ABE fermentation is not economically viable (Liu *et al*., 2005).

Table 2.1 physicochemical properties of *n*-butanol (Lee *et al*., 2008)

Properties	**Value**
Molecular formula	$CH_3(CH_2)_3OH$
Molar mass	74.122 g/mol
Appearance	colorless liquid
Density	0.8098 g/cm^3 (20 °C)
Melting point	−89.3 °C
Boiling point	117.7 °C
Solubility in water	7.7 g/100 mL (20 °C)
Viscosity	3 cP (25 °C)
Flash point	365 °C
Autoignition temperature	345 °C
Critical temperature	287 °C

2.1.1 Butanol as an alternative liquid fuel

Butanol can be used as an intermediate in chemical synthesis and as a solvent for a wide variety of chemical and textile industry applications. Moreover, butanol has been considered as a potential fuel or alternative liquid fuel. Biobutanol has sufficiently similar characteristics to gasoline to be used directly in any gasoline engine without modification or substitution (Table 2.2) (Lee *et al*., 2008). In comparison to gasoline and ethanol, butanol is hard to ignite and it burns with a cleaner flame. It is combustible but not dangerously flammable as is gasoline and ethanol. Furthermore, again in contrast to ethanol, butanol can be shipped through existing oil pipelines without causing damage (Ramey, 2007).

Table 2.2 Quality characteristics of gasoline and alcohol fuels (Lee *et al*., 2008)

Properties of fuels	**Butanol**	**Gasoline**	**Ethanol**	**Methanol**
Energy density (MJ/L)	29.2	32	19.6	16
Air-fuel ratio	11.2	14.6	9	6.5
Heat of vaporization (MJ/kg)	0.43	0.36	0.92	1.2
Research octane number	96	91-99	129	136
Motor octane number	78	81-89	102	104

Moreover, Butanol is superior to ethanol as a fuel additive in many regards: higher energy content, lower volatility, less hydroscopic (thus does not pick up water), and less corrosive (Durre, 2007). Also, branched chain 4-carbon alcohols including isobutanol, 2-methyl-1-butanol and 3-methyl-1-butanol have higher octane numbers compared with n-butanol (Atsumi *et al*., 2008), and thus are good candidates as fuel additives. However, butanol is in its infancy and many unanswered questions remain.

2.1.2 Butanol production by chemical synthesis

There are three most important processes for the chemical butanol industry including Oxo synthesis, Reppe synthesis, and Crotonaldehyde hydrogenation (Figure 2.2). The Oxo-synthesis was discovered in 1938 by Otto Roelen at Ruhrchemie that has marked the birth of the large scale industrial application of homogeneous catalysis by organometallic complexes. The term Oxo-synthesis, also known as hydroformylation, denotes the synthesis of oxygenates by

hydro-carbonylation of olefins (Drent and Budzelaar, 2000). Carbon monoxide and hydrogen are added to a carbon–carbon double bond using catalysts such as Co, Rh, or Ru substituted hydrocarbonyls (Figure 2.2a). In the first reaction step, aldehyde mixtures are obtained and followed by production of butanol by hydrogenation reaction. Different isomeric ratios of butanol are obtained which depend upon reaction conditions (pressure, temperature) as well as the catalyst (Lee *et al*., 2008). In Reppe process developed in 1942, propylene, carbon monoxide and water are made to react under pressure in the presence of a catalyst (tertiary ammonium salt of polynuclear iron carbonlyl hydrides) (Figure 2.2b). The difference between this process and hydroformylation is that at low temperature (100 °C) and pressure alcohol is formed directly (Bochman *et al*., 1999). Nevertheless, this process has not been commercially successful in spite of certain advantages it offered compared to conventional oxo process.

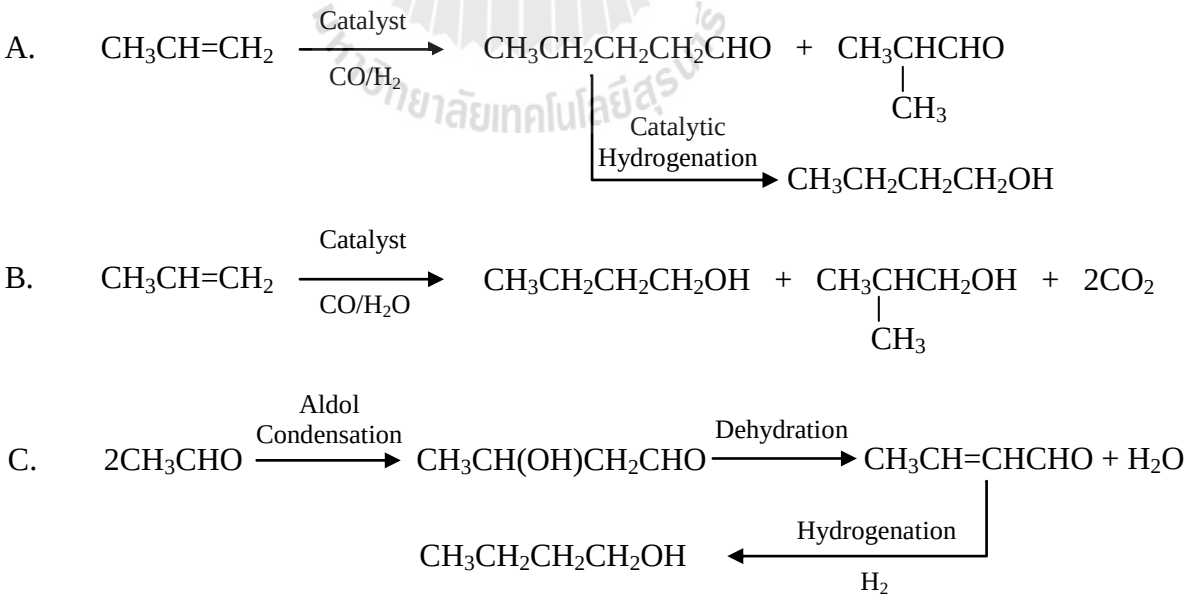


Figure 2.2 Chemical synthesis of butanol: (a) Oxo synthesis, (b) Reppe process, (c) Crotonaldehyde hydrogenation

Moreover, this process is attributed to more expensive process technology. Until the mid 1950s, *n*-butanol was produced based on acetaldehyde process using crotonaldehyde hydrogenation (Figure 2.2c) and also was the preferred process. Acetaldehyde is produced consisting of aldol condensation, dehydration, and hydrogenation at normal temperature and pressure (Bochman *et al*., 1999). In addition, for tropical countries with large supplies of cheap biomass as well as for the more developed countries of the third world who do not have their own oil resources, this process is alternated to Oxo process. In this case, the plants producing butanol from alcohol have been generally located near alcohol distillation.

2.1.3 Biotechnological butanol production

One of the oldest industrial fermentation with a history of more than 100 years is known as ABE fermentation which it was first reported by Pasteur using microbial fermentation from his landmark anaerobic cultivation in 1861 (Jones and Woods, 1986). In 1911, biomass such as potatoes was fermented by Fernbach to produce butanol using isolated culture. This research was promoted by the synthetic rubber industry, which used precursors such as butadiene and isoprene obtained from butanol (Ranjan and Moholkar, 2009). This was followed by Chaim Weizmann, who works at Manchester University, isolated cultures of *Clostridium acetobutylicum*. The result shows that it had capability of fermenting starchy substrate, with higher butanol yield than the cultures of Fernbach. The era of World War I and II saw the largest growth of ABE fermentation industry in Europe and USA, as a source of acetone for manufacture of cordite, a smokeless powder used in ammunition. In 1945, two thirds of industrially used butanol was produced by fermentation in U.S. However, the ABE

fermentation process had lost competitiveness by 1960s due to the increase of feedstock costs and advancement of the petrochemical industry except in Russia and in South Africa, where the substrate and labor costs were low (Lee *et al*., 2008). The ABE fermentation processes in South Africa and Russia continued to operate until the late 1980s to early 1990s (Zverlov *et al*., 2006). More than two decades later, the interest of the scientific community and industry in the process has revived due to depleting oil reserve and highly fluctuating crude oil price. The basic research is now directed towards improvement of the complete process by use of genetically manipulated strains, alternate cheaper fermentation substrate, better cultivation techniques, and efficient product removal. The successful industrial level of butanol fermentation in the countries mentioned above can provide guidelines to our current effort to produce butanol in large scale.

2.2 ABE fermentation

The saccharolytic butyric acid producing Clostridia are microorganism to process in ABE fermentation (Jones and Woods, 1986). The most popular and extensively implemented strain for the production of acetone and butanol are now generally classified as *Clostridium acetobutylicum*. In addition, several other species of butanol producing clostridia have also been recognized such as *Cl. beijerinckii* (*Cl. butylicum*) produces solvents in approximately the same ratio as *Cl. bcetobutylicum*, but isopropanol is produced in place of acetone, while *Cl. aurantibutyricum* produces both acetone and isopropanol in addition to butanol (George and Chen, 1983). A newly isolated species which produces almost equimolar amounts of butanol and ethanol but no other solvents is also known as *Cl. tetanomorphum* (Gottwald *et al*.,

1984). Schuster *et al*. (1998) reported that *C. acetobutylicum* showed marked change in the cell morphology during the course of the cultivation (Figure 2.3). During early growth and the acid production phase, only rod-shaped cells, which sometimes formed chains, were observed. Later, at or just prior to the solvent shift, clostridial forms appeared, containing granulose. As the fermentation proceeded, the cellular granulose content reached a peak, which coincided in most experiments with the maximum solvent productivity, after which it decreased.

These butanol-producing Clostridia exhibit very similar metabolic pathways. During fermentation, three major classes of products are produced by *Cl. acetobutylicum*: (i) solvents (acetone, ethanol and butanol); (ii) organic acids (acetic acid, lactic acid and butyric acid); (iii) gases (carbon dioxide, and hydrogen). The biosyntheses of acetone, butanol and ethanol share the same metabolic pathway from glucose to acetyl-CoA but branches into different pathways thereafter (Figure 2.4) (Zheng *et al*., 2009). There are five enzymes including acetoacetyl-CoA thiolase, 3-hydroxybutyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase and aldehyde/alcohol dehydrogenase, which are needed to complete the conversion of acetyl-CoA to butanol. Moreover, nowadays, higher selectivity for butanol as well as higher overall yield of ABE solvents have also developed by researchers at University of Illinois using the mutant strain named *Cl. beijerinckii* BA101, which has the ratio of 3:16:1 with total solvent yield of 33 g/L (Annous and Blaschek, 1991). This result is completely differences to the typical batch fermentation which has the ratio of ABE solvents produced by *Cl. acetobutylicum* is 3:6:1 with the maximum concentration of 20 g/L. However, the choice of strain for a particular process depends upon the nature of substrate and ratio of the end products required.

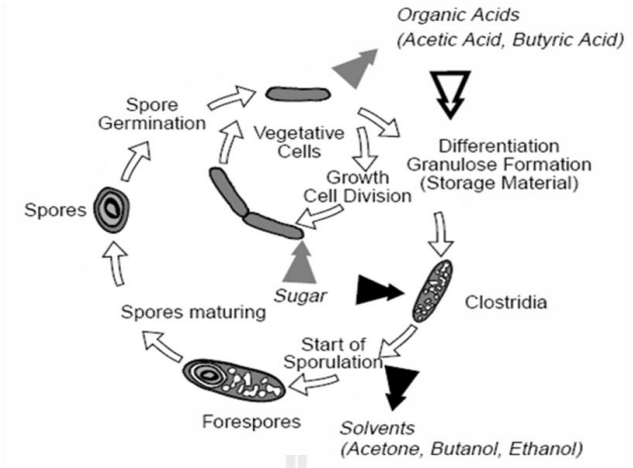


Figure 2.3 Cell cycle of *Clostridium acetobutylicum* (Schuster *et al*., 1998)

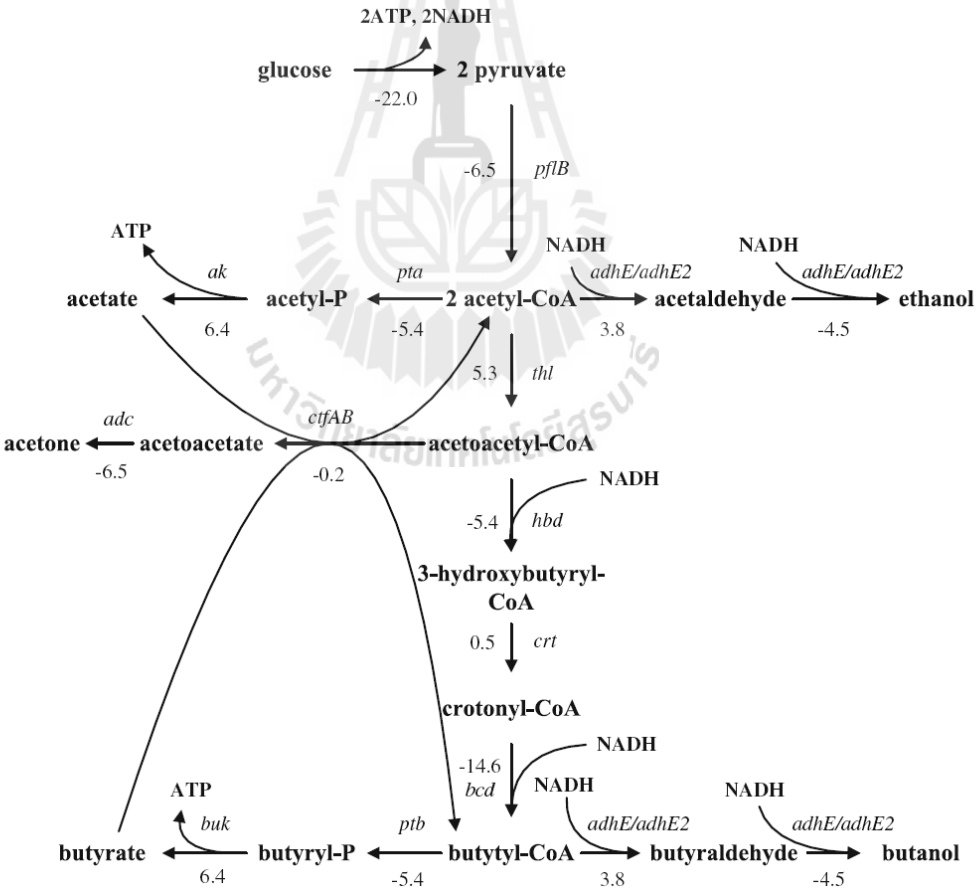


Figure 2.4 Metabolic pathways of *C. acetobutylicum* for acetone, butanol, and ethanol production (Zheng *et al*., 2009)

The various conventional substrates for ABE fermentation have been used including molasses, whey permeate, corn (Ezeji *et al*., 2007; Jones and Woods, 1986), and starch with origin such as maize, wheat, rye etc. (Gibbs, 1983; Lenz and Moreira, 1980). However, these substrates have been utilized for other purposes such as cattle feed. Therefore, a main factor impacting overall economy of the butanol production is the substrate cost and hence, extensive research has been in recent past on the variety of cheaper substrates which can substitute for the conventional substrates (Table 2.3). More recently, many several other carbon sources have been tried to develop the suitable condition such as liquefied corn starch that provides yield of 81.3 g/L ABE solvents under fed batch mode, wheat straw that yield 12 g/L ABE solvents with simultaneous saccharification and fermentation, and corn fiber hydrolysate with sulfuric acid treatment achieve yield of 9 g/L ABE solvents (Ranjan and Moholkar, 2009). Another sufficient raw material for fermentation is lignocelluloses biomass with 20-40% of hemicelluloses which it contains important pentose sugar, especially xylose. Hemicelluloses is fermented by *Clostidium Acetobutylicum*, but with lower yield of about 28%. A new method, biomass is the direct utilized by mixed cultures of microorganisms, which have enzymes capable of hydrolyzing cellulose and hemicelluloses (Soni *et al*., 1982).

Furthermore, excess carbon is used with limited nitrogen in order to achieve high levels of solvent production (Madihah *et al*., 2001). Iron is an important mineral supplement since the conversion of pyruvate to acetyl-CoA involves a ferredoxin oxidoreductase iron-sulfur protein (Kim *et al*., 1988). Another very important parameter for biphasic acetone–butanol fermentation is pH of the medium. In acidogenesis, acetic and butyric acids are formed rapidly by decrease in pH. In solventogenesis, it will

Table 2.3 Butanol Production by different substrates using *C. Beijerinckii* BA101 (Ezeji *et al*., 2007)

Parameters	Glucose	Corn starch	Malto-dextrin	Soy molasses	Agricultural waste	Cassava starch*
Acetone (g/L)	4.3	7.7	6.8	4.2	4.8	3.6
Butanol (g/L)	19.6	15.8	18.6	18.3	9.8	16.9
Ethanol (g/L)	0.3	1.2	0.7	0.3	0.2	0.5
Tatol ABE (g/L)	24.2	24.7	26.1	22.8	14.8	21.0
ABE productivity (g/L h)	0.34	0.34	0.37	0.19	0.22	0.44
ABE yield (g/g)	0.42	0.44	0.50	0.39	-	0.41

* Simple batch fermentation using *C. saccharoperbutylacetonicum* N1-4 (Thang *et al*., 2009)

be started when pH reaches a critical point, beyond which acids are reassimilated and butanol and acetone are produced. Therefore, low pH is a prerequisite for solvent production (Kim *et al*., 1984). However, if the pH decreases below 4.5 before enough acids are formed, solventogenesis will be brief and unproductive. A simple way to increase growth and carbohydrate utilization is increasing of buffering capacity of the medium (Bryant and Blaschek, 1988). Conventional ABE fermentation takes 2–6 days for completion a batch fermentation depending on the condition and the type of substrate employed. In batch fermentation, the final total concentration of solvents is produced in range of 12 to 20 g/L, which can be recovered from the fermentation broth by various methods.

2.2.1 Biobutanol production by batch fermentation

In the biotechnological industry, batch fermentations were used widely to produce biobutanol due to simple operation and reduced risk of contamination.

