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นางสาวสุมาลี ศรีสุโน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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PURIFICATION OF SUCCINIC ACID FROM FERMENTATION BROTH USING VAPOR PERMEATION-ESTERIFICATION

Sumalee Srisuno

A Thesis Submitted in Partial Fulfillment of the Requirements for the

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PURIFICATION OF SUCCINIC ACID FROM FERMENTATION BROTH USING VAPOR PERMEATION-ESTERIFICATION

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

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กรดซักซินิกถูกนำมาทำบริสุทธิ์ ด้วยเทคนิคการแยกไอผ่านแผ่นใยนาโนเซรามิค โดยการกรองแบบนาโนฟิลเตรชั่น และการแยกไอผ่านเยื่อแผ่น การทดสอบการแยกสารใช้ทั้งสารละลายต้นแบบ และน้ำหมัก โดยศึกษาค่าการกักกันกรดอินทรีย์ เพื่อหาฟังก์ชันของความดัน ความเข้มข้น และพีเอช ของสารละลาย สำหรับผลการทดลองที่เกิดขึ้นในน้ำหมักนั้น การกรองแบบนาโนฟิลเตรชันสามารถแยกโปรตีน และสีของน้ำหมักได้ดี แต่ไม่สามารถแยกกรดอินทรีย์ที่ละลายอยู่ออกจากน้ำหมัก ในช่วงเริ่มต้นของการศึกษาปฏิกริยาเอสเตอริฟิเคชั่น ระหว่างสารละลายกรดซักซินิกกับเอทานอลนั้น พบว่าผลผลิตยีสต์ของไดเอทธิลซักซิเนท มีความสัมพันธ์กันกับอัตราส่วนของสารตั้งต้น ในขณะเดียวกันอุณหภูมิในการทดลองก็มีบทบาทสำคัญ ต่อปริมาณผลิตภาพในกระบวนการกรองแบบนาโนฟิลเตรชั่น เพื่อแยกกรดซักซินิกออกจากน้ำหมักที่ได้จากการใช้ *Actinobacillus succinogens* ATCC 55618 เป็นจุลินทรีย์ผู้ผลิตกรดซักซินิกในการหมักนั้น ปริมาณผลผลิตยีสต์ และปริมาณผลิตภัณฑ์ไดเอทธิลซักซิเนทที่ได้จากน้ำหมัก ขึ้นอยู่กับขั้นตอนในการแยกน้ำ จากการทดลองแสดงให้เห็นว่า ในการเปลี่ยนจากกรดซักซินิกไปเป็นไดเอทธิลซักซิเนทเกิดขึ้นในช่วงท้ายของขั้นตอน ที่มีการใช้การแยกไอผ่านเยื่อแผ่น ร่วมกับปฏิกริยาเอสเทอริฟิเคชั่น หลังจากผ่านขั้นตอนการกลั่นลำดับส่วน และไฮโดรไลซิสแล้ว จะได้กรดซักซินิกที่มีความบริสุทธิ์สูง

สาขาวิชาเทคโนโลยีชีวภาพ ลายมือชื่อนักศึกษา _____

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SUMALEE SRISUNO : PURIFICATION OF SUCCINIC ACID FROM FERMENTATION BROTH USING VAPOR PERMEATION-ESTERIFICATION. THESIS ADVISOR : ASSOC. PROF. APICHAT BOONTAWAN, Ph.D., 73 PP.

EXTRACTIVE FERMENTATION/ETHANOL/VACUUM FRACTIONATION/ INHIBITION EFFECT/ETHANOL PRODUCTION

An integrated membrane process that consists of nanofiltration (NF) and vapor permeation (VP) was employed as a series of purification process for fermentation-derived succinic acid. Separation performance of a ceramic NF membrane was examined for both model solutions and fermentation broth. Rejection of organic acids was investigated for model solutions as a function of feed pressure, feed concentration and pH. For fermentation broth, the NF showed its usefulness for protein and color removal but not separation of organic acids. The esterification reactions of succinic acid with ethanol were initially investigated using model solutions. The yield of diethyl succinate (DES) was the function of initial reactant ratio whilst the operating temperature played an important role for productivity. Realistic purification was performed with NF-treated fermentation broth using *Actinobacillus succinogens* ATCC 55618 as the succinic acid producer. The yield and volumetric productivity of DES strongly depended on the dehydration rate. Experimental results showed that most succinic acid was converted into DES at

the end of the VP-assisted esterification reaction. After fractionation and hydrolysis, a high purity of succinic acid was obtained.