Typical capacities of these fermenters were 200 to 800 m^3. However, the productivity achievable in a batch reactor is low due to the lag phase, product inhibition as well as down time for cleaning, sterilizing, and filling. The industrial process used 8–10% corn mash, which was cooked for 90 min at 130–133 °C (Ezeji *et al*., 2007). Sugar cane molasses was also used to produce biobutanol in a commercial plant in South Africa until the early 1980s. An example, the main substrate, molasses, containing 55% w/w fermentable sugar and 30% w/w non-fermentable solids, was diluted to 60 g/L sugar and mixed with other nutrients in feed tank. The fermentation period is 30 h and the broth contains 13.7 g/L butanol, 5.4 g/L acetone, 1.5 g/L ethanol, 0.2 g/L butyric acid, 0.3 g/L acetic acid and 3 g/L cells (Ranjan and Moholkar, 2009). Typically, conventional butanol fermentation should be noted that at a maximum concentration of approximately 20 g butanol/L, cell growth inhibition and premature termination of the fermentation occurs. Low butanol concentration in the reactor can cause by product inhibition or toxicity. In addition, the use of a dilute sugar solution results in large process volumes. Because of these constraints, the commercial biobutanol production on a large scale has been considered to be uneconomical. Several new process designs have been investigated to overcome these problems. The preparation time and lag phase can be eliminated using continuous culture and the problem of product inhibition can be solved using an *in situ* product removal system (Lee *et al*., 2008).

2.2.2 Fed-batch fermentation

Due to the complication of ABE fermentation with problems of culture stability, the use of continuous culture for the industrial production of solvents has

been well established. Fed-batch fermentation is carried out by like manner to a batch culture, but it is continuously fed with substrates and without removal of fermentation broth. Leung and Wang (1981) demonstrated the production of 15.9 g/L by *C. Acetobutylicum* ATCC 824, with a yield of 0.32 g/g and a productivity of 1.5 g/L/h in a glucose-limited (50 g/L) complex medium at a dilution rate of 0.1/h. At a dilution rate of 0.22/h, a maximum productivity of 2.55 g/L/h was obtained, but the solvent yields and concentration were reduced to 12 g/L. Although, researches have shown that continuous cultures can be utilized with high rate of productivity, the total solvent concentration and yield stay in same efficiency as batch cultures (Jones and Woods, 1986). Multistage continuous culturing is a remedy, which gives separation of propagation and production phase. Dyr *et al*., (1958) employed a series of five fermenters which the first fermenter gives maximum growth. Acid and solvent products were formed in the second fermenter and the last three fermenters, respectively. Nevertheless, two or multi-stage fermentation have been less selected due to there are major problem of continuous culture including loss of solvent production, and its effect on pH and butanol production.

2.2.3 Immobilized and cell recycle in continuous fermentation

Generally, cell concentration in a conventional batch reactor does not exceed 48 g/L and hence, the biobutanol productivity rarely exceeds 0.59 g/L/h (Ranjan and Moholkar, 2009). Immobilized cell systems may be more suitable for solvent production than continuous culture utilizing free cells. It is an easy way to solves above limitations by immobilizes microbial cells and recycles them. Advantages of immobilized cell systems are distinct included (Jones and Woods,

1986): (1) the physical retention of the cells in the matrix, facilitating the separation of the cells from the products; (2) high cell densities per reactor volume; (3) high cell concentrations, allowing smaller reactor volumes and greater productivity; (4) use of packed columns or fluidized-bed reactors, resulting in maximum reaction rates; (5) minimum nutrient depletion and product inhibition; (6) better mass transfer through decreased feed viscosity and increased differential velocities and; (7) simpler non-growth media when stationary-phase cells are immobilized.

Several research have related to immobilized cells. Huang *et al*., (2004) immobilized cell using *Cl. acetobutylicum* in a fibrous support and used these in a continuous reactor to produce ABE; a productivity of 4.6 g/L/h was obtained. Qureshi and Maddox, (1995) immobilized cells of *Cl. acetobutylicum* by adsorption onto bonechar, and used in a packed bed or fluidized bed reactor for continuous production of ABE from whey permeate. At dilution rates in the range 0.35-1.10 h^{-1}, ABE productivity values of 3.0 to 4.0 g/L/h were observed, but lactose utilization values were poor. Another research, solvent production immobilized in calcium alginate gels have been investigated by Haggstrom and Molin (1980) using vegetative cells and spores of *Cl. acetobutylicum*. The maximum levels of solvents obtained in batch and continuous column operations varied between 1.44 and 4.53 g/L, with productivities of 57 to 67 g of butanol/L/day and yield coefficients of 0.176 to 0.209 g of butanol per g of glucose. In addition, major unfavorable properties associated with this technique were high gas hold up or accumulation of the bubbles in the immobilization matrix because the continuous phase or fermentation broth was not in complete contact with the matrix. This reason, mass transport of substrate and products were limited and activity loss. Therefore, the system is not suitable for continuous

operation in fixed bed mode. Other design alternatives such as fluidized bed or continuous stirred tank mode have also been limited by the inhibitory butanol concentration.

2.3 Downstream processes

In biotechnological industries, the process is mostly operated under unsuitable condition in order to achieve high productivity of biocatalytic products and keeping with physiological limitations (Lye and Woodley, 1999; Schugerl, 2000). Dilute product streams, low productivities, and high recovery costs are the consequences of these limitations. High product recovery cost is a major limitation in biobutanol production. In order to produce biobutanol, the traditional recovery process is the distillation but it is still suffered from a high operation cost due to the low concentration of butanol in the fermentation broth. To solve this problem as well as the solvent toxicity problem during fermentation, *in situ* recovery systems have been introduced. The concept of an integrated fermentation/product recovery process, also is known as extractive fermentation, is the selective continuous removal of inhibitory product from a reactor or reaction site as soon as it is formed and hence, permits full advantage to be taken of highly productive reactor systems and can also provide further benefits for the subsequent downstream processing (Qureshi and Maddox, 1995). *In situ* product removal (ISPR) methods can increase the productivity or yield of a biocatalytic reaction by any of the following means (Chauhan and Woodley, 1997); (1) overcoming inhibitory or toxic effects, (2) shifting unfavourable reaction equilibria, (3) minimizing product losses owing to degradation or uncontrolled release, and (4) reducing the total number of downstream-processing steps. The various bases for

Table 2.4 Quantitative comparison of ISPR techniques of biocatalytic process (Lye and Woodley, 1999)

Separation basis (driving forces)	**Operating methods**	**Comments**
Physical properties		
Volatility	Distillation Gas stripping	Few example with these properties
Molecular weight or size	Membrane processes Centrifugation Size exclusion Pervaporation Perstraction	The difference between substrate and product is frequently small
Solubility	Extractions Precipitation Crystallization	High capacity but low selectivity
Chemical properties		
Charge	Ion-exchange Electrodialysis	High selectivity but low capacity
Hydrophobicity	Chromatograghy Adsorption	
Specific elements	Affinity methods	

quantitative comparison of ISPR techniques of a biocatalytic process are summarized in Table 2.4. Indeed, some extractive fermentation processes including pervaporation, perstraction, liquid–liquid extraction, gas stripping, and reverse osmosis have been developed to improve recovery performance and reduce costs the ABE fermentation process.

2.3.1 Gas stripping

Gas stripping is a simple technique, but efficient for butanol recovery, that can be applied for *in situ* butanol recovery during the ABE fermentation. Normally, CO_2 and H_2 is generated during the ABE fermentation. These fermentation gases are used to recover butanol during simultaneous fermentation. In a process to recover the biobutanol from the ABE fermentation broth is simpler and more economical (Ezeji *et al*., 2007). Figure 2.5 show a schematic diagram of a typical process of solvent removal by gas stripping. The fermentation gas is bubbled through the fermentation broth, and then passed through a condenser for solvent recovery. It captures ABE which is subsequently condensed and collected in a receiver vessel. Once the solvents are condensed, the stripped gas is then recycled back to the fermentor to capture more ABE and the process continues until all the sugar in the fermentor is completely utilized. For advantages of this technique, it enables the use of a concentrated sugar solution in the fermentor and a reduction in butanol inhibition and high sugar utilization. There are wide literatures have been worked in order to develop the ABE processing. (Ezeji *et al*., 2003) investigated an integrated process of ABE fermentation-recovery using *C. Beijerinckii* BA101. A batch control reactor *C. Beijerinckii* BA101 utilized 45.4 g glucose/L and produced 17.7 g total ABE/L, while in the integrated process it utilized 161.7 g glucose/L and produced total ABE of 75.9 g/L. Another process was produced in an integrated fed-batch and continuous fermentation by (Ezeji *et al*., 2004a). Gas stripping product recovery system was attempted in a process and using *C. Beijerinckii* BA101 with H_2 and CO_2 as the carrier gases. In a fed-batch process, 500 g glucose was consumed and 233 g solvent was produced with the productivity of 1.16 g/L/h and the yield of 0.47 g/g. In

addition, a continuous butanol fermentation was recovered by gas stripping where feed and effluent were continuous. In this process, 460 g of total solvent was produced from 1,163 g glucose with the productivity of 0.91 g/L/h (Ezeji *et al*., 2004b). This suggests that can be successfully applied to ABE fermentation.

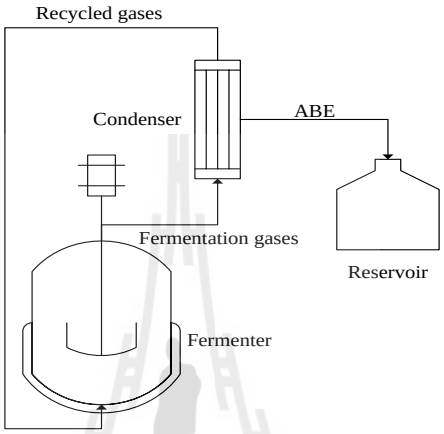


Figure 2.5 Schematic diagram of biobutanol production and recovery by gas striping (Lee *et al*., 2008)

2.3.2 Reverse osmosis

Reverse osmosis (RO) is a recovery technique that relies on membranes. Reverse osmosis starts to dewater the fermentation liquor by rejecting solvents but allowing water to pass through the membrane. Consequently, the products are concentrated, and the volume of liquid to be distilled is dramatically reduced (Zheng *et al*., 2009). The polyamide membranes were used as filter which exhibited rejection rates as high as 98%, and that optimum rejection of butanol in the fermentation liquor occurred at recoveries of 20–45% (Garcia Iii *et al*., 2004). However, this process needs to operate under high energy consumption and high pressure (50 bar) will be

applied as well. In addition, it is necessary to remove the suspended vegetative organisms using a hollow-fiber ultrafilter before the reverse osmosis is carried out.

2.3.3 Adsorption

Adsorption is a simple technique that can be used to remove butanol from the fermentation broth energy efficiently. In this manner, butanol is first adsorbed by adsorbents from the fermentation broth and then desorbed by heat treatment or displacers to give concentrated butanol solutions as final products. A variety of materials can be used as adsorbents for butanol recovery, but silicalite is the one used most often. Silicalite, a form of silica with a zeolite-like structure and hydrophobic properties, can selectively adsorb small organic moleculars like C1–C5 alcohols from dilute aqueous solutions (Zheng *et al*., 2009). Milestone and Bibby (1981) investigated the Adsorbing 1-butanol from a 0.5% solution by drying the silicalite at 40°C, and then heating to 150°C. The result shown that a condensate contains 98% (w/v) butanol. However, this process still needs the regeneration steps, hence if we need to process with the ISPR method, the process should be automatically operated.

2.3.4 Liquid-liquid extraction

Another efficient technique to recover or remove inhibitory products from the fermentation broth is liquid–liquid extraction. This approach takes advantage of the differences in the distribution coefficients of the chemicals in organic solvents. In a process, an extractant (extraction solvent) is mixed with the fermentation broth. The products (acetone, butanol, and ethanol) will be extracted into the extractant because the products are more soluble in the extractant (organic phase) than in the

fermentation broth (aqueous phase) and hence, it is selectively concentrated in the extractant. Finally, it is recovered by back extractant into another extractant or by distillation. Due to the early report on the extractive butanol process by Wang *et al.* (1979), there are many reports on the use of numerous extraction solvents for extractive butanol fermentation. Roffler *et al.* (1988) successfully investigated to increasing the productivity of the acetone-butanol fermentation by continuously removal during fed-batch fermentation which containing viable cells of *Clostridium acetobutylicum*. Acetone and butanol were extracted into oleyl alcohol flowing counter-currently through the column. The concentrated feed solution containing 300 g/L glucose was fermented at an overall butanol productivity of 1.0 g/L h, 70% higher than the productivity of normal batch fermentation.

However, this technique still has critical problems such as the toxicity of the extractant to the cell and emulsion formation (Lee *et al*., 2008). In order to overcomes these constrains, it can be successful if the fermentation broth and extractant are separated by a membrane that provides surface area for solvent exchange between the two immiscible phases. On the other hand, there are many extractant choice to take place in continuous removal of fermentation products, a important extractant has been oleyl alcohol because it is relatively non-toxic, and being a good extractant as well (Ezeji *et al*., 2007).

2.3.5 Perstraction

As earlier mentioned on liquid-liquid extraction, several problems are associated with liquid–liquid extraction, such as cell toxicity, loss of extraction solvent, formation of an emulsion, and the accumulation of microbial cells at the

extractant and fermentation broth interphase. To solve these problems, perstraction technique was developed to recover the products. In a perstractive separation, the extractant and the fermentation broths are separated by a membrane, which allows butanol to diffuse into the extractant phase. The existence of the membrane greatly reduces, if not eliminates, the toxicity of the extractants, but the rate of butanol extraction is limited, because the membrane presents a physical barrier between the extractant phase and the fermentation broth (Ezeji *et al*., 2007). In term of perstraction that shown on figure. *Cl. acetobutylicum* has been cultivated in a continuously operated membrane bioreactor connected to a four-stage mixer-settler cascade (Eckert and Schugerl, 1987). In this system, butanol was extracted with n-decanol (extractant) from the cell-free fermentation broth, which was re-fed into the fermentor. This system enabled production of solvents with a high productivity of 3.08 g/L/h.

2.3.6 Pervaporation

Pervaporation is a membrane-based process that is used to remove volatile compounds from model solution/fermentation broth by using a selective membrane. Pervaporation is considered to be the best potential separation technology to recover *n*-butanol, ethanol and acetone from the ABE fermentation broth because of its efficiency and energy-saving capabilities if a high performance (selectivity and permeability) membrane is available (Huang and Meagher, 2001). Additional advantages include no harmful effects on the microorganisms or removal of medium ingredients from the reaction mixture. Usually, the compounds diffuse through a solid membrane leaving behind nutrients, sugar, and microbial cells. As a process, one side of the membrane is in contact with the fermentation broth, and the volatile or organic

component selectively diffuses through the membrane matrix as a vapor which is induced by the application of a vacuum pump or an inert carrier gas on the other side of the membrane. The compound is then recovered by condensation. The concentration of solvents across the membrane depends upon membrane composition and membrane selectivity, which is a function of operating conditions (Ezeji *et al*., 2004b). In addition, the mechanism by which a volatile/organic component is removed by pervaporation is called solution-diffusion model as shown in Figure 2.6. The transport mechanism for the pervaporation system can be explained using the solution-diffusion model which involves three major steps. The first step involves absorption of chemical molecules into the membrane surface. The second step is the diffusion across the membrane matrix due to concentration and/or pressure difference. In the third step, the chemical compound then vaporize somewhere in the membrane, and can be obtained as a vapor under vacuum or swept out by an inert carrier gas before being collected in a cold trap. Pervaporation functions independent of the vapor/liquid equilibrium, and the permeate must be volatile under the operating conditions. The effectiveness of pervaporation can be measured by two parameters: the selectivity (a measure of the selective removal of volatiles) and flux (the rate at which an organic/volatile passes through the membrane per unit area) (Ezeji *et al*., 2007).

Polydimethylsiloxane membranes and silicon rubber sheets are generally used for the pervaporation process as shown in Figure 2.7. To develop a stable membrane having a high degree of selectivity, Qureshi *et al*., (1999) synthesized a silicon-silicalite-1 composite membrane which showed a 2.2-fold improvement in selectivity. In the same manner, a membrane made with a silicalite to polymer ratio of 1.5:1 (g:g) gave butanol selectivities of 100–108 and a flux of 90 g/m^2/h at feed butanol

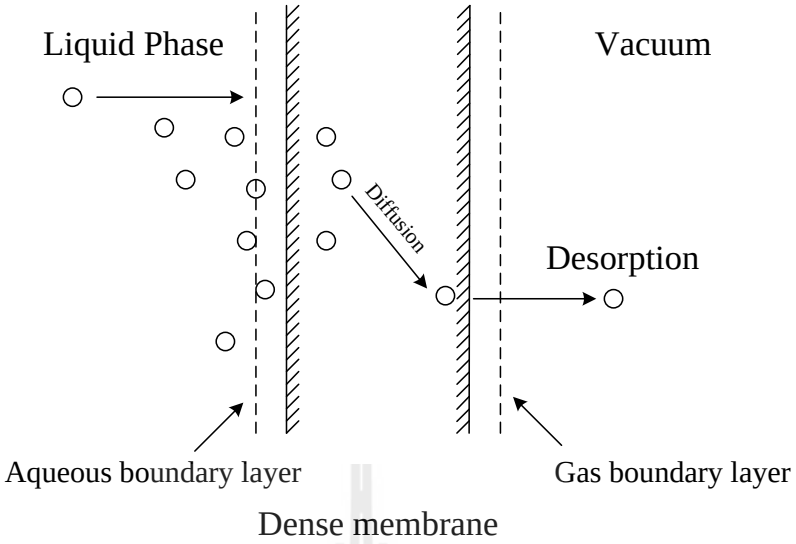


Figure 2.6 Schematic diagram of pervaporation system (Huang, 1991).

concentration of 5–9 g/L, while a silicone membrane at the same conditions had a selectivity and flux of 30 and 12.5 g/m^2/h, respectively (Qureshi *et al*., 2001). Moreover, Huang and Meagher (2001) developed a dense silicone–silicalite membrane by incorporating 1–3 μm silicalite-1 particles into silicone with the thickness of 100–300 μm. Under certain conditions the membrane had a selectivity of 100–200 depending on the feed concentration of *n*-butanol. The averaged flux was 100 g/m^2/h at 78◦C and was dependent on the feed *n*-butanol concentration and temperature. This membrane has the desired *n*-butanol selectivity, but lacks the flux rate necessary for a commercial process. The best way to increase membrane flux is to decrease the membrane active layer thickness via a thin-film composite membrane structure.

The other application of pervaporation for continuous recovered butanol fermentation has been described by several investigators. Qureshi and Blaschek (1999) applied an integrated batch-pervaporation process with *Cl. beijerinckii* BA101

to recover butanol fermentation broth. In this process, which was initiated with 151.2 g/L glucose solution, 51.5 g/L ABE was produced. *Cl. beijerinckii* BA101 was not negatively affected by the pervaporation conditions. Since the membrane permeate contains acetone, butanol, and ethanol, distillation is still required for further purification. Furthermore, butanol fermentation and recovery were also performed in a fed-batch reactor where a 500 g/L glucose solution was used to feed the reactor. In this fed-batch pervaporation system, 165.1 g/L of total ABE was produced. ABE productivity was increased from 0.35 to 0.98 g/L/h due to the reduction in product inhibition (Qureshi and Blaschek, 2000). Recently, the overall solvent productivity in continuous fermentation of *Cl. acetobutylicum* was increased up to 2.34 g/L/h by integrating with a pervaporation system using an ionic liquid polydimethylsiloxane ultrafiltration membrane (Izak *et al*., 2008).

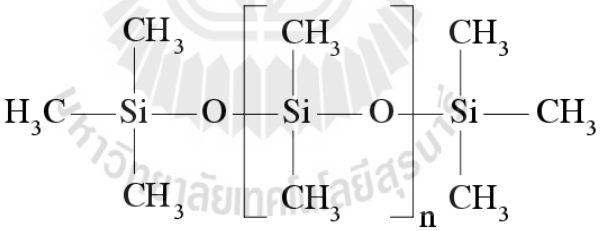


Figure 2.7 Chemical structure of polydimethyl siloxane (PDMS)

2.4 Hollow fiber membrane

Almost of the membrane techniques described above were originally developed to produce flat-sheet membrane. Currently, flat sheet membranes have been widely employed for vapor permeation regardless of its limited area and low packing density. Leemann *et al*. (1996) suggested that the unavailability of commercial membrane with sufficiently high packing density and insufficient solvent stability of the existing

membranes are among the major difficulties encountered in the development of vapour permeation membrane technology. However, most techniques can be adapted to produce membrane in the form of thin tube or fiber. Formation of membrane into hollow fibers has a number of advantages, one of the most important of which is the ability to form compact modules with very high surface areas. This advantage is offset, however, by the lower flux of hollow fiber membranes compared to flat-sheet membranes made from the same materials. Nonetheless, the development of hollow fiber membranes by Mahon and the group at Dow Chemical in 1960, and their later commercialization by Dow Chemical, DuPont, Monsanto and others, represents one of the major events in membrane technology.

Hollow fibers are usually on the order of 25-200 μm in diameter. They can be made with a homogeneous dense structure, or more preferably as a microporous structure having a dense permselective layer on the outside or inside surface. The dense surface layer can be integral or separately coated. The fiber are packed into bundles and potted into tubes to form a membrane module. More than a kilometer of fiber is required to form a membrane module with surface area of one square meter. Since on breaks or defects are allowed in a module, this requires very high standards of reproducibility and quality control. Hollow fiber fabrication methods can be divided into two classes. The most common is solution spinning, in which a 20-30 % polymer solution is extended and precipitated into a bath of nonsolvent. Solution spinning allows fibers with the asymmetric structure to be made. An alternative technique is melt spinning, in which a hot polymer melt is extruded from an appropriate die and is then cooled and solidified in air or a quench tank. Melt spun fiber are usually dense and have lower fluxes than solution spun fibers, but, because

the fiber can be stretched after it leave the die, very fine fibers can be made. Melt spinning can also be used with polymers such as poly(trimethylpentene) which are not soluble in convenient solvents and are therefore difficult to form by wet spinning.

The previous research project (Panvichit, Kanchanatawee *et al*., 2006) has been successful for fabrication of composite hollow fiber membrane using the PVDF (polyvinylidene fluoride) as support layer coated with PDMS as selective layer. In this case, the membrane was used to separate the ethanol from dilute fermentation broth by perstraction system. The result show that the overall mass transfer coefficient rang from 3.0×10^{-7} to 4.21×10^{-6} m/s. In the future, development of hollow fiber is necessary to provide more surface area of the membrane with improvement the selective surface layer or coating technology.

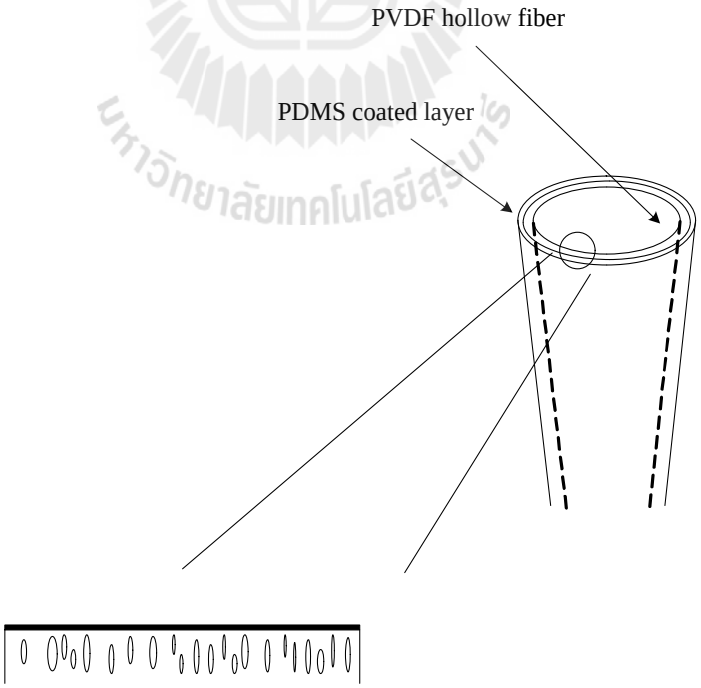


Figure 2.8 Structure of composite hollow fiber membrane with coating of PDMS

2.5 Natural rubber latex

The commercial source of natural rubber latex is the para rubber tree (*Hevea brasiliensis*), a member of the spurge family, Euphorbiaceae. This is largely because it responds to wounding by producing more latex. Natural rubber (NR) is an elastomer (an elastic hydrocarbon polymer) that was originally derived from latex, a milky colloid found in the sap of some plants. The plants would be tapped, an incision made into the bark of the tree and the latex sap collected and refined into a usable rubber. The purified form of natural rubber is the chemical polyisoprene, which can also be produced synthetically. Natural rubber is used extensively in many applications and products, as is synthetic rubber. Generally, the natural rubber latex has approximate density of 0.975 – 0.980 g/ml, pH 6.5 - 7.0, and viscosity of 12 – 15 cP. The total compositions of natural rubber latex are shown in Table 2.5.

Table 2.5 Composition of natural rubber latex (Blackley, 1997)

Compositions	**percentages**
Total solid cotent, TSC	27 – 48
Dry rubber content, DRC	25 – 45
Protein	1 – 1.5
Resin	1 – 2.5
Ash	>1
Carbohydrate	1
Water	remainder

During the last few decades, the importance of polymer blends has increased, since it is possible to tailor desirable properties by simple blending of polymers.

Natural rubber was also considered as potential rubber that can use to fabricate the membrane in downstream bioprocess. Polymer blending has already been established as an effective means for constructively altering the transport properties of polymeric materials. Johnson and Thomas (1998) investigated the pervaporation separation and the swelling behavior of chlorinated hydrocarbon/acetone mixtures using natural rubber (NR) and epoxidized natural rubber (ENR) membrane with 25 and 50 mol% epoxidation, respectively. The membranes were found to be permselective to chlorinated hydrocarbons from acetone-chlorinated hydrocarbon mixtures. The flux decreases with increase in epoxidation level, whereas the separation factor increases. The permeation decreases and separation factor increases with increase in the acetone feed concentration. However, many of these polymer blends are incompatible or immiscible. They are characterised by narrow interface and weak interfacial interaction, and often exhibit poor mechanical properties (Paul, 1976).

Table 2.6 The common properties of natural and synthetic rubbers (Pongsathorn, 2005)

Properties	Type of rubbers					
	NR	**IR**	**SBR**	**BR**	**NBR**	**EPDM**
Tensile strength (without additive)	1	2	5	6	5	5
Tensile strength (with additive)	1	2	2	4	2	3
Tear resistance	2	2	3	5	3	3
Heat resistance	5	5	4	4	3	2
Acid resistance	3	3	3	3	4	1
Base resistance	3	3	3	3	4	1
Gas permeability	5	5	4	4	2	4

*1 = excellent, 6 = poor: Natural rubber (NR), Synthetic polyisoprene (IR), Styrene-butadiene Rubber (SBR), Polybutadiene (BR), Nitrile rubber (copolymer of polybutadiene and acrylonitrile, NBR), ethylene propylene diene monomer (EPDM)

CHAPTER III

MATERAILS AND METHODS

3.1 Apparatus

Bioreactor: Sartorius, Germany

Electronic digital scale: Sartorius, Germany

Gas Chromatography: SRI Instruments, INC., USA

High performance Liquid chromatography: Agilent technologies 1200 series, USA

Hot plate: V.Go, USA

Membrane modules: Biofuel Production from Biomass Research Unit, School of Biotechnology, Suranaree University of Technology, Thailand)

Membrane spinning machine: Biofuel Production from Biomass Research Unit, School of Biotechnology, Suranaree University of Technology, Thailand)

Peristaltic pump: Cole-Parmer, USA

pH combined electrode: EUTECH Instruments, Singapore

pH meter: Sartorius, Germany

Syringe pump: Cole-Parmer, USA

Thermostat: Julabo, Germany

Vacuum pump: Osaka Air Machine INC., Japan

3.1 Materials and chemicals

Analytical grade *n*-butanol, acetone, and ethanol (Sigma-Aldrich, Singapore) were used together with de-ionized water to prepare the aqueous feed solutions for the pervaporation studies on membrane performances. The membranes used in this work were three different membrane materials. A commercial Polydimethyl siloxane (PDMS) composite flat-sheet membrane was supplied by Sulzer Chemtech GmbH, Switzerland, and two composite hollow fiber membranes, Polyvinylidene fluoride (PVDF) membranes were spun for the supportive layer in our laboratory prior they were kindly provided the coating with Natural rubber (NR) and Carboxylated Styrene-Butadiene Rubber (XSBR) for the active layer by the Membrane Science and Technology Research Center, Prince of Songkla University (PSU), Thailand. In order to evaluate the effects of operating conditions on the separation performance, All of membranes mentioned previously were used for the separation of butanol from binary solution (butanol/water) with butanol concentration in the range of 1.25-10.0 % v/v and quaternary mixture solution (acetone/butanol/ethanol/ water) containing 3.0 g/L acetone, 10 g/L n-butanol, 1 g/L ethanol that encountered to the fermentation broth. Both the binary and quaternary solutions were performed at varying temperature of 35-80 °C for 1 h.

3.1 Preparation of composite hollow fiber membranes

3.1.1 Fabrication of asymmetric PVDF hollow fiber membranes

PVDF hollow fiber membranes used as the supportive membrane in this study were spun by using the dry–wet phase inversion method. The dope composition consist of 15 wt.% PVDF (Kynar K760) in 85 wt.% *N*-methyl pyrrolidone (NMP) (Merck, Synthesis grade) and 4 g of lithium chloride (LiCl) were added in every 100 g

of PVDF–NMP solution as non-solvent additive. The detailed spinning procedure used in this study was followed to Yeow *et al.* (2005). A desired amount of PVDF was pre-dried for 24 h in oven dried at 50 °C and then was weighed and poured into pre-weighed NMP solvent contained in 1 L Duran bottles. The mixture was subjected to vigorous shaking so as to ensure thorough wetting of polymer pellets, prior to the addition of LiCl. The polymer dope mixtures were then placed to continuous stirring using vigorous shaking at 300 rpm for 96 h. The fully dissolved polymer solution was transferred into a stainless steel reservoir, allowed to stand and degassed for 24 h at room conditions prior to hollow fiber spinning. An experimental setup of the spinning apparatus is shown in Figure 3.1.

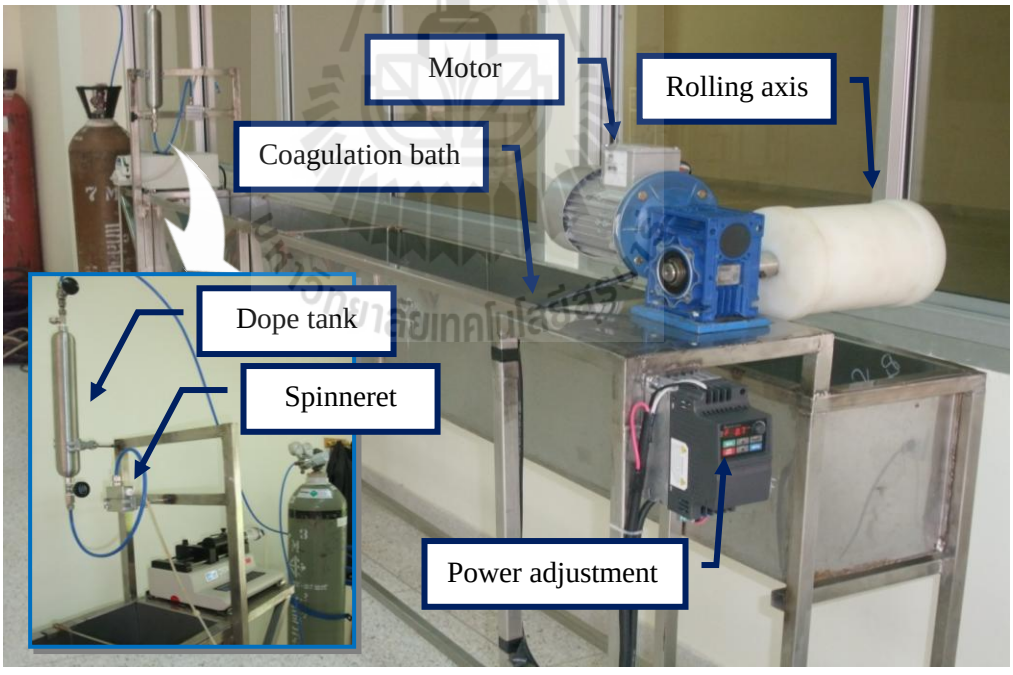


Figure 3.1 An experiment setup of spinning apparatus using dry-wet phase inversion

The dissolved polymer solution was pressurized through a spinneret with a controlled extrusion rate and coagulated in the coagulation bath before land into

final collection bath to complete the solidification process. The hollow fibers prepared were immersed in the final water bath for a period of 3 days, with daily change of water, so as to ensure thorough removal of residual solvent and additives prior to final membrane drying in ambient conditions.

3.1.2 Coating of asymmetric PVDF hollow fiber membranes

Various polymers can be used for coating the spun hollow fibers. This research focus on the natural rubber (NR) that it was used as the coating solution. In membrane coating process, we were kindly assisted from the Membrane Science and Technology Research Center. There were four major steps for the overall coating of composite membrane, pre-treatment with hexane or coating solution, heat treatment at 50 °C, vacuum coating and finally post-crosslinking at 50 °C. Briefly, the spun hollow fibers were cutted and assembled into housing with the length of 20-25 cm. In the pre-treatment step, the hollow fiber bundles were immersed in hexane for 60 s at room condition (25 °C, 60% RH), followed by heat treatment at 50 °C for 4 h. After that, the treated hollow fibers were cooled at room temperature, followed by vacuum coating whereby it was immersed in the coating solution with vacuum pressure applied at the fiber lumen side for an intended duration, ranging from 30 s to 4 min. This will be followed by post-crosslinking at 50 °C for 24 h.

3.2 Pervaporation experiments

The experimental setup of the pervaporation apparatuses were performed with two different membrane modules, flat-sheet and hollow membranes, as shown in Figure 3.2 and 3.3. The first apparatus, a composite flat-sheet membrane was installed in a stainless steel module. 2 L of the feed in a 3 L stirred tank reactor was circulated

at 10 L/h through the membrane module and returned to reactor as retentate by using a peristaltic pump (Masterflex® Peristaltic Pump, Cole parmer, USA). For the second apparatus, a composite hollow membrane was immersed directly inside the bioreactor. For both of the experiment, the feed temperature was controlled by a circulating thermostat water bath (Julabo, Germany). The feed side was kept at atmospheric pressure, whereas the permeate pressure was maintained below 5 mbar using a vacuum pump coupled with a pressure controller. Permeates were condensed using two glass cold traps filled with liquid nitrogen to ensure that all permeates were fully collected. Both the feed and permeate samples were collected at a fixed interval (0.5-1 h for aqueous solution, and 6 h for *in situ* product removal, respectively). The total flux (g/m^2 h) and selectivity were calculated by the following equations.

$$Total\ flux = \frac{W}{A \times t} \tag{3.1}$$

$$Selectivity = \frac{y/(1-y)}{x/(1-x)} \tag{3.2}$$

Where W is the weight of the permeate in grams, A is the membrane area in m^2 and t is the time in hour for the sample collection. The x and y represent the weight fraction of components in feed and permeate samples, respectively.