School of Biotechnology Student's Signature _____

Academic Year 2015 Advisor's Signature _____

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LIST OF ABBREVIATIONS

α	=	Separation factor
K_i'	=	Substrate inhibition constant
K_s'	=	Saturation constant
P_m'	=	Maximum product concentration
°C	=	Degree Celsius
cm	=	Centimeter
CO_2	=	Carbon dioxide
DES	=	Diethylsuccinate
MES	=	Monoethylsuccinate
et al.	=	And others
EtOH	=	Ethanol
g	=	Gram
g/L	=	Gram per liter
g/mL	=	Gram per milliliter
CG	=	Gas chromatography
H	=	Hour
HPLC	=	High Pressure liquid chromatography
kPa	=	Kilopascal
mM	=	Milli molar
Mn	=	Manganese
P	=	Pressure

LIST OF ABBREVIATIONS (Continued)

SEM	=	Scanning electron microscope
T	=	Temperature
VLE	=	Vapor/liquid equilibrium
VVM	=	Volume per minute
Zn	=	Zinc

CHAPTER I

INTRODUCTION

1.1 Background

Currently, there is the most considerable interest on development of biodegradable plastics (bioplastics) because of the global warming problem. Many countries all over the world including Thailand require adding more utilization and market share of bioplastics to reduce the effect from global warming crisis. Thailand is a large producer of raw materials to produce succinic acid such as cassava starch and sugar cane. Therefore, the country has high potential to develop succinic acid to support bioplastic industry. Succinic acid is an important platform chemical used widely in a wide range of industries. Until recently, the commercial scale production of succinic acid is petroleum-derived. However, it can also be produced from renewable feedstock by biological route using wild-type and engineered micro-organisms.

For the production of succinic acid by fermentation, more than 50% of the production costs are typically attributed to separation and purification processes (Cheng *et al*., 2012). The primary challenges are low concentration in the fermentation broth, the presence of by-products especially other organic acids, and the requirement of pH control during fermentation that leads to the succinic acid being present in the salt form. Different recovery techniques have been introduced including precipitation, solvent extraction, ion exchange, membranes and esterification, distillation followed by hydrolysis. However, the last choice seems to be the most

efficient method it can completely remove other organic acids by products by altering their boiling point of the corresponding esters. Esterification reactions are characterized by thermodynamic limitations on the conversion yield. Higher ester yields can be obtained by shifting the equilibrium towards product formation using hybrid processes such as reactive distillation and membrane-assisted reactors instead of using a large excess of alcohol. In combination with a reactor, a membrane can be used to continuously remove water to shift the reaction equilibrium in order to improve yield and volumetric productivity.

In this work, fermentation of succinic acid was investigated using a commercial strain of succinic acid-producing bacterium. Fermentation broth was pre-treated using nanofiltration process prior to esterification with ethanol. In addition, a commercial NaA zeolite tubular ceramic membrane was employed for the dehydration of the esterification reaction. The effects of different operating parameters on esterification reaction were investigated including the temperature and initial molar ratio of the reactants, respectively. Finally, the vapor permeation-esterification of pre-treated fermentation broth with ethanol followed by distillation, and hydrolysis was attempted in order to obtain a high purity succinic acid.

1.2 Research objectives

1.2.1 To investigate fermentation of succinic acid by the commercial strain *Actinobacillus. succinogenes* ATCC 55618 using glucose as the carbon source.