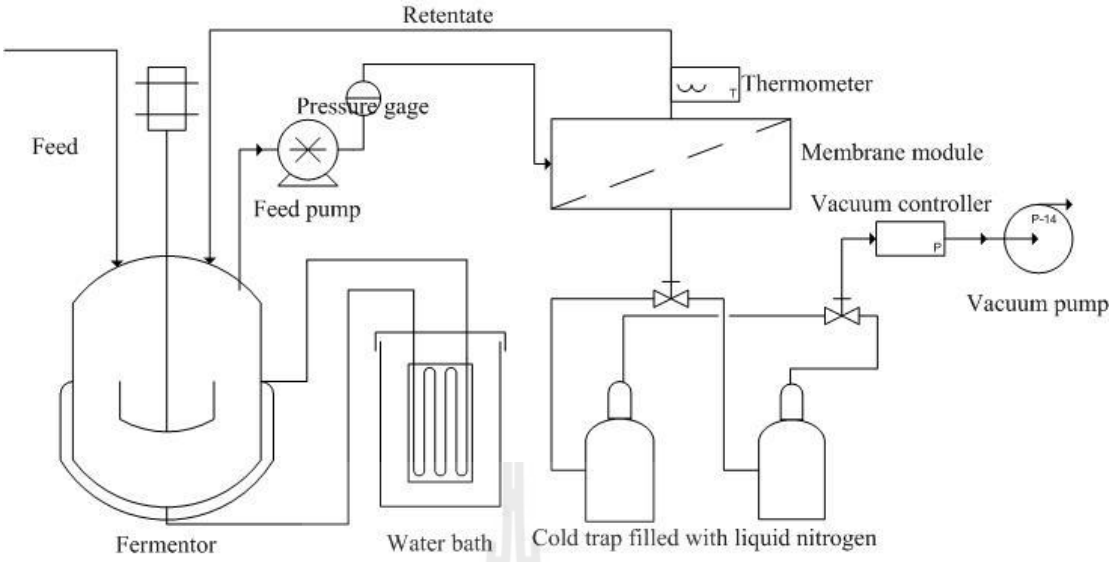


Figure 3.2 Schematic diagram of pervaporation by a composite flat-sheet membrane

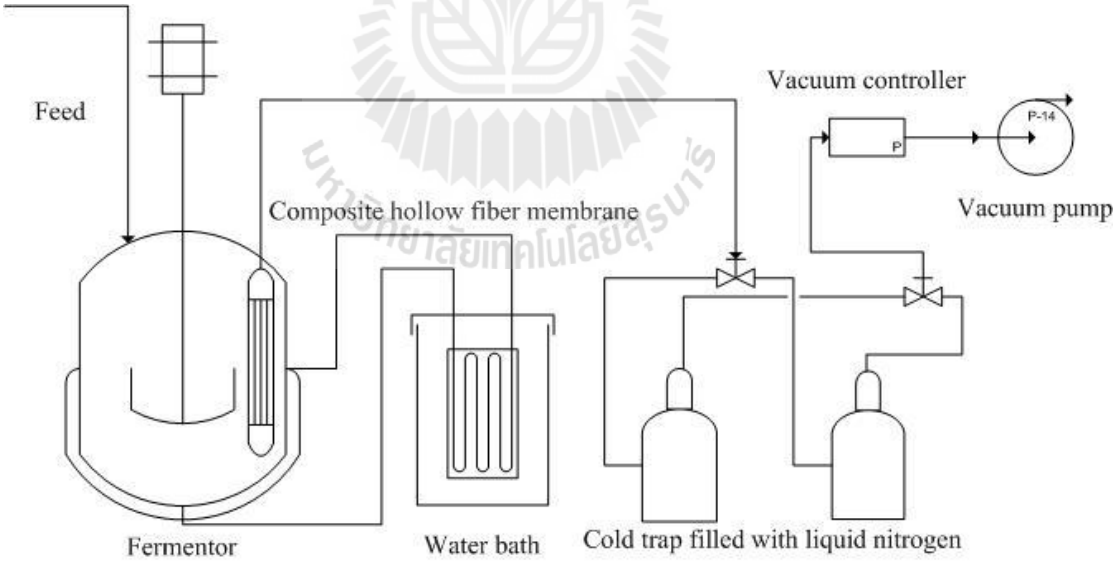


Figure 3.3 Schematic diagram of pervaporation by a composite hollow fiber membrane

3.3 ABE fermentation

3.3.1 Microorganism and inoculums

Clostridium acetobutylicum TISTR 1462 was obtained from Thailand Institute of Scientific and Technological Research (TISTR). The culture was stored as a spore suspension in sterile distilled water at 2–4 °C. An inoculum media was prepared as follows: 2.5 g cooked meat medium was soaked in 20 ml distilled water for 20 minutes in 25 ml capped volumetric flask and then 0.2 g glucose was added. The inoculum media was sterilized in an autoclave for 15 minutes at 121 °C and followed by cooling to 75 °C in an anaerobic chamber. To the tube 0.2 – 0.3 ml spore suspension was added and heat shocked at 75 °C for 2 minutes and followed by cooling in ice-cold water for 1.5 minutes. The heat shocked spores were then incubated in an anaerobic jar at 35 °C for 18-24 hours. When growth appeared, 2 – 3 ml of cell culture was added to 230 ml of inoculums medium.

3.3.2 Fermentation media

Culture medium, consisting of: glucose 50 g/L, yeast extract 5 g/L, ammonium acetate 2 g/L, KH_2PO_4 0.75 g/L, K_2HPO_4 0.75 g/L, $MgSO_4 \cdot 7H_2O$ 0.40 g/L, $MnSO_4 \cdot 7H_2O$ 0.01 g/L and $FeSO_4 \cdot 7H_2O$ 0.01 g/L, was contained in 1500 ml screw capped bottle. Before inoculation, the medium was autoclaved at 121 °C for 15 minutes (0.01 g/L *p*-aminobenzoic acid and 0.001 g/L biotin were filtered through 0.45 µm filter prior to adding to the medium after cooled down to 35°C) and cooled to 35 °C in an anaerobic chamber. Growth proceeded in an anaerobic reactor at 35 °C for 4 – 5 days.

3.3.3 Fermentation process

3.3.3.1 Batch fermentation

Batch ABE fermentations were performed in a 3 L fermentor (Sartorius, Germany). The bioreactor containing 2.07 L of medium was inoculated with 0.23 L of inoculum from a 18-24 hour culture. All experiments were conditioned at optimal growth temperature of 35 °C, the pH of the broth was adjusted to 6.2 at the beginning of fermentation. Surface flushing by oxygen-face nitrogen gas was limited to the fermenter before and after inoculation for 30 min, and the agitation speed was set at 100 rpm (in order to make the broth homogeneously under an anaerobic environment). Samples were taken aseptically at regular interval times for further analyses.

3.3.3.2 The ABE fermentation with *in situ* product removal (ISPR)

ABE fermentation was performed in the manner of integrated production, and separation process at the same time with total medium of 2.3 L in a 3 L bioreactor (Sartorius, Germany) by using a PDMS composite membrane as shown in Figure 3.4. All experiments were conditioned similarly to batch fermentation described above. The fermentation was initially run for 24 h without separation before the ABE were continuously removed by using pervaporation process. The fermentation broth was re-circulated through a feed channel of the membrane module before returned back to the bioreactor. The vacuum pressure was supplied by a vacuum pump. This experiment was performed for 102 h with collection of retentate and permeate samples as well as changing the glass cold traps every 6 h.

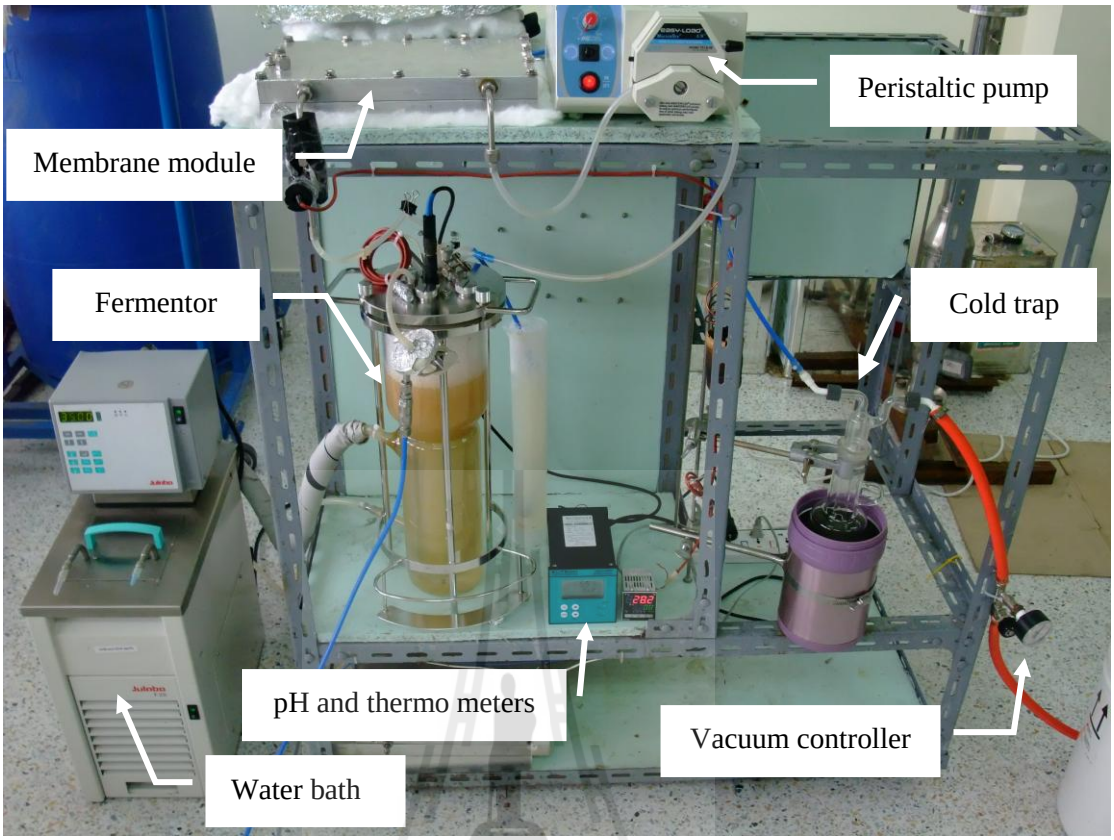


Figure 3.4 Experimental setup of ABE fermentation with ISPR process using PDMS composite membrane

3.4 Analytical procedures

3.4.1 Fermentation broth

The cell concentration in the fermentation broth was determined by optical density at 600 nm (OD600) by a spectrophotometer. ABE productivity was calculated from total ABE concentration (g/L) divided by fermentation time (h). Fermentation time was defined as the time period when a maximum ABE concentration was obtained. ABE yield, which does not have a unit, was calculated from total ABE produced (g) divided by total glucose utilized (g).

3.4.2 Solvent concentrations

Solvent concentrations taken from the aqueous solutions and fermentation broth in the feed and permeate samples were analyzed by using a SRI 8610C gas chromatography equipped with a Carbowax® column (Restek, USA) of 30 m x 0.32 mm x 0.25 µm and a flame ionization detector (FID). Helium, 99.99% pure, was used as carrier gas with flow rate of 20 mL/min. The temperature of injector and detector were set at 50, and 200 °C, respectively. The oven temperature was programmed at 50 to 200 °C with the rate of 15 °C/min.

3.4.3 Glucose and organic acid concentrations

Samples containing cell or suspended solids were centrifuged at 14,000 rpm for 2 min in a microcentrifuge. Glucose and organic acids (acetic and butyric acid) in the fermentation broth were measured using high performance liquid chromatography (HPLC) with RI detector (Model 1200 series, Agilent technology, USA) and 4 mM sulfuric acid was used as the mobile phase. The temperature of the column was operated at ambient temperature with a flow rate of 1.0 mL/min.

3.4.4 Calculation of permeance, *Q*

In order to convert flux in terms of permeance, the UNIFAC method and Antione equation were used in this approach. Following the solution–diffusion mechanism, the basic transport equation for pervaporation can be written as:

$$J_i = (P_i/l)\Delta P_i \tag{3.3}$$

and

$$\Delta P_i = x_i\gamma_i p^{sat} - y_i P^p \tag{3.4}$$

where P_i is the membrane permeability, which is a product of diffusivity and solubility coefficients, l is the membrane thickness, ΔP is the partial pressure difference, x_i is the mole fraction in the feed, y_i is the permeate mole fraction and γ_i is the activity coefficient calculated by the UNIQUAC equation (J.M.Smith *et al*., 2005) (APPENDIX B). The saturated vapor pressure p^{sat} can be determined from the Antoine equation (Qiao *et al*., 2005) (APPENDIX A) and P^p is the permeate pressure.

The term (Pi/l) is known as permeance that can be determined by rearranging the above equation:

$$Q_i = \frac{J_i}{x_i \gamma_i p^{sat} - y_i P^p} \tag{3.5}$$

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Pervaporation study on membrane performances

4.1.1 Effect of feed butanol concentration on total and partial permeation fluxes

The separation of butanol by pervaporation technique using three different membrane materials were firstly investigated from binary butanol/water solution with varying feed butanol concentration from 1.25 – 10 % v/v at a set different temperature of 35 – 80 °C. At a given temperature, with an increase in butanol concentration in the feed solution, the PDMS composite membrane showed that water flux increased slightly at the beginning of an increasing the feed concentration, prior leveled off at the higher feed concentration. However, the butanol flux increased proportionally with increasing the feed butanol concentration as showed in Figure 4.1. The linearity of the butanol flux–concentration relationship suggested that constant butanol permeability could be assumed in the dilute feed concentration range studied. Figure 4.2 showed that the NR composite hollow fiber membrane conferred almost linear increasing of butanol flux similar to flat-sheet membrane, except at the lowest temperature showed slight increasing. Moreover, the total flux of this membrane increased with an increase in feed butanol concentration as well as the permeation flux of water, but at the high temperature (70 and 80 °C), the water flux decreased slightly at the highest feed butanol concentration used in this study. To consider the

pervaporation of the XSBR composite hollow fiber membrane, the total and partial permeation fluxes were illustrated in Figure 4.3. The result revealed that the total and water flux showed the linear increase with increasing the feed butanol concentration at 1.25 – 2.5 % v/v, then leveled off at higher concentration. However, the butanol flux was found similar trend to the previous membrane mentioned. From the results of the three membranes, the phenomenon occurred could be explained on the basis of membrane-permeant interactions. This is understandable as butanol sorbed into the membrane will swell the membrane, resulting in an increased free volume and polymer chain flexibility that will facilitate water permeation through the membrane. The internal surface of the three membrane was hydrophobic, and the coating material in the membrane did not affect water solubility in the membrane. In addition, the observed increase in the water flux was primarily due to the increased permeability of the membrane. On the other hand, the permeation of butanol was expected to be affected by both the feed concentration and the membrane permeability. Coating materials are known to have a strong affinity to butanol (Fouad and Feng, 2009). Because of the organophilicity of coating materials in the membrane, butanol sorption in the membrane will be enhanced, which is the rationale of using the coating materials to improve the membrane permselectivity. This was consistent with the results of using of pervaporation to separate butanol from dilute aqueous solutions through poly(ether-block-amide) membranes (Fouad and Feng, 2008).

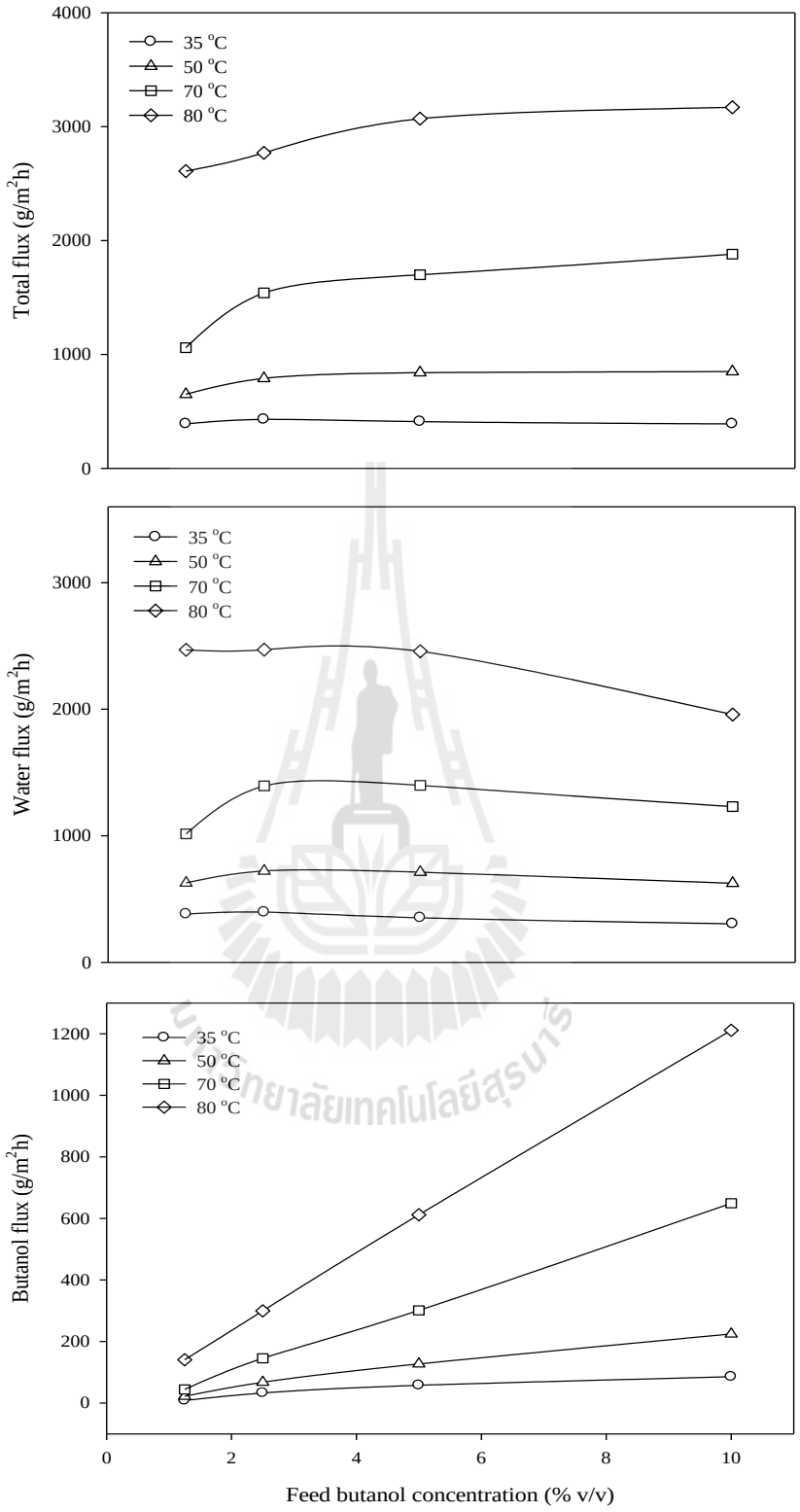


Figure 4.1 Effect of feed butanol concentration on total and partial permeation fluxes of pervaporation using PDMS composite mambrane

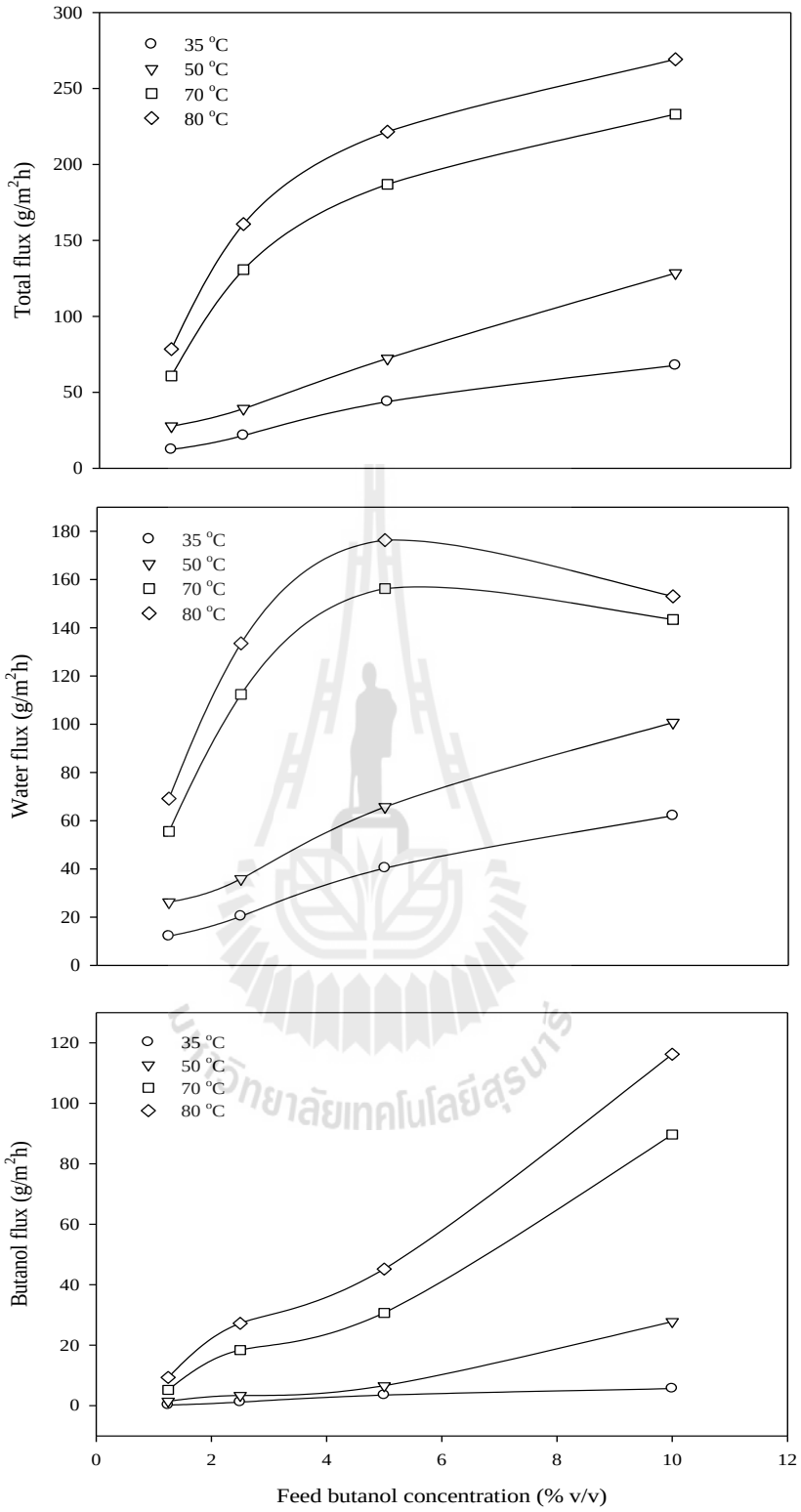


Figure 4.2 Effect of feed butanol concentration on total and partial permeation fluxes of pervaporation using NR composit hollow fiber membrane

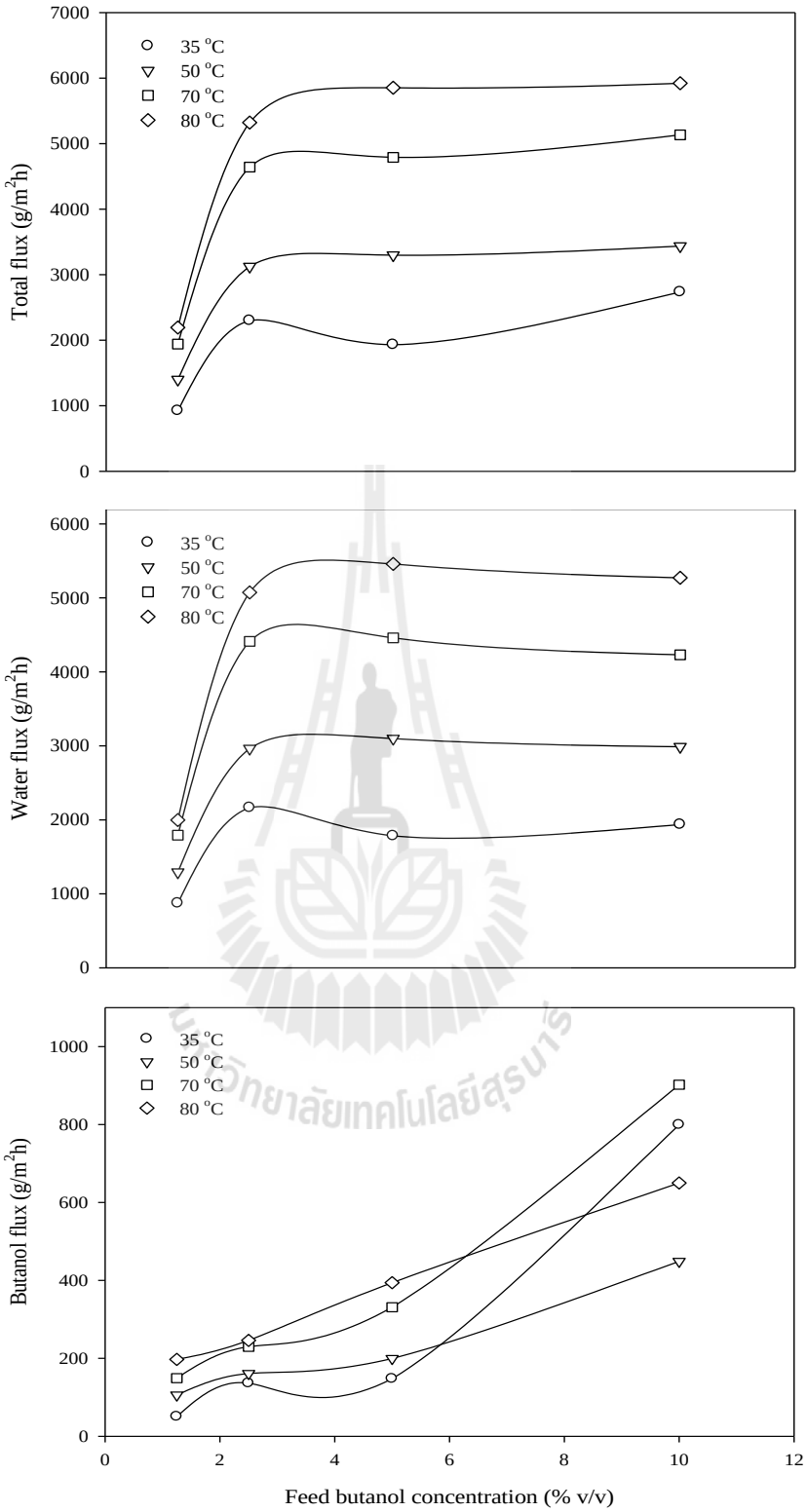


Figure 4.3 Effect of feed butanol concentration on total and partial permeation fluxes of pervaporation using XSBR composite hollow fiber membrane

4.1.2 Effect of feed butanol concentration on butanol concentration in permeate

The butanol concentration in the permeate of the three different membranes was shown in Figure 4.4, where the PDMS composite membrane gave good permselectivity for butanol/water separation as demonstrated in Figure 4.4a. At the given feed butanol concentration, the butanol concentration in permeate increased linearly and at a feed butanol concentration of 10 % v/v, a permeate butanol concentration of as high as close to 400 g/L can be obtained. An increase in permeate butanol concentration was also found in pervaporation system using NR composite hollow fiber membrane but slightly increased at lowest operating temperature as shown in Figure 4.4b. *n*-Butanol was a strong polar solvent and thus had a strong effect to the hydrophobic membrane. This would increase the effect on butanol permeation flux, resulted in a increase in the butanol concentration in permeate. However, pervaporation of butanol/water using XSBR composite hollow fiber membrane showed the inverse trend to the other membrane as shown in Figure 4.4c. At the feed butanol concentration of 1.25 – 5.0 % v/v showed insignificant difference in permeate butanol concentration and at highest feed butanol concentration (10 % v/v) revealed linear increase in permeate butanol concentration with the inverse trend (increased with decrease in feed temperature) to the previous membranes. This feature could be attributed to the coating particles in the membrane that cause competitive sorption to the permeating species.

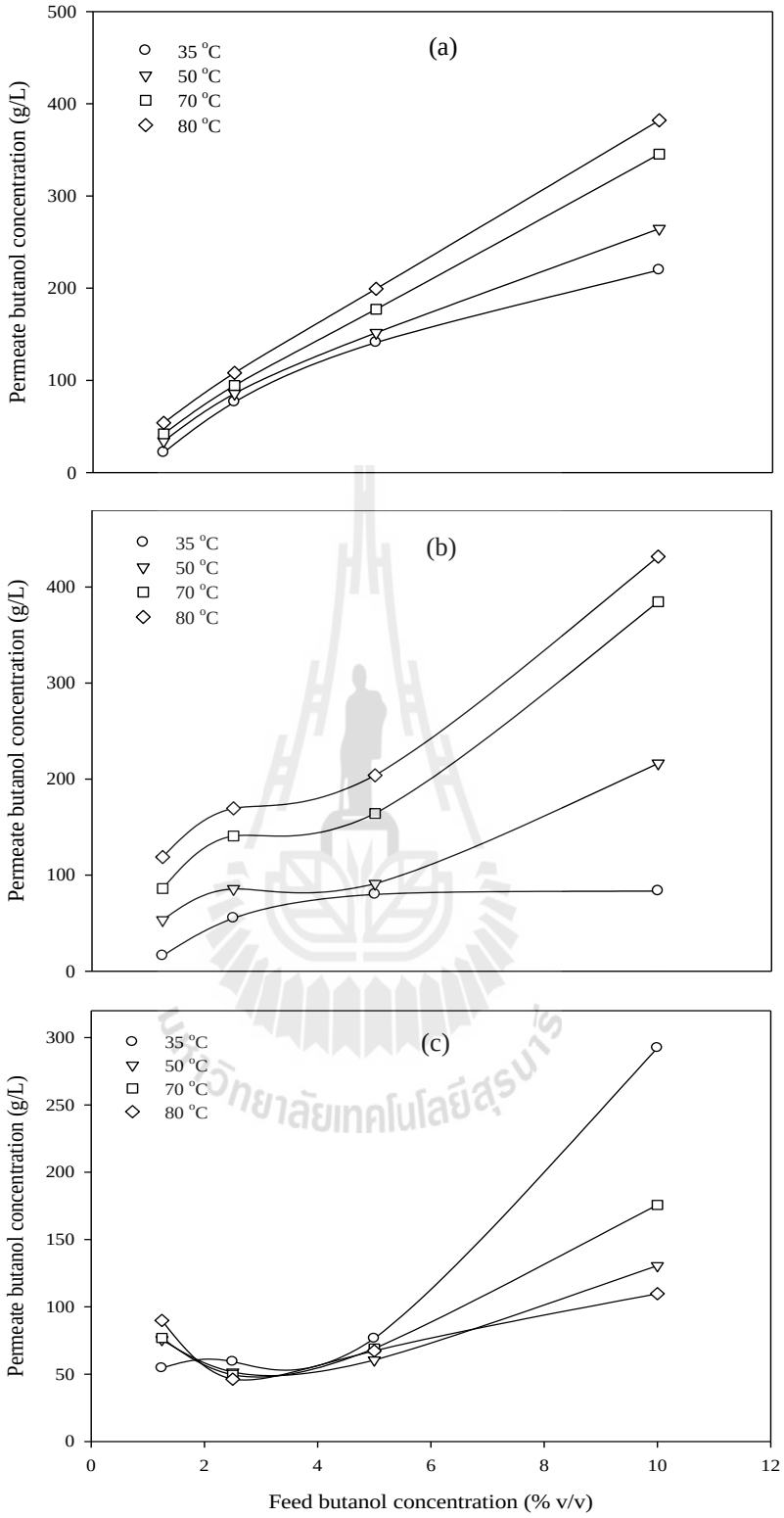


Figure 4.4 Effect of feed butanol concentration on permeate butanol concentration of pervaporation using: (a) PDMS, (b) NR, and (c) XSBR composite membranes

4.1.3 Effect of feed butanol concentration on butanol selectivity

Figure 4.5 illustrated the effect of butanol concentration in feed on butanol selectivity over a varying operating temperature using different membrane materials. the butanol selectivity, which characterized the degree of enrichment of the permeate product relative to the feed, was shown to decrease with the feed butanol concentration when using XSBR composite hollow fiber membrane (see Figure 4.5c), and the rate at which it decreased tails off and/or slight increased at higher concentrations of butanol in the feed solution. These results were similar to those of pervaporation using NR composite hollow fiber membrane as shown in Figure 4.5b. This phenomenon could be described similarly to the previous results, due to butanol was a strong polar solvent and hence had a strong cohesion effect in water due to strong hydrogen bonding. This would increase the coupling effect on permeation between water and butanol, resulting in a decrease in the selectivity. This trend was consistent with previous reports for the separation of ABE from dilute aqueous solutions (Liu *et al*, 2005). In contrast, the PDMS composite membrane revealed to increase in butanol selectivity at primary increasing the feed butanol concentration prior to slight increased and/or decreased at the higher feed butanol concentration depending upon the operating temperature used (Figure 4.5a). This result suggested that the PDMS composite membrane provided the best membrane performance in term of permeability at high temperature.

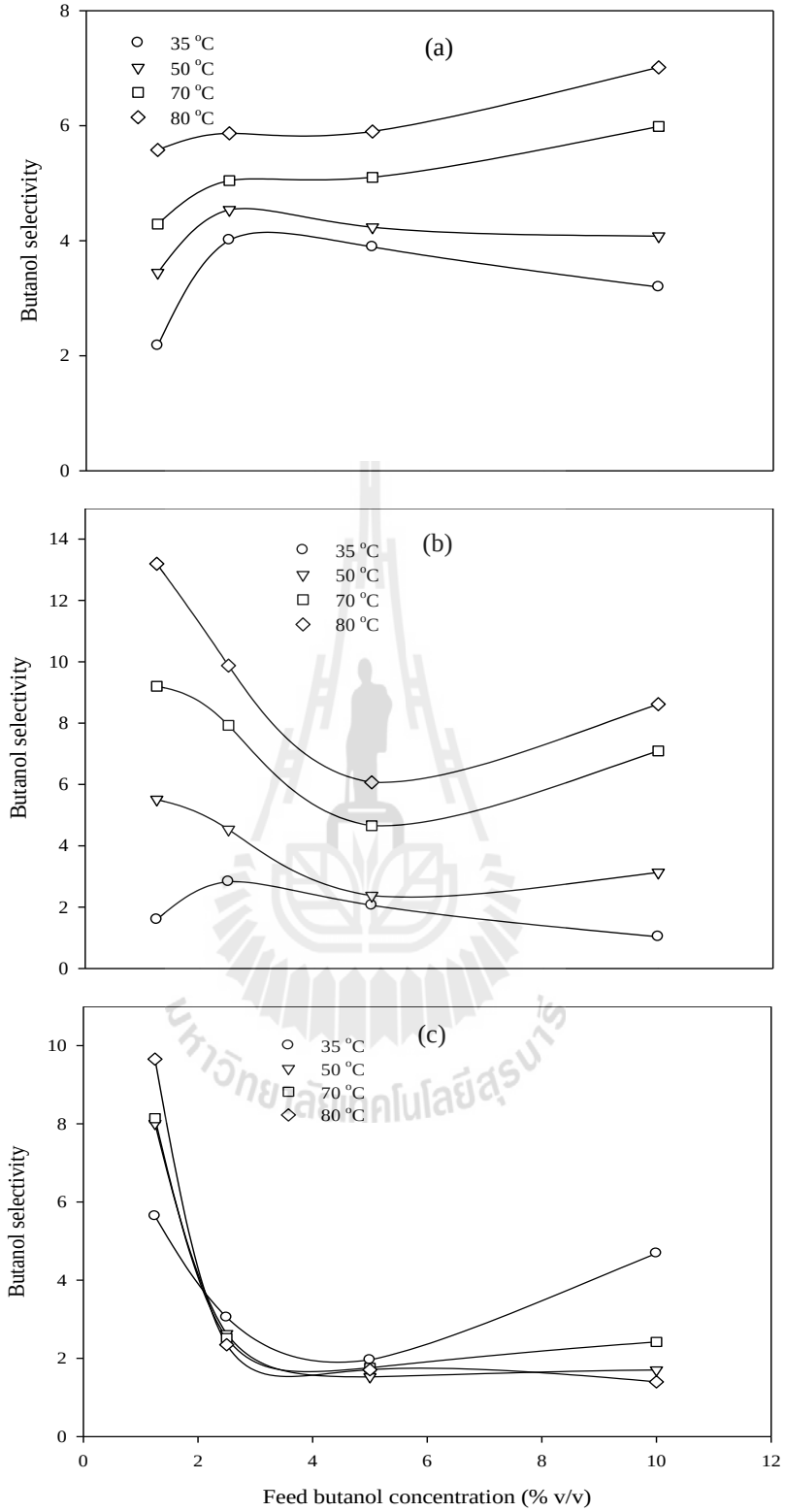


Figure 4.5 Effect of feed butanol concentration on butanol selectivity of pervaporation using: (a) PDMS, (b) NR, and (c) XSBR composite membrane

4.1.4 Effect of operating temperaure on permeation fluxes and selectivity characterizing by the activation energy

The above data showed that at a given feed concentration, increasing the operating temperature would increase both the permeation flux and selectivity of both the PDMS and NR composite membrane, and inverse trend in XSBR composite membrane. However, temperature has a significant kinetic effect on reaction. Variation of the rate constant *k* with temperature is described by the Arrhenius equation:

$$k = A\, e^{-E/RT} \tag{4.1}$$

Where *k* is the rate constant, *A* is the Arrhenius constant, *E* is the activation energy for the reaction, *R* is the ideal gas constant, and *T* is absolute temperature.

The temperature dependency of the permeation fluxes for the PDMS composite membrane were found to follow an Arrhenius type of relationship, as shown in Figure 4.6 and 4.7, where the partial permeation fluxes of butanol and water were plotted against reciprocal temperature. It appeared the butanol flux was more sensitive to temperature than water flux over the feed concentration range studied. Moreover, The temperature dependency of the permeation fluxes for the NR composite membrane were also found the similar trend to that shown in Figure 4.8 and 4.9.