1.2.2 To purify succinic acid based on nanofiltration, esterification and hydrolysis methods.

CHAPTER II

LITERATURE REVIEWS

2.1 Succcinic acid

Succinic acid or butanedioic acid (IUPAC systematic name) is an organic acid having the molecular formula of $C_4H_6O_4$. It is a dicarboxylic acid of four carbon atoms. It occurs naturally in plant and animal tissues. It is very important for body because it is used in the Krebs cycle (citric acid cycle) and involved in the intermediary metabolic process. There is growing interest in the production of succinic acid from renewable resources by microbial fermentation because succinic acid can be used in numerous applications. Application of succinic acid is wildly in many industries such as food industry, pharmaceuticals industry, agriculture industry, cosmetic, photography and textile. Physico-chemical properties and chemical structure of succinic acid are shown in Table 2.1 and Figure 2.1 respectively.

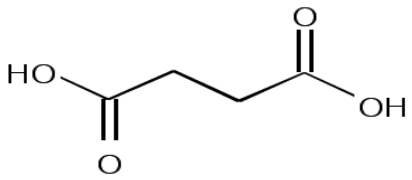

Figure 2.1 The chemical structure of succinic acid.

Table 2.1 Physiochemical properties of succinic acid.

Properties	
Physical state	Odorless and colorless white crystals
Molar mass	118.09 $g.mol^{-1}$
Density	1.56 $g.cm^{-3}$
Melting point	184 °C (363 °F)
Boiling point	235 °C (455 °F)
Solubility in water	58 $g.L^{-1}$ (20 °C)
Acidity (pKa)	$pK_a1 = 4.2$, $pK_a2 = 5.6$

The current worldwide use of succinic acid is around 20,000 to 30,000 tons per year and this increase approximately 10 percents per year (Kidwell, 2008). In the present, succinic acid could become a future commercial replacing petrochemicals in many applications as illustrated in Figure 2.2. Succinic acid can be used as a precursor of many industrial chemicals such as adipic acid, 1,4-butanediol, tetrahydrofuran, N-methyl pyrrolidinone, 2-pyrrolidinone, succinate salts and gamma-butyrolactonesuccinic acid. More importantly, it is used to the synthesis of biodegradable polymers such as polybutelene succinate (PBS) and polyamide to produce bioplastics. Bioplastics are a form of plastics derived from renewable biomass sources. Nowadays, the most utilization of bioplastic production is polylactic acid or polylactide (PLA) and the estimated potential market for PBS is expected at 270,000 tons per year (McKinlay *et al*., 2007).