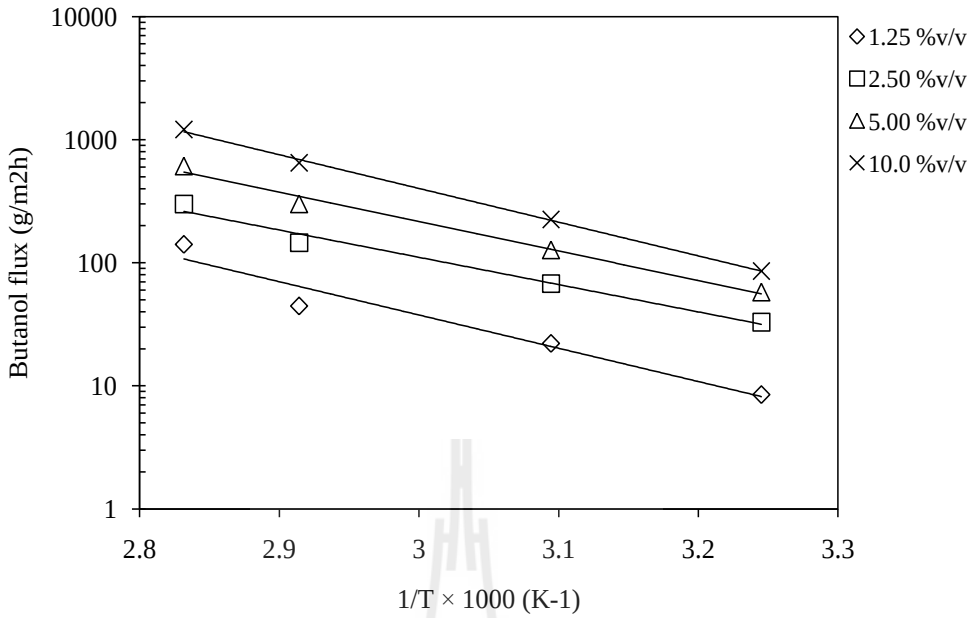


Figure 4.6 The temperature dependence of butanol flux at given feed butanol concentration using PDMS composite membrane

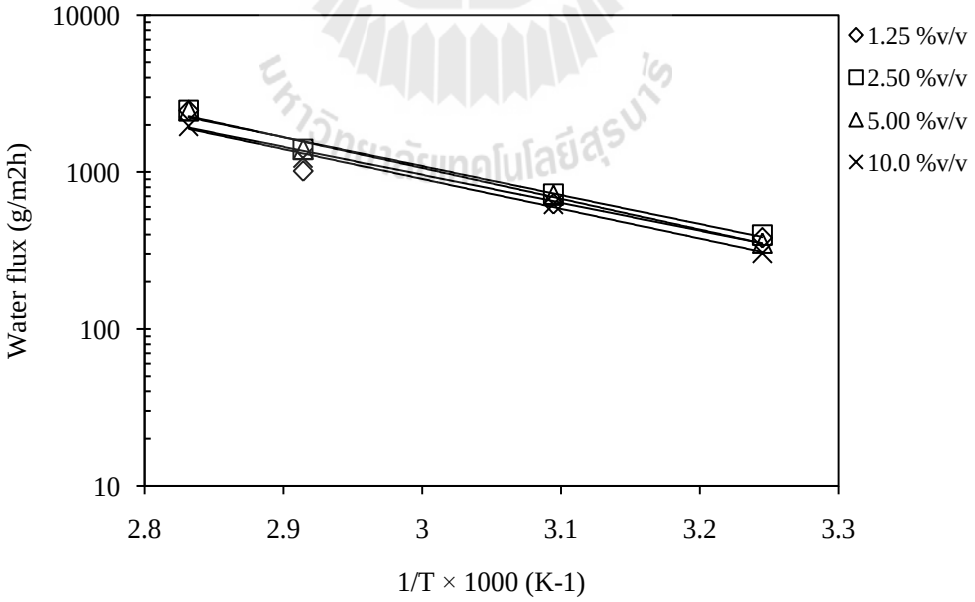


Figure 4.7 The temperature dependence of water flux at given feed butanol concentration using PDMS composite membrane

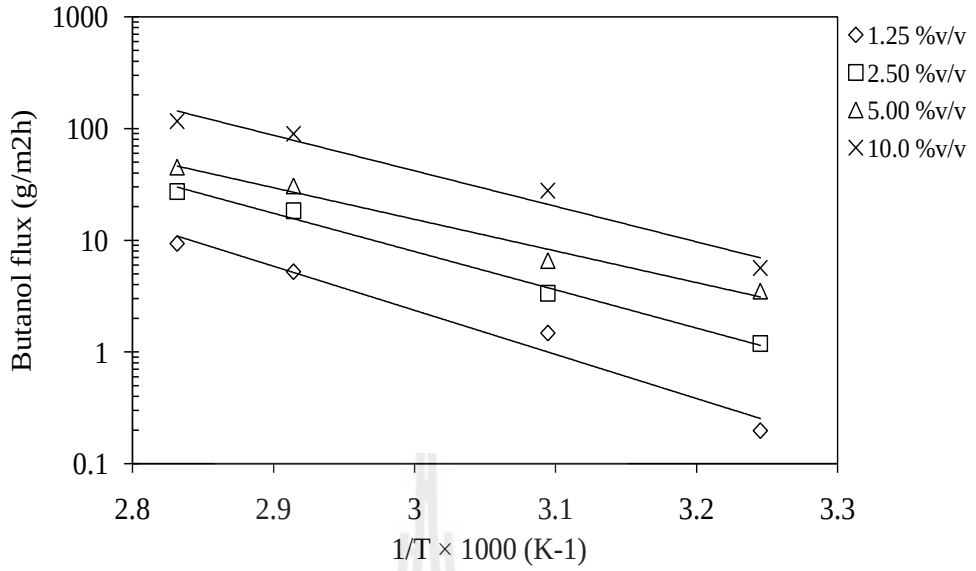


Figure 4.8 The temperature dependence of butanol flux at given feed butanol using NR composite hollow fiber membrane

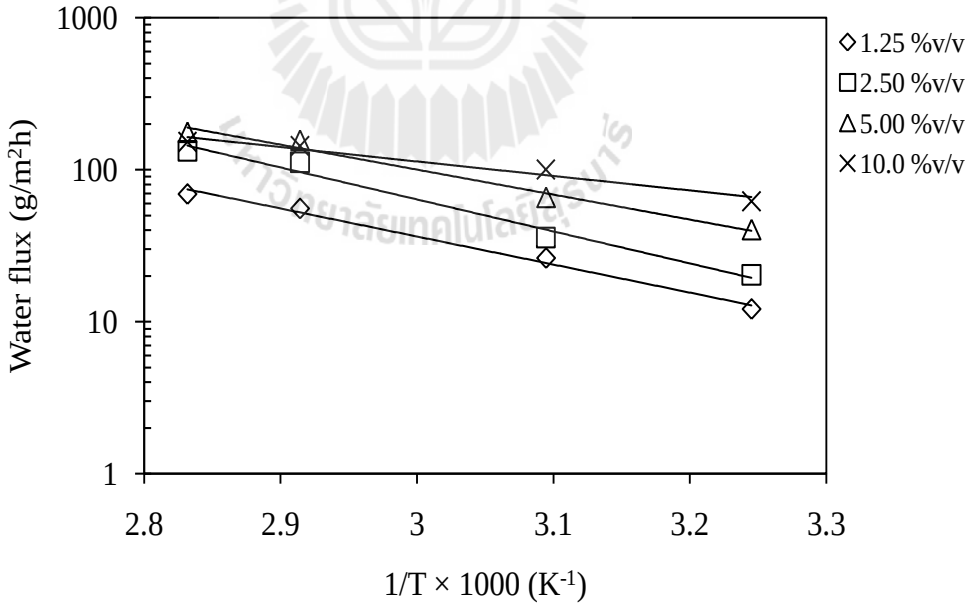


Figure 4.9 The temperature dependence of water flux at given feed butanol concentration using NR composite hollow fiber membrane

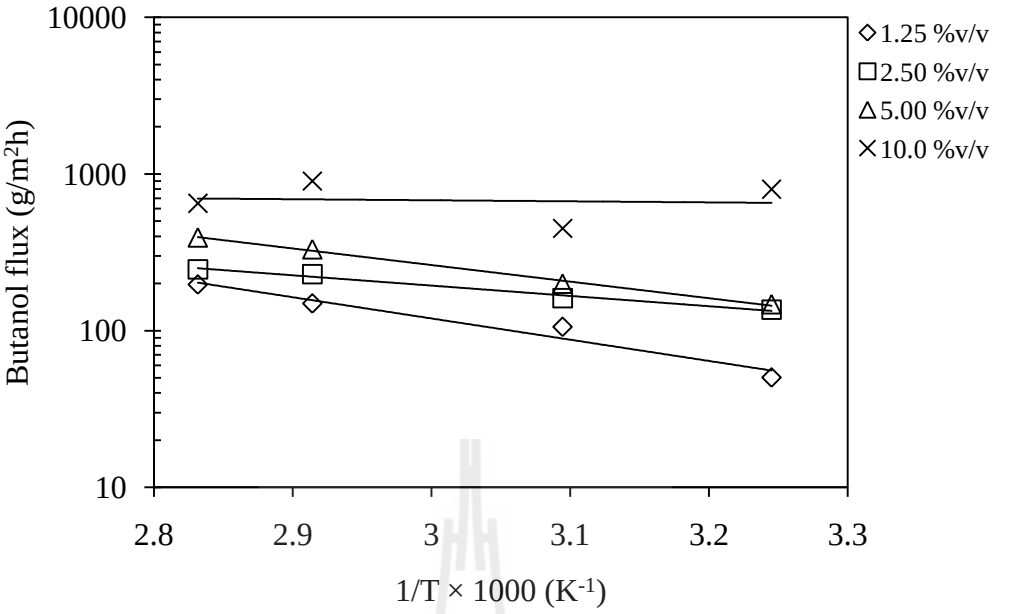


Figure 4.10 The temperature dependence of butanol flux at given feed butanol concentration using XSBR composite hollow fiber membrane

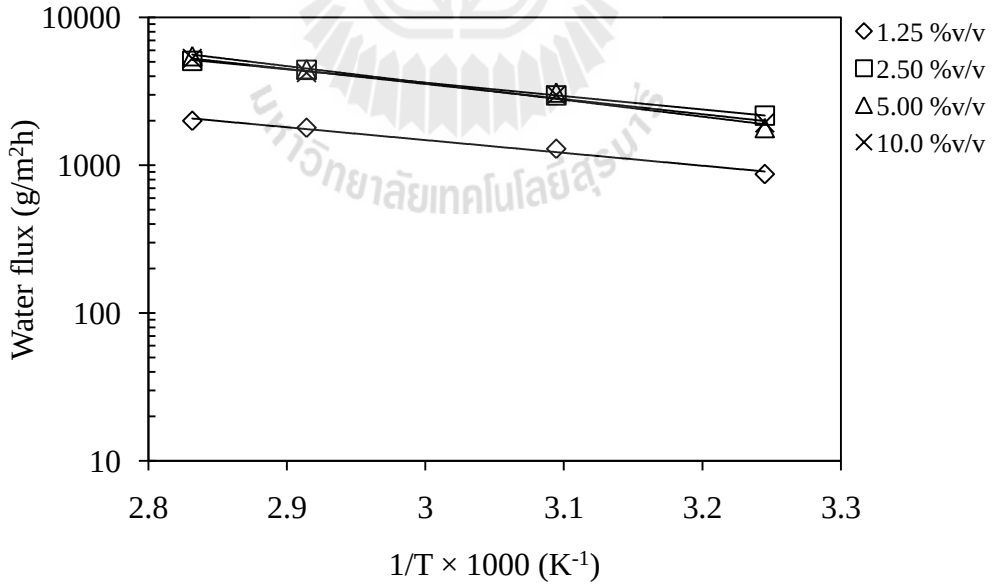


Figure 4.11 The temperature dependence of water flux at given feed butanol concentration using XSBR composite hollow fiber membrane

According to the Arrhenius equation, as T increases, k increases exponentially. Taking the natural logarithm of both side of Eq. (4.1):

$$\ln k = \ln A - \frac{E}{RT} \tag{4.2}$$

Thus, a plot of ln *k* versus 1/*T* gave a straight line with slope *–E/R*. For many reactions the value of *E* is positive and large, indicating a rapid increase in reaction rate with temperature. The apparent activation energies characterizing the temperature dependencies of the permeation fluxes, which could be obtained from the slopes of the straight lines of Arrhenius relation, were shown in Figure 4.12 for PDMS, NR, and XSBR, respectively. The activation energy for the butanol permeation of PDMS and NR membrane were in the range of 42.5 – 52.4 and 54.2 – 75.6 J/mol, respectively, which was higher than the activation energy for the water permeation of the same membrane (34.2 – 37.6 and 18.2 – 40.3 J/mol, respectively). This explained that the butanol selectivity of both the PDMS and NR membrane increased with an increase in temperature as noticed previously (see Figure 4.5).

However, it was interesting to note that the effects of temperature on the permeation of both the butanol and water for the XSBR composite membrane differed from above mentioned, as shown in Figure 4.10 and 4.11, where the partial permeation fluxes were also plotted versus reciprocal temperature. The activation energies for the butanol and water permeation of XSBR composite membrane presented in Figure 4.12c. The activation energies for permeation of butanol tended to decrease as the feed butanol concentration increases, and the temperature dependence of butanol flux was more significant at only feed butanol concentrations below 1.25 % v/v. The result indicated that at higher operating temperature, the butanol

selectivity was shown to decrease. As butanol concentration increases, the amount of butanol sorbed in the polymer will increase, making the sorptive sites more saturated with butanol molecules and the polymer chains more flexible. As a result, the energy barrier that needed to be overcome by the permeant molecules for permeation to occur will be lowered. In pervaporation, temperature affects the permeation flux in three aspects: the solubility, diffusivity, and the driving force for permeation (i.e., vapor pressure). The activation energy had accounted for the effect of temperature on the driving force for permeation, which could roughly be measured by heat of evaporation. In principle, the temperature effects on driving force and membrane permeance could be separated on the basis of solution-diffusion model using partial vapor pressure difference across the membrane as the driving force (Du *et al*., 2008).

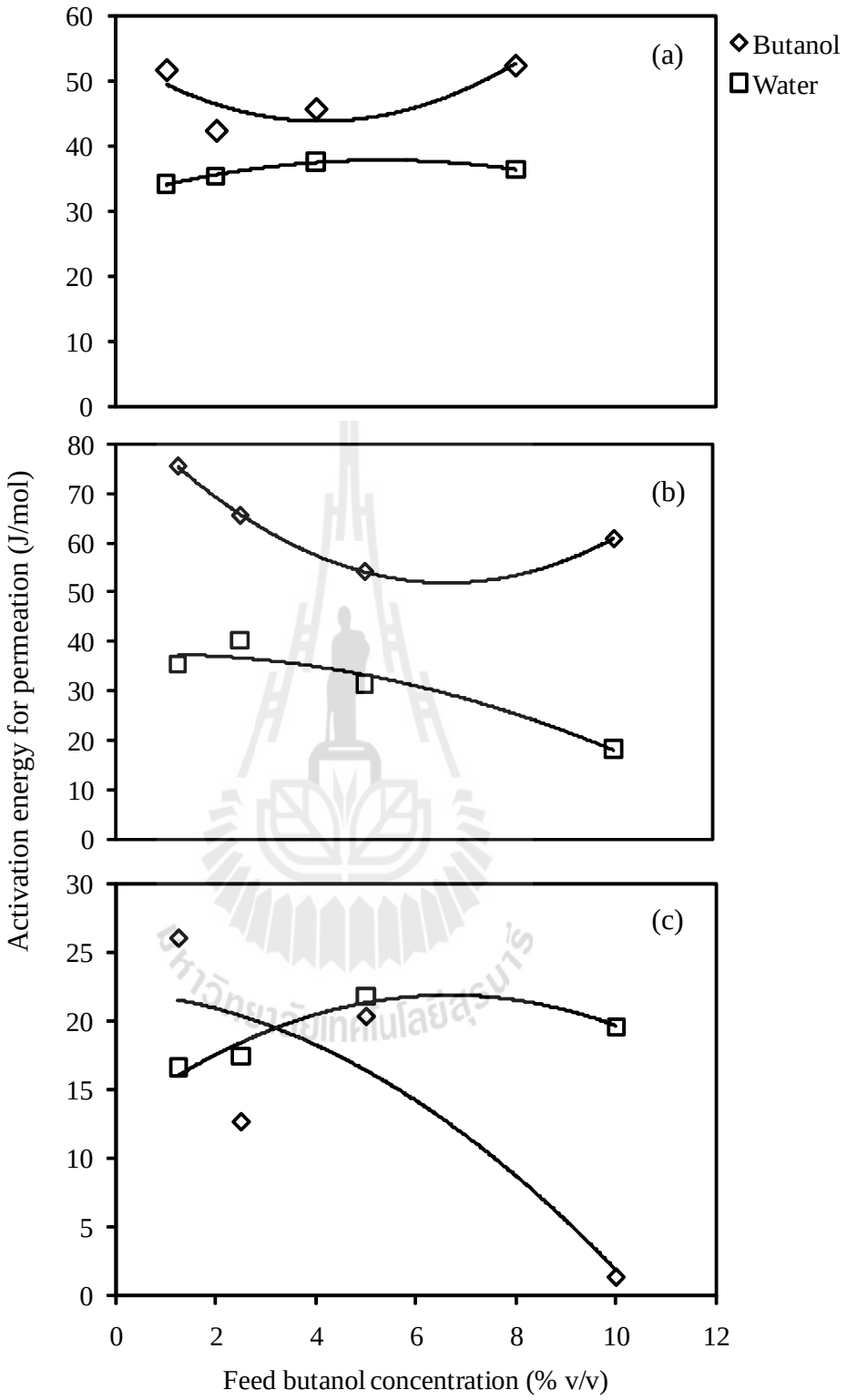


Figure 4.12 Activation energy for butanol and water permeation by pervaporation using: (a) PDMS, (b) NR, and (c) XSBR composite membranes

4.2 Comparison of membrane performances for PDMS, NR, and XSBR composite membranes

The comparison of pervaporative properties of the three different membrane materials (two differences in membrane module) were listed in Table 4.1. The result indicated that the PDMS composite membrane showed significantly better results in term of butanol flux. With active layer of 2 μm, the PDMS membrane offered butanol flux higher than that of the NR and XSBR composite hollow membranes when using feed butanol concentration of 10 g/L at temperature of 35-80 °C. Moreover, at optimum temperature (35 °C) of ABE fermentation, the butanol flux of NR composite hollow fiber membrane was almost absent and it was not significantly different from the butanol flux of XSBR composite hollow membrane. However, NR and XSBR composite hollow membrane showed significantly higher performance in term of selectivity when compared to the PDMS composite membrane. It could be seen that incorporating the supportive layer with the dense NR and XSBR active layer make both membrane layers much thicker than 2 μm of PDMS active layer coated on PVDF supportive layer. The thinner membrane exhibited a higher permeation flux and a lower selectivity than the thicker membrane. Therefore, all experiments mentioned above indicated that the permeability of butanol across the membrane was found to follow the same relationship with membrane thickness, feed temperature and butanol concentration.

Table 4.1 Summary of the three different membranes for butanol separation by pervaporation using 10 g/L butanol/water aqueous solution

Membranes	**Active layer thickness (μm)**	**temperature (°C)**	**Butanol flux (g/m^2h)**	**Butanol Selectivity**
PDMS composite	2	35	14.69	2.9
memebrane		50	27.60	4.1
		70	68.06	5.7
		80	112.98	7.2
NR composite	N/A	35	0.20	4.1
hollow membrane		50	1.48	9.4
		70	5.23	11.1
		80	9.33	11.2
XSBR composite	N/A	35	1.04	5.7
hollow membrane		50	4.56	9.2
		70	14.96	11.2
		80	45.54	13.1

However, the above comparison may not be fully completed, the butanol permeance of the three different membrane were compared as a function of temperature as illustrated in Figure 4.13. The result implied that the butanol permeace of PDMS composite membrane offered significantly higher value than that of NR and XSBR composite hollow membrane. One may notice that the effects of temperature on butanol permeance of PDMS composite membrane did not follow instinctive

predictions, butanol permeance did not increase proportionally with an increase in temperature. The butanol permeance versus operating temperature in these three membrane systems followed the order of the highest at PDMS, followed by XSBR, and then NR membrane. However, the butanol permeance of both the NR and XSBR shown the trend which observed in most gas separation membranes, the permeance increased with increasing the temperature.

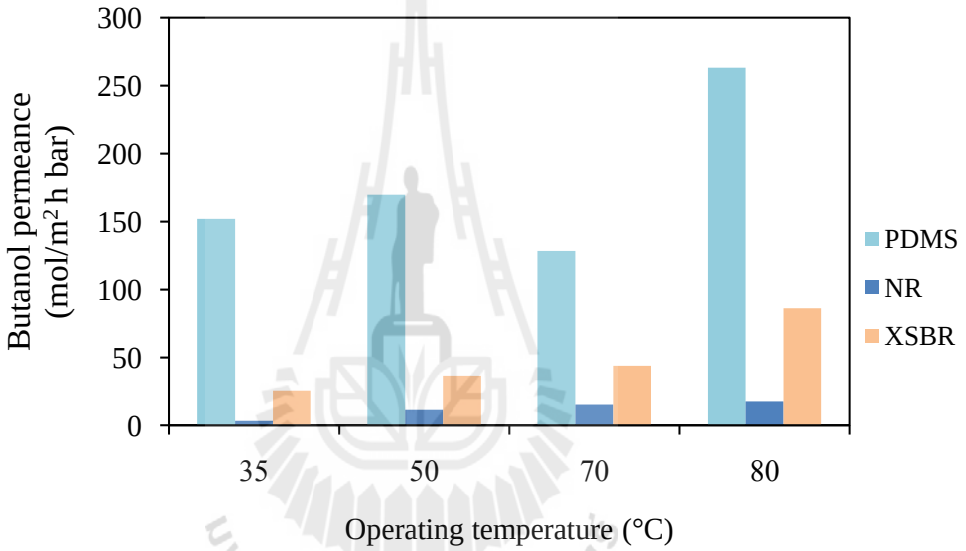


Figure 4.13 Comparison of butanol permeance for pervaporation by three different membranes using feed butanol concentration at 10 g/L

The relatively dependence of butanol permeances of three membranes arise from the fact that permeance is defined as permeantion flux divided by driving force (Guo *et al*., 2004). The driving force combines two temperature dependent factors, butanol activity coefficients; γ^i and saturated partial pressure; p^{sat}, which were external factors outside the membrane. As shown in Table 4.2, the values of γ^i and p^{sat} were different at different temperatures. The permeation flux of the three membrane

were also much more different (see Table 4.1) and thus, the permeantion flux played a more important role than the γ^i and p^{sat} in these systems. A higher permeation flux resulted in a larger divider of Eq. (3.5) and consequently a larger permeance. In addition, A higher temperature resulted in a higher γ^i and p^{sat}, a larger denominator and consequently a smaller permeance as well.

Table 4.2 The comparison of butanol activity coefficients and saturated vapr pressure at different temperatures for pervaporation of 10 g/L butanol solution calculated by the UNIFAC method and Antione equation

Temperature (°C)	**Butanol activity coefficient, γ**	p^{sat} **(bar)**
35	17.23175	0.018276
50	15.75063	0.046116
70	14.12938	0.135263
80	13.43975	0.218825

4.3 Pervaporative separation of ABE from synthetic model solution using the PDMS composite membrane

Pervaporation of the ABE solvents from aqueous solutions through the PDMS/PVDF composite flat-sheet membrane was investigated with varying operating temperatures of 35 – 80 °C. The concentration of the organic solvents in the feed solution was prepared at the encountered to the fermentation broth with 10 g/L butanol, 3 g/L acetone, and 1 g/L ethanol in the total solution volume of 2 L. The total flux and solvent selectivities of the membrane was exposed in Figure 4.14 over the

different temperature. Total flux of the PDMS composite membrane increased rapidly from 515 to 1,665 g/m^{2}h with increasing the feed temperature from 35 to 80 °C. This phenomenon could be also traditionally explained by the increase of solubility and diffusivity of organic solvent (particularly butanol) and water in membrane as well as the increase of sorption and desorption rate of permeant molecules in membrane matrix. As the temperature increased to 80 °C, the butanol selectivity increased to 7.2 and the acetone selectivity also increased with increase the temperature. Moreover, this membrane could also separate the ethanol from dilute aqueous solution that shown slight increases in ethanol selectivity with an increase operating temperature due to low concentration in the feed (1 g/L). In contrast, the water selectivity decreased slightly with increasing the operating temperature due to the increase of solvent diffusivity through the membrane at higher temperature.

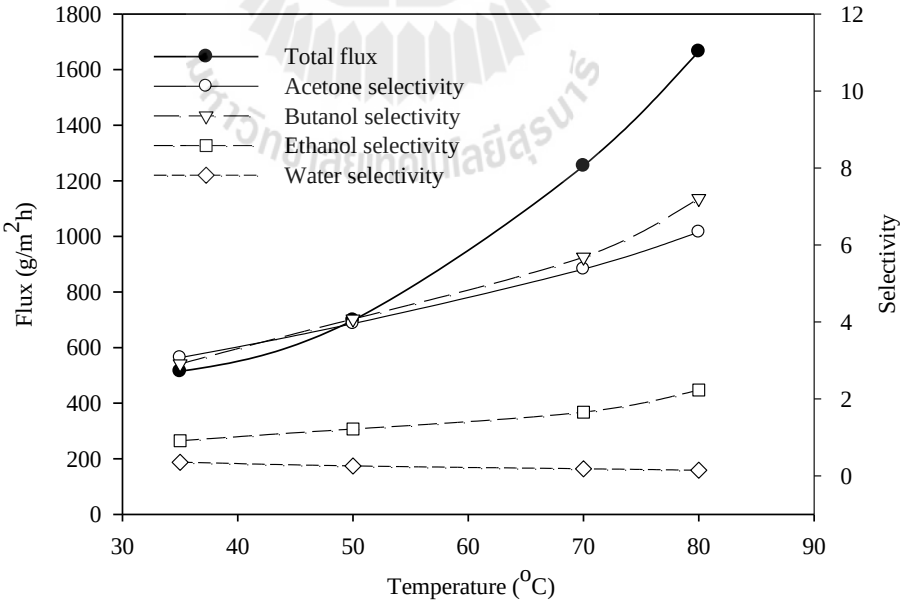


Figure 4.14 Flux and selectivity versus operating temperatures by pervaporation

4.4 ABE Fermentation and pervaporative separation process

4.4.1 ABE batch fermentation

Production of ABE from glucose was shown in Figure 4.15. Glucose concentration in both cases was around 50 g/L. It could be seen in general that the fermentation profiles were similar. After 24 h, maximum concentration of dry cell weight of 2.5 g/L was reached in fermentation both. The initial pH 6.2 of fermentation broth decreased to pH 5.1 after 24 h and then increased to final pH 5.8 after 72 h. At the same time, glucose in fermentation both was rapidly consumed until the final glucose concentration was about 6.3 g/L. During the fermentation, maximum ABE concentration of 14.38 g/L was produced in fermentation broth. This included 2.7 g/L acetone, 10.8 g/L butanol, and 0.88 g/L ethanol. A solvent productivity of 0.30 g/L h and solvent yield of 0.32 g/g was achieved in this experiment. At the end of fermentation, the total concentration of acids was 0.7 g/L.

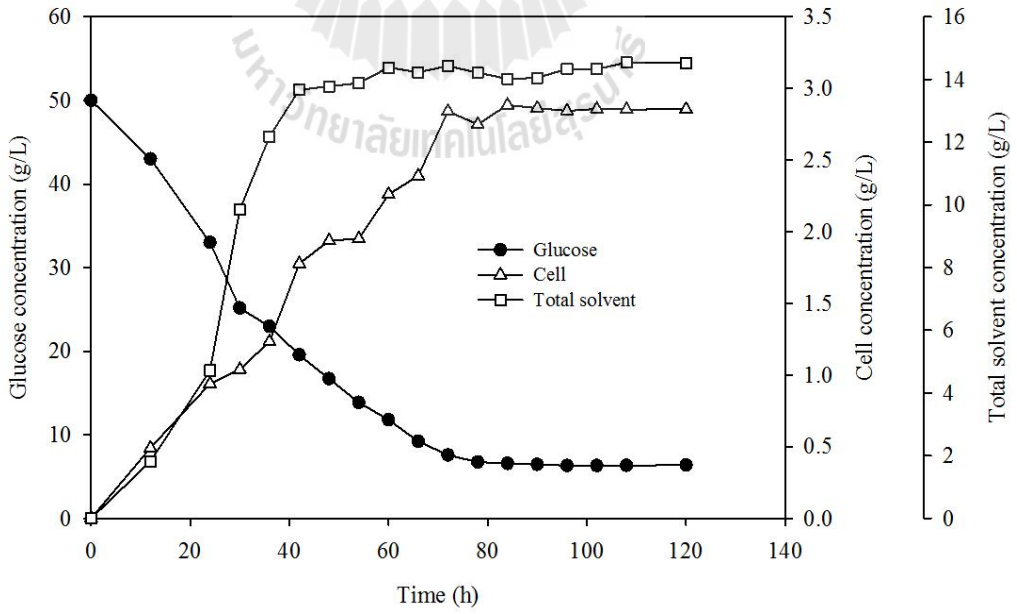


Figure 4.15 Glucose, cell, and total solvent concentration in ABE fermentation using *Cl. acetobutylicum* TISTR 1462

4.4.2 ABE production with *in situ* product removal (ISPR)

As described earlier, 2.3 L broth was fermented in a 3 L bioreactor. After 24 h of fermentation time, whole ABE fermentation broth was circulated directly through a module of PDMS composite membrane in order to begin the separation process at the same time. The permeate molecules were condensed and collected by using a glass cold trap immerged in liquid nitrogen and retentate molecules were flown back to the reaction side by using a peristaltic pump. For the total fermentation time of 102 h, 17.94 g/L of total solvent concentration produced in a fermentation broth, total solvent yield of 0.37 g solvents/g glucose, and productivity of 0.44 g/L h were obtained in the ABE production with ISPR system (Table 4.3). It could be seen that all the parameters mentioned above shown higher than that of ABE fermentation in traditional batch production. Throughout the ABE fermentation, cell concentration in the bioreactor still kept increasing with the time while glucose was consumed rapidly until the end of fermentation as shown in Figure 4.16. It could be seen that once the inhibitory products were produced, the ISPR process can simultaneously removed the products which affected to the cell in the reaction side. Similarly, Chauhan and Woodley (1997) reported that ISPR method could increase the productivity or yield of a biocatalytic reaction by any of the following means: (1) overcoming inhibitory or toxic effects, (2) shifting unfavourable reaction equilibria, (3) minimizing product lost owing to degradation or uncontrolled release, and (4) reducing the total number of downstream-processing steps. These reasons gave the higher productivity and total solvent concentration as well as the production yield when compared with traditional batch carried out in our laboratory.

Table 4.3 ABE fermentation with ISPR process using *Cl. acetobutylicum*

	Batch with ISPR process	**Batch fermentation***
Acetone (g/L)	3.3	2.7
Butanol (g/L)	14.3	10.8
Ethanol (g/L)	0.34	0.88
Total solvents (g/L)	17.94	14.38
Solvent productivity (g/L h)	0.44	0.30
Solvent yield (g/g)	0.37	0.32
Glucose utilized (%)	96.6	87.4

*ABE production in batch carried out in our laboratory with the same condition

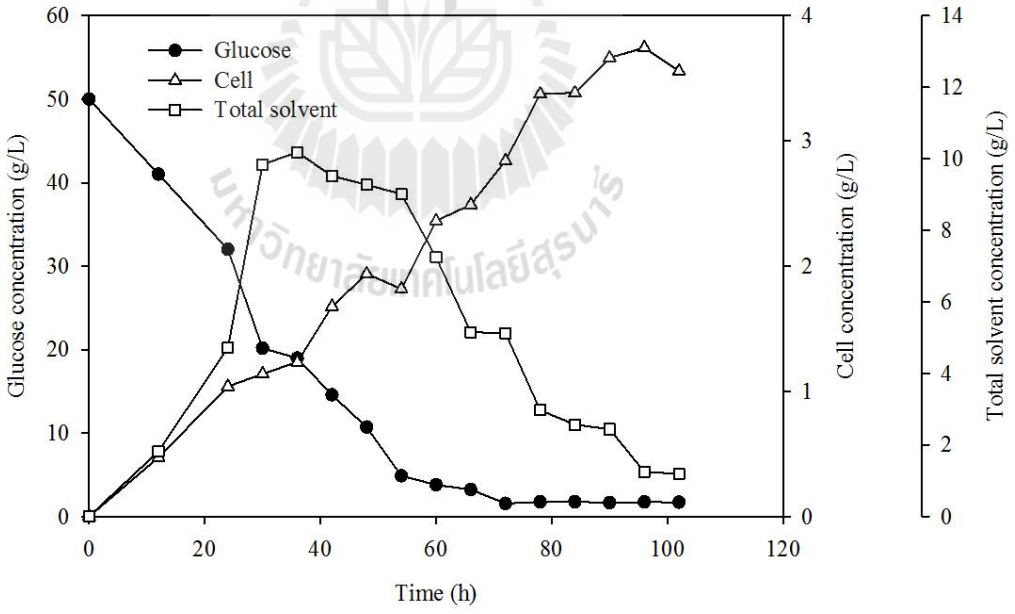


Figure 4.16 Glucose, cell, and total solvent concentration in ABE fermentation with ISPR process using *Cl. acetobutylicum* TISTR 1462 and PDMS composite membrane

For the pervaporation results of acetone, butanol, and ethanol produced in the fermentation broth were shown in Figure 4.17. In the production side, total solvent concentration increased linearly at the beginning until fermentation time of 30 h followed by stationary phase with the highest concentration of 10.2 g/L during pervaporation experiment. Compared to the permeate side, the total solvent concentration showed similar trend to the concentration in the reaction side as well as the total flux. The result indicated that the rate of removal of solvent strongly depended upon solvent concentration, particularly on butanol. Lower concentrations in broth resulted in lower concentration in permeate and flux as well.

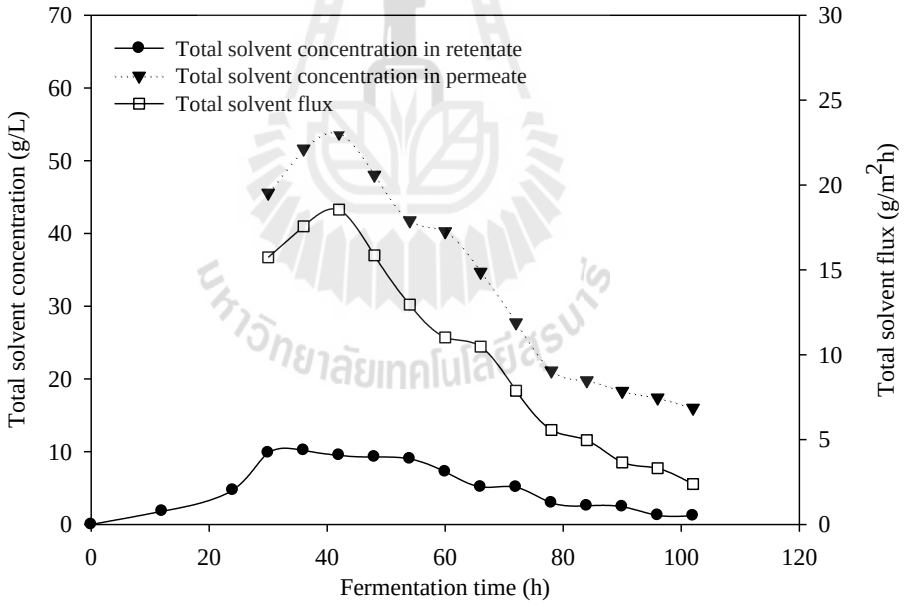


Figure 4.17 Total flux and solvent concentration in reaction side and permeate in ABE fermentation with ISPR using *Cl. acetobutylicum* and PDMS composite membrane

CHAPTER V

CONCLUSION

The separation of *n*-butanol from aqueous binary solution by pervaporation using the three different membrane materials, (PDMS composite membrane, NR, and XSBR composite hollow fiber membrane), were investigated for separation performances in term of permeation fluxes and selectivity. The effect of feed butanol concentrations and operating temperatures were studied and compared individually. The results revealed that an increase in feed butanol concentration in the range of 1.25 – 10 % v/v resulted in rapid increasing of butanol flux and permeate butanol concentration with inverse trend of butanol selectivity which tended to decrease at higher feed butanol concentration. This result showed the similar trend for the three different membranes. On the other hand, the permeation flux and butanol selectivity of the PDMS composite membrane and NR composite hollow membrane increased with increasing the operating temperature from 35 – 80 °C and they were found the inverse trend in XSBR composite hollow fiber membrane which indicated that at higher operating temperature, the butanol selectivity was shown to decrease. In comparison of pervaporative properties, the three different membranes were also used to separate butanol at concentration of 10 g/L in binary solution with varying temperature of 35 – 80 °C. The PDMS composite membrane with a thinner active layer offered the significantly higher butanol flux when compared to the NR and XSBR composite hollow fiber membranes. However, the thicker active layer of NR

and XSBR composite hollow fiber membranes showed the higher performance in term of butanol selectivity, but they did not work efficiently with low temperature (insignificantly low flux was found at 35 °C) which it was the optimal temperature of ABE production. In addition, butanol permeace characterizing the membrane permeability of PDMS composite membrane implied significantly highest values in this experiment. The PDMS composite membrane was therefore chosen to perform the *in situ* product removal (ISPR) system due to its higher permeation flux and butanol permeance. ABE production with ISPR revealed that the total solvent and production yield were higher (17.94 g/L and 0.37 g/g, respectively) than that of traditional batch fermentation (14.38 g/L and 0.32 g/g, respectively) as well as 1.5 times more productivity were achieved in this system. Moreover, the ISPR equipped with ABE production affected positively on fermentation time and downstream processing step.