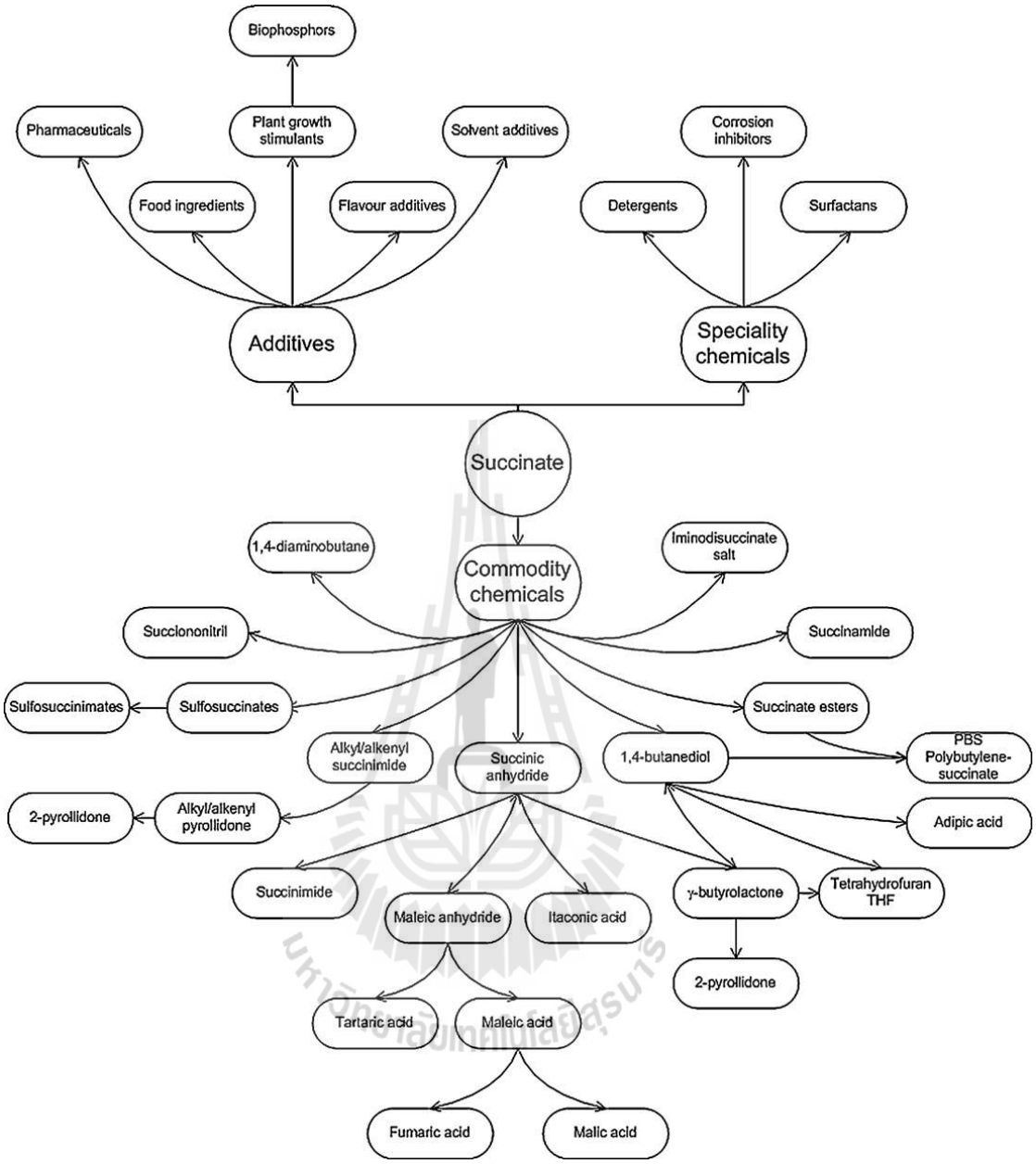

Figure 2.2 Various chemicals and products that can be synthesized from succinic acid (Beauprez *et al*., 2010).

Succinic acid can be produced in difference ways including petrochemical-based synthesis, and fermentation route. The first encounters with high construction and operation cost. More than 15,000 tons of industrial succinic acid is produced

from butane through maleic anhydride. It is sold at a spot price of about U.S. $ 5.90±8.80/kg depending on its purity (Zeikus *et at*., 1999). The latter route is fermentation method. There are many succinic acid producers including *Actinobacillus succinogenes*, *Anaerobiospirillum succiniciproducens*, *Mannheimia succiniciproducens* and recombinant *Escherichia coli*. At present only natural succinic acid sold in the food market is produced by fermentation.

2.1.1 Production of succinic acid by chemical process

Succinic acid produced from petrochemical resources is derived from maleic anhydride, which is produced from n-butane through oxidation over vanadium-phosphorous oxide catalysts. The simplified reaction pathway of n-butane to maleic anhydride is shown in Figure 2.3 (Zhang *et al*., 2009). The reaction from maleic anhydride to succinic acid begins by hydrolysis, breaking one of the single bonds between carbon and oxygen, forming maleic acid. The addition of hydrogen breaks the carbon-carbon double bond and completes the reaction, forming succinic acid. However, succinic acid produced from fossil fuels is not being a natural product.