Recommendation for further studies

According to the results, NR composite hollow fiber membrane offered the higher butanol selectivity when compared to the flat-sheet membrane under the higher operating temperature. Therefore, it was a challenge and might get good outcome if the system (ISPR) equipping with NR composite hollow membrane will be performed with ABE production under the high temperature by using proficiency in bio-process engineering. In addition, the ABE production should be carried out with the higher efficiency process, i.e. fed-bath fermentation with ISPR, in order to meet high production yield, productivity, and particularly butanol concentration.

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APPENDICES

APPENDIX A

Antione equation

Chemists often use the Clausius-Clapeyron equation to estimate the vapor pressures of pure liquids or solids. Several of the assumptions made in the derivation of the equation fail at high pressure and near the critical point, and under those conditions the Clausius-Clapeyron equation will give inaccurate results. Chemists still like to use the equation because it's good enough in most applications and because it's easy to derive and justify theoretically.

Chemical engineers sometimes need more reliable vapor pressure estimates. The Antoine equation is a vapor pressure equation and describes the relation between vapor pressure and temperature for pure components. It is a simple 3-parameter fit to experimental vapor pressures measured over a restricted temperature range:

$$LogP = A - \frac{B}{T + C} \tag{A.1}$$

where *A*, *B*, and *C* are "Antoine coefficients" that vary from substance to substance. Sublimations and vaporizations of the same substance have separate sets of Antoine coefficients, as do components in mixtures. The Antoine equation is accurate to a few percent for most volatile substances (with vapor pressures over 10 Torr).

Example 1. Calculate the vapor pressure of ethanol at 78.32 °C by using the Antoine equation, *A* = 8.20417, *B* = 1642.89, and *C* = 230.300.

*Solution***:**

$$log_{10}P = 8.20417 - \frac{1642.89}{230.300 + 78.32}$$

P = 760 mmHg

The coefficients of Antoine's equation are normally given in mmHg even today where the SI is recommended and pascals are preferred. The usage of the pre-SI units has only historic reasons and originates directly from Antoine's original publication. It is however easy to convert the parameters to different pressure and temperature units. For switching from degree Celsius to kelvins it is sufficient to subtract 273.15 from the C parameter. For switching from millimeters of mercury to pascals it is sufficient to add the common logarithm of the factor between both units to the *A* parameter:

$$A_{Pa} = A_{mmHg} + log_{10}\frac{101325}{760} \quad \text{(A.2)}$$

$$= A_{mmHg} + 2.124903 \quad \text{(A.3)}$$

Example 2. Calculate the vapor pressure (in Pa) of ethanol at 351.47 K by using the Antoine equation.

Solution: The parameters for °C and mmHg for ethanol

A	B	C
8.20417	1642.89	230.300

are converted for K and Pa to

A	B	C
10.32907	1642.89	-42.85

The calculation with Eq. (6) becomes

$$log_{10}P = 10.3291 - \frac{1642.89}{351.47 - 42.85}$$

P = 101328 Pa

APPENDIX B

UNIFAC Method

In concept, the UNIFAC method follows the ASOG method, wherein activity coefficients in mixtures are related to interactions between structural groups. The molecular activity coefficient is separated into two parts: one part provides the contribution due to differences in molecular size and shape, and the other provides the contribution due to molecular interactions. In ASOG, the first part is arbitrarily estimated by using the athermal Flory-Huggins equation; the Wilson equation, applied to functional groups, is chosen to estimate the second part. Some of this arbitrariness is removed by combining the solution-of-groups concept with the UNIQUAC equation; first, the UNIQUAC model contains a combinatorial part, essentially due to differences in size and shape of the molecules in the mixture, and a residual part, essentially due to energy interactions, and second, functional group sizes and interaction surface areas are introduced from independently obtained, pure-component molecular structure data. The UNIQUAC equation often gives good representation of vapor-liquid and liquid-liquid equilibrium for binary and multicomponent mixtures containing a variety of nonelectrolytes such as hydrocarbons, ketones, esters, water, amines, alcohols, nitriles, etc. In a multicomponent mixture, the UNIQUAC equation for the activity coefficient of (molecular) component *i* is

$$ln\,\gamma_i = ln\gamma_i^C + ln\gamma_i^R$$

Combinatorial *Residual*

(B.1)

where

$$ln\,\gamma_i^C = 1 - J_i + lnJ_i - 5q_i\left(1 - \frac{J_i}{L_i} + ln\frac{J_i}{L_i}\right)$$

(B.2)

$$ln\,\gamma_i^R = q_i\left(1 - ln\,s_i - \sum_j \theta_j \frac{\tau_{ij}}{s_j}\right)$$

(B.3)

where in addition

$$J_i = \frac{r_i}{\sum_j r_j x_j}$$

(B.4)

$$L_i = \frac{q_i}{\sum_j q_j x_j}$$

(B.5)

$$s_i = \sum_l \theta_l \tau_{li}$$

(B.6)

Subscript *i* identifies species, and *j* and *l* are dummy indices. All summations are over all species, and $\tau_{ij} = 1$ for $i = j$. In these equations r_i (a relative molecular volume) and q_i (a relative molecular surface area) are pure-species parameters.

The UNIFAC method for estimation of activity coefficients depend on the concept that a liquid mixture may be considered a solution of the structural units from

which the molecules are formed rather than a solution of the molecules themselves. These structure units are called *subgroups*, and a few of them are listed in the Table B.1. A number, designated *k*, identifies each subgroup. The relative volume *R* and relative surface area *Q* are the properties of the subgroups, and values are listed in columns 4 and 5 of Table B.2. Also shown (column 6) are examples of molecular species. When it is possible to construct a molecule from more than one set of subgroup, the set containing the least number of different subgroups is correct set. The great advantage of the UNIFAC method is that a relatively small number of subgroups combine to form a very large number of molecules.

Table B.1 UNIFAC Group Specifications and Sample Group Assignments (Hansen *et al.*, 1991)

Group numbers		Name	Volume	Surface	Sample Assignments = (Number of Occurrences) × (Secondary Group Number)
Main	Secondary		*R*	Area *Q*	
1	1	CH_3	0.9011	0.848	Hexane = (2)(1) + (4)(2)
	2	CH_2	0.6744	0.540	2-Methylpropane = (3)(1) + (1)(3)
	3	CH	0.4469	0.228	Neopentane = (4)(1) + (1)(4)
	4	C	0.2195	0.000	2,2,4-Trimethylpentane = (5)(1) + (1)(2) + (1)(3) + (1)(4)
2	5	CH_2═CH	1.3454	1.176	3-Methyl-1-hexene = (2)(1) + (2)(2) + (1)(3) + (1)(5)
	6	CH═CH	1.1167	0.867	Hexene-2 = (2)(1) + (2)(2) + (1)(6)
	7	CH_2═C	1.1173	0.988	2-Methyl-1-butene = (2)(1) + (1)(2) + (1)(7)
	8	CH═C	0.8886	0.676	2-Methyl-2-butene = (3)(1) + (1)(8)
	70	C═C	0.6605	0.485	2,3-Dimethylbutene = (4)(1) + (1)(70)
3	9	ACH	0.5313	0.400	Benzene = (6)(9)
	10	AC	0.3652	0.120	Styrene = (1)(5) + (5)(9) + (1)(10)
4	11	$ACCH_3$	1.2663	0.968	Toluene = (5)(9) + (1)(11)
	12	$ACCH_2$	1.0396	0.660	Ethylbenzene = (1)(1) + (5)(9) + (1)(12)
	13	ACCH	0.8121	0.348	Cumene = (2)(1) + (5)(9) + (1)(13)
5	14	OH	1.0000	1.200	Ethanol = (1)(1) + (1)(2) + (1)(14)
6	15	CH_3OH	1.4311	1.432	Methanol = (1)(15)
7	16	H_2O	0.9200	1.400	Water = (1)(16)
8	17	ACOH	0.8952	0.680	Phenol = (5)(9) + (1)(17)
9	18	CH_3CO	1.6724	1.488	Methylethylketone = (1)(1) + (1)(2) + (1)(18)
	19	CH_2CO	1.4457	1.180	Ethylphenylketone = (1)(1) + (1)(19) + (5)(9) + (1)(10)
10	20	CHO	0.9980	0.948	Hexanal = (1)(1) + (4)(2) + (1)(20)
11	21	CH_3COO	1.9031	1.728	Butyl acetate = (1)(1) + (3)(2) + (1)(21)
	22	CH_2COO	1.6764	1.420	Methyl propionate = (2)(1) + (1)(22)
12	23	HCOO	1.2420	1.188	Ethyl formate = (1)(1) + (1)(2) + (1)(23)

Table B.2 UNIFAC Group-Group Interaction Parameters, a_{mn}, in Kelvins

Main group	n = 1	2	3	4	5	6	7	8	9
m = 1	0.0	86.02	61.13	76.50	986.5	697.2	1318	1333	476.4
2	−35.36	0.0	38.81	74.15	524.1	787.6	270.6	526.1	182.6
3	−11.12	3.446	0.0	167.0	636.1	637.4	903.8	1329	25.77
4	−69.70	−113.6	−146.8	0.0	803.2	603.3	5695	884.9	−52.10
5	156.4	457.0	89.60	25.82	0.0	−137.1	353.5	−259.7	84.00
6	16.51	−12.52	−50.00	−44.50	249.1	0.0	−181.0	−101.7	23.39
7	300.0	496.1	362.3	377.6	−229.1	289.6	0.0	324.5	−195.4
8	275.8	217.5	25.34	244.2	−451.6	−265.2	−601.8	0.0	−356.1
9	26.76	42.92	140.1	365.8	164.5	108.7	472.5	−133.1	0.0
10	505.7	56.30	23.39	106.0	529.0	−340.2	480.8	−155.6	128.0
11	114.8	132.1	85.84	−170.0	245.4	249.6	200.8	−36.72	372.2
12	329.3	110.4	18.12	428.0	139.4	227.8	NA	NA	385.4
13	83.36	26.51	52.13	65.69	237.7	238.4	−314.7	−178.5	191.1
14	−30.48	1.163	−44.85	296.4	−242.8	−481.7	−330.4	NA	NA
15	65.33	−28.70	−22.31	223.0	−150.0	−370.3	−448.2	NA	394.6
16	−83.98	−25.38	−223.9	109.9	28.60	−406.8	−598.8	NA	225.3
17	1139	2000	247.5	762.8	−17.40	−118.1	−341.6	−253.1	−450.3
18	−101.6	−47.63	31.87	49.80	−132.3	−378.2	−332.9	−341.6	29.10
19	24.82	−40.62	−22.97	−138.4	185.4	162.6	242.8	NA	−287.5
20	315.3	1264	62.32	89.86	−151.0	339.8	−66.17	−11.00	−297.8
21	91.46	40.25	4.680	122.9	562.2	529.0	698.2	NA	286.3
22	34.01	−23.50	121.3	140.8	527.6	669.9	708.7	NA	82.86
23	36.70	51.06	288.5	69.90	742.1	649.1	826.8	NA	552.1
24	−78.45	160.9	−4.700	134.7	856.3	709.6	1201	10000	372.0
25	106.8	70.32	−97.27	402.5	325.7	612.8	−274.5	622.3	518.4
26	−32.69	−1.996	10.38	−97.05	261.6	252.6	417.9	NA	−142.6
27	5541	NA	1824	−127.8	561.6	NA	360.7	NA	−101.5
28	−52.65	16.62	21.50	40.68	609.8	914.2	1081	1421	303.7
29	−7.481	NA	28.41	19.56	461.6	448.6	NA	NA	160.6
30	−25.31	82.64	157.3	128.8	521.6	NA	23.48	NA	317.5

Activity coefficients depend not only on the subgroup properties *R* and *Q*, but also on interactions between subgroups. Here, similar subgroups are assigned to a main group, as shown in the first two columns of Table B.2. All subgroups belonging to the same main group are considered identical with respect to group interactions. Therefore parameters characterizing group interactions are identified with pairs of main groups. Parameter values a_{mk} for a few such pairs are given in Table B.2.

The UNIFAC method is based on the UNIQUAC equation, for which the activity coefficients are given by Eq.(B.1). When applied to a solution of groups, Eqs.(B.2) and (B.3) are written:

$$ln\gamma_i^C = 1 - J_i + lnJ_i - 5q_i\left(1 - \frac{J_i}{L_i} + ln\frac{J_i}{L_i}\right) \tag{B.7}$$

$$ln\gamma_i^R = q_i\left[1 - \sum_k \left(\theta_k \frac{\beta_{ik}}{s_k} - e_{ki} ln\frac{\beta_{ik}}{s_k}\right)\right] \tag{B.8}$$

The quantities J_i and L_i are still given by Eq.(B.4) and (B.5). In addition, the following definitions apply:

$$r_i = \sum_k v_k^{(i)} R_k \tag{B.9}$$

$$q_i = \sum_k v_k^{(i)} Q_k \tag{B.10}$$

$$e_{ki} = \frac{v_k^{(i)} Q_k}{q_i} \tag{B.11}$$

$$\beta_{ik} = \sum_m e_{mi} \tau_{mk} \tag{B.12}$$

$$\theta_k = \frac{\sum_i x_i q_i e_{ki}}{\sum_j x_j q_j} \tag{B.13}$$

$$s_k = \sum_m \theta_m \tau_{mk} \tag{B.14}$$

$$\tau_{mk} = exp \frac{-a_{mk}}{T} \tag{B.15}$$

Subscript *i* identifies species, and *j* is a dummy index running over all species. Subscript *k* identifies subgroups, and *m* is dummy index running over all subgroups. The quantity $v_k^{(i)}$ is the number of subgroups of type *k* in molecule of species *i*. Values of the subgroup parameters R_k and Q_k and of the group interaction parameters a_{mk} come from tabulations in the literature.

The equation for UNIFAC method are presented here in a form convenient for computer programming. In the following example we run through a set of hand calculation to demonstrate their application.

Example 3. For the binary system *n*-butanol(1)/water(2) at 343.15 K, find γ_1 and γ_2 when $x_1 = 0.0019$, $x_2 = 0.9981$

Solution: the subgroup involved are indicated by chemical formular:

CH_3-$(CH_2)_3$-OH [1] / H_2O [2]

n-butanol water

-The following below table shows the subgroups, their identification numbers *k*, Values of the subgroup parameters R_k and Q_k (from Table B.1), and the number of each subgroup in each molecule:

subgroups	***k***	R_k	Q_k	v_{ki}	
				[1]	**[2]**
CH3	1	0.9011	0.848	1	0
CH2	2	0.6744	0.540	3	0
OH	14	1.0000	1.200	1	0
H2O	16	0.9200	1.400	0	1

-By Eq.(B.9), $r_1 = 1(0.9011) + 3(0.6744) + 1(1) + 0(0.9200) = 3.924$

-Similarly $r_2 = 0.920$

-In like manner, by Eq.(B.5),

$q_1 = 3.668$, $q_2 = 1.400$

-The r_i and q_i values are molecular properties, independent of composition. Substituting known values into Eq.(B.11) generates the following table for e_{ki}:

subgroups	k	R_k	Q_k	v_{ki}		e_{ki}	
				[1]	[2]	[1]	[2]
CH3	1	0.9011	0.848	1	0	0.231	0.000
CH2	2	0.6744	0.540	3	0	0.442	0.000
OH	14	1.0000	1.200	1	0	0.327	0.000
H2O	16	0.9200	1.400	0	1	0.000	1.000

-The following interaction parameters are found from Table B.2

	a_{mk}			
m	1(1)	2(1)	14(5)	16(7)
1(1)	0	0	986.5	476.4
2(1)	0	0	986.5	476.4
14(5)	156.4	156.4	0	84.0
16(7)	300.0	300.0	-229.1	0

-Substitution of these values into Eq.(B.15), with T=323.15K

	τ_{mk}			
m	1	2	14	16
1(1)	1	1	0.056	0.249
2(1)	1	1	0.056	0.249
14(5)	0.634	0.634	1	0.783
16(7)	0.417	0.417	1.95	1

-Application of Eq.(B.12), leads to the values of β_{ik}

	β_{ik}			
i	**1**	**2**	**14**	**16**
[1]	0.88	0.88	0.365	0.424
[2]	0.417	0.417	1.95	1

-Find θ_{ik} values from Eq.(B.13), yield:

θ_1= 0.268, θ_2 = 0.325, θ_{14} = 0.266, and θ_{16} = 0.141

-And by Eq.(B.14), :

s_1= 0.887, s_2 = 0.887, s_{14} = 0.379, and s_{16} = 0.482

-The activity coefficients may now be calculated by Eq.(B.7) and (B.8)

i	**[1]**	**[2]**
$\ln\gamma_i^C$	0.749	1×10^{-5}
$\ln\gamma_i^R$	1.913	3×10^{-5}
$\ln\gamma_i$	2.663	5×10^{-5}
γ_i	14.33	1

BIOGRAPHY

Mr. Wirat Inthavee was born on August 18, 1986 in Sisaket Province, Thailand. In 2005, he studied in Department of Food Technology, Faculty of Agriculture, Ubon Ratchathani University. He participated in the Co-operative Education Program to work as production, and quality control supervisor in Better Food Co., Ltd. He graduated Bachelor Degree of Science (Food Technology) from Ubon Ratchathani University in 2009. After graduation, He continued his graduate studies in the Biotechnology Program, School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand. During Master Degree study at School of Biotechnology, He participated as a speaker in the International Conference on Agriculture and Agro-Industry (ICAAI2010), 19-20 November 2010, at Mea Fah Luang University, Chiang Rai, Thailand, and presented the work under the title of "Development of composite tubular membranes for separation of acetone-butanol-ethanol (ABE) from fermentation broth using pervaporation technique".