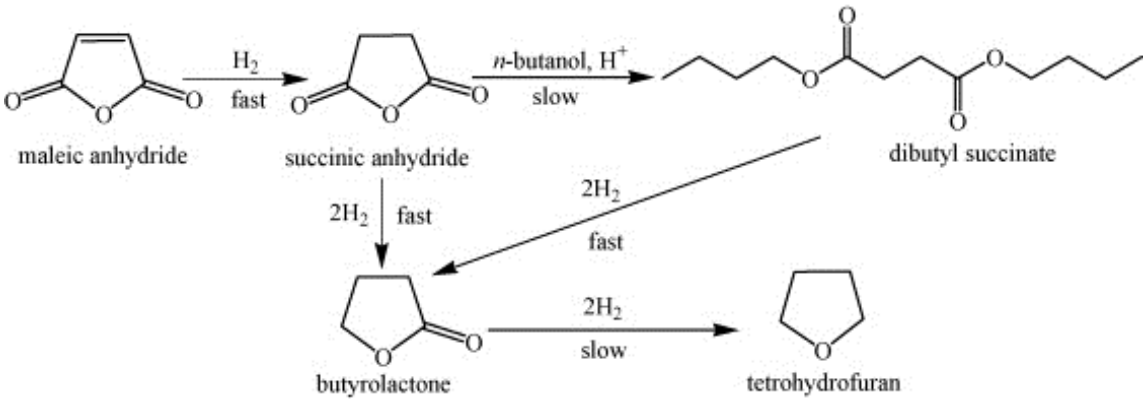

Figure 2.3 Chemical route of the production of succinic acid from maleic anhydride (Zhang *et al*., 2009).

2.1.2 Production of succinic acid by fermentation process

Succinic acid can be produced from the most abundant sugars in plants biomass including glucose, fructose, arabinose, and xylose, respectively. Some anaerobic bacteria, such as *E. coli*, *An. succiniciproducens, M. succiniciproducens, Corynebacterium glutamicum* and *A. succinogenes* are capable to produce succinic acid as a major fermentation product of their metabolism. However, *A. succinogenes* is the most promising strain due to its high volumetric productivity, high succinic acid titer, and less by-products formation. The strain was originally isolated from bovine ruminal contents. This bacterium is a facultative anaerobic and Gram-negative rod or occasionally filamentous bacterium. *A. succinogenes* grows at optimum temperature and pH of 37 °C and 6.8, respectively. At the optimum condition, glucose can be metabolized to produce succinate, formate, acetate, and ethanol as the major products. The components in succinic acid fermentation broth by different bacteria species using glucose as carbon source are shown in Table 2.2.

A. succinogenes is a moderate osmophile and has good tolerance to a high concentration of glucose and can metabolize glucose to succinic acid, acetic acid, and formic acid. Initial glucose concentration can affect cell viability and distribution of fermentation products as well as the level of CO_2 (Lee *et al*., 1999). The highest succinic acid productivity was 1.8 g l^{-1} h^{-1} when 40 g l^{-1} glucose was used (Lee *et al*., 1999). It can produce succinic acid from various carbon sources such as arabinose, fructose, galactose, glucose, lactose, maltose, mannitol, mannose, sorbitol, sucrose, xylose or salicin under anaerobic condition (Zeikus *et al*., 1999). *A. succinogenes* tolerates to a high glucose concentration of 150 $g.L^{-1}$ (Lin *et al*., 2008). A simplified map of wild type *A. succinogenes* succinate-producing metabolism is given in Figure 2.4.

Table 2.2 The components in succinic acid fermentation broth using different bacteria species (McKinlay *et al*., 2007).

Strains	Components in broth (gL^{-1})													Substrate	Reference
	Suc	Mal	Pyr	Ace	For	Lac	Cit	Eth	Gly	Glu	Xyl	Ara	Pro		
A. succinogenes FZ53 (based on ATCC55618)	105.8	-	2.3	18.1	0.7	-	-	-	-	-	-	-	1.9	Glucose	Guettler *et al*. 1996
A. succinogenes FZ6 (based on ATCC55618)	70.6	-	2.3	2.8	0.3	-	-	-	-	2.4	1.4	0.9	3	Corn fiber hydrolysate	Guettler *et al*. 1996
A. succinogenes CGMCC2650	97.8	-	-	17.4	22.5	5.1	-	-	-	-	-	-	-	Glucose	Li *et al*. 2010
An. Succiniciproducens ATCC53488	50.3	-	-	13.6	1.3	-	-	-	-	1.9	-	-	-	Glucose	Glassner and Datta 1992
An. Succiniciproducens ATCC29305	19	-	-	0.6	-	-	-	-	7.5	-	-	-	-	Glucose	Lee *et al*. 2001
M. succiniciproducens KCTC 0769BP	8.8	-	-	3.9	3.6	1	-	-	-	-	-	-	-	Glucose	Song *et al.* 2007
M. succiniciproducens KCTC 10626BP	52.4	12.3	11.7	0.8	-	0.3	-	-	-	-	-	-	-	Glucose	Lee *et al.* 2006
S. cerevisiae SUC-200 (based on CEN.PK113-6B)	34.5	7.8	-	-	-	-	-	4.5	7.7	-	-	-	-	Glucose	Graaf *et al*. 2011

Table 2.2 (Continued).

Strains	Components in broth (gL^{-1})													Substrate	Reference
	Suc	Mal	Pyr	Ace	For	Lac	Cit	Eth	Gly	Glu	Xyl	Ara	Pro		
S. cerevisiae SUC-297 (based on CEN.PK113-6B)	43	-	-	-	-	-	-	16.4	14.9	-	-	-	-	Glucose	Graaf *et al.* 2011
E. coli AFP111-*pyc* (based on ATCC202021)	99.2	-	-	9.5	-	-	-	4.8	-	4.2	-	-	-	Glucose	Vemuri *et al.* 2002
E. coli KJ073 (based on ATCC8937)	86.5	5.2	-	15	-	0.2	-	-	-	-	-	-	-	Glucose	Jantama *et al*. 2008
E. coli KJ060 (based on ATCC8937)	78.8	15.8	4.8	11	-	-	-	-	-	-	-	-	-	Glucose	Jantama *et al.* 2008
E. coli SBS550MG-PHL413 (based on ATCC47076™)	45	-	0.1	-	0.8		4.7	-	-	-	-	-	-	Glucose	Graaf *et al*. 2011
E. coli SD121 (based on ATCC12435)	57.8	-	-	8.2	-	-	-	1.6	-	-	-	-	-	Corn stalk enzymatic hydrolysate	Wang *et al*. 2011
C. glutamicum *Δldh*A-pCRA717 (based on FERMP18976)	146	-	-	16	-	-	-	-	-	10	-	-	-	Glucose	Okino *et al.* 2008

Suc, succinic acid; *Mal*, malic acid; *Pyr*, pyruvic acid; *Ace,* acetic acid; *For*, formic acid; *Lac,* lacti acid; *Cit*, citric acid; *Eth*, Ethanol; *Gyl*, glycerol; *Glu,* glucose; *Xyl*, xylose; *Ara*, arabinose; and *Pro*, propionic acid.

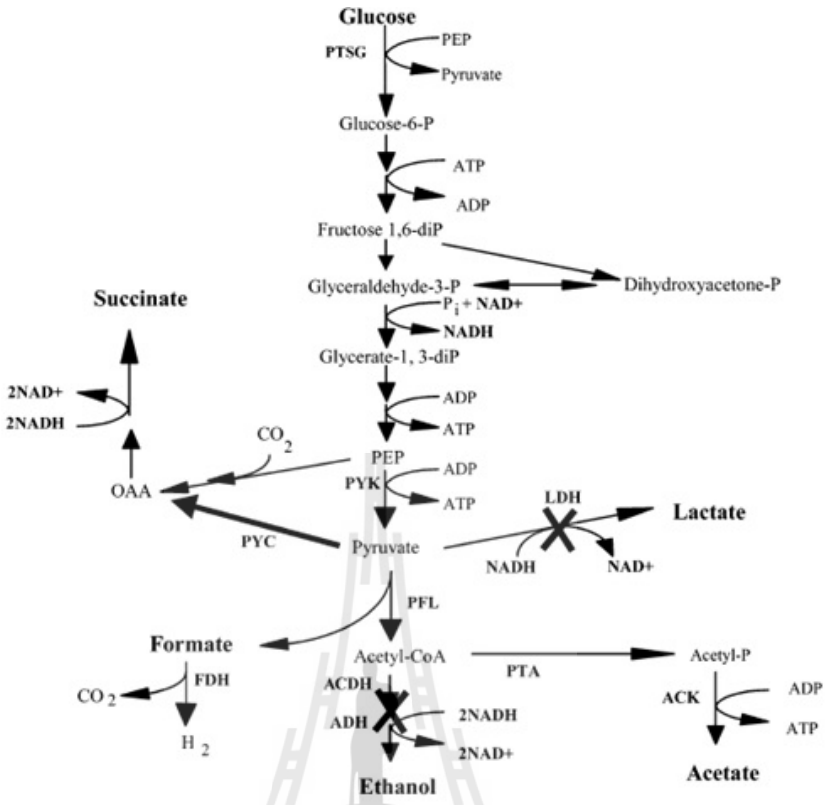

Figure 2.4 Catabolic pathway of succinic acid production of wild-type *A. succinogens* (Sánchez *et al*., 2005).

The metabolism of *A. succinogenes* was originally investigated using in vitro enzymes assays, and by examining fermentation profiles under different growth conditions. Five key enzymes are responsible for succinic acid production including phosphoenolpyruvate carboxykinase (PEPCK), malate dehydrogenase (MDH), malic enzyme (MEnz), fumarase (Fm) and fumarate reductase (Frd) (Song and Lee, 2006). Phosphoenolpyruvate (PEP) carboxylation is the crutial step for succinic acid production in rumen bacteria. It is the major CO_2-fixing enzyme which produces oxaloacetatic acid (OAA) before being converted to succinate by the reductive TCA branch, also called C_4 pathway. The pathway is defined as PEP → OAA → malic acid (Mal) → fumaric acid (Fum) → succinic acid (Suc). The activity of the enzyme is

strongly regulated by pH and CO_2 concentration in the fermentation broth. In theory, a mol of CO_2 is required to form a mol of succinic acid. The higher CO_2 level resulted in an increased succinic acid production at the expense of ethanol and formic acid. The pathway that produces formate, acetate, lactate and ethanol is called the C_3 pathway defined as PEP → pyruvic acid (Pyr) → acetyl CoA (AcCoA) → acetic (Ace) + ethannol (EtOH), and also includes Pyr → lactic acid (Lac).

A. succinogenes is able to produce relatively more succinic acid than other microorganisms (Samuelov *et al*., 1991) and concomitant production of metabolic by-products such as acetic, formic, and lactic acids are problematic because it reduces the succinic acid yield and makes the purification process difficult and costly (Lee *et al*., 2006). As a result, the yield of succinic acid can be increased by disrupting the carbon fluxes to lactate, formate, and acetate by inactivating lactate dehydrogenase (LDH), pyruvate formate-lyase (PFL), phosphotrans acetylase (PTS), and acetate kinase (AK), respectively.

2.2 Conventional fermentation and purification of succinic acid

Biological or fermentation process using microorganisms has no detrimental effect to the environment and this technology route has been wildly researched. Moreover, production of succinic acid by bacterial fermentation can solve technical problems and the chemicals used in fermentation process can be renewable thus reduce environmental problems as less as possible. The optimal pH for generate succinic acid was adjusted and reducing cell toxicity by added calcium hydroxide as shown in Figure 2.5. The major role of adding a calcium ion source is for neutralizing the fermentation broth and to precipitating the succinate as calcium succinate because of its low solubility in water. Isolation of calcium succinate can be achieved by using

a filtration method before treating it with sulfuric acid to form calcium sulfate (gypsum) and succinic acid. Further treatment of the succinic acid can be done with a strong acidic ion ex-changer follow by a weak basic ion ex-changer in order to remove impurities, and obtain a highly purified succinic acid product. In a preferred embodiment, the calcium succinate is isolated from the fermentation broth by filtration; the filtrate is heated to precipitate additional calcium succinate and the spent filtrate which contains nutrients is recycled to the bioreactor. In addition in fermentation process may have produced by-products as calcium lactate. However, all impurities have to be removed in order to make a high purity succinic acid to be used for the plastics industry.

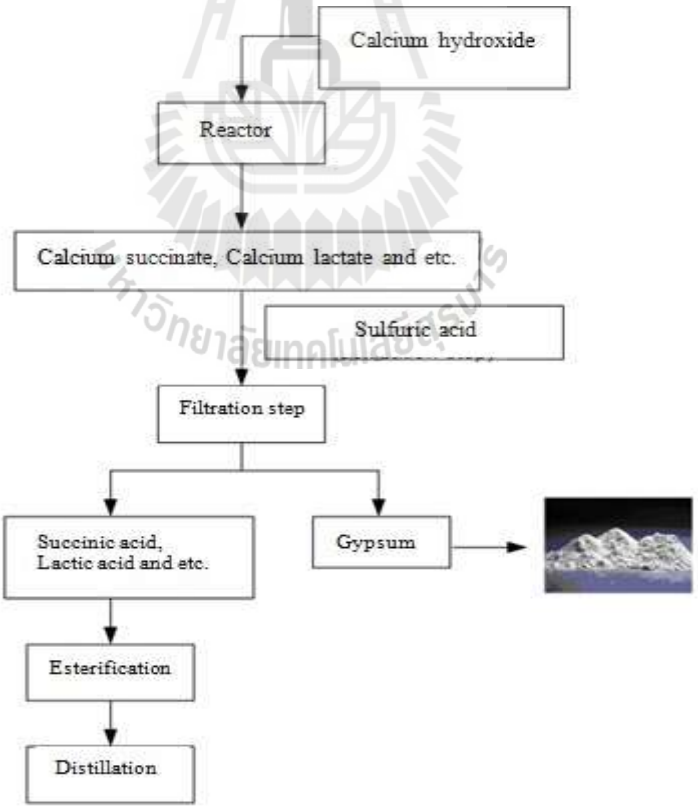

Figure 2.5 Steps in purification of succinic acid from fermentation broth based on esterification and distillation methods.

2.3 Downstream processing of succinic acid

The downstream processing step is defined as the step to recover and purify the product after fermentation including the recycling of salvageable components and the proper treatment and disposal of waste. Both downstream processing and bio-separation refer to the separation or purification of biological products but at different scales of operation and for different purposes. A typical process for the production of a bioproduct like succinic acid by microbial fermentation consists of seed cultivation, fermentation, product recovery, concentration and purification. Considering that the downstream purification cost in the fermentation based process normally accounts for more than 50% of the total production cost (Cheng *et al*., 2012). As a result, it is crucial to develop an economical purification process of succinic acid from fermentation broth. In the case of succinic acid purification, separation of by-products including acetic, formic, lactic and pyruvic acids is the most crucial. Several methods for the purification of succinic acid including precipitation/crystallization, adsorption, membrane separation processes, solvent extraction and esterification were reported. Detailed explanation of each technique can be given below.

2.3.1 Precipitation/crystallization

A traditional method for the organic acid isolation from broth is by precipitation with $Ca(OH)_2$ or $CaCO_3$. Isolation of lactic acid or citric acid using this method has already been commercialized and the technology is already mature. Isolation of succinic acid by precipitation was also studied in previous study (Yuzbashev *et al*., 2010). After adding $Ca(OH)_2$ or $CaCO_3$, the calcium succinate is filtrated from the broth before reacts with sulfuric acid in order to liberate free succinic acid. Further purification can be carried out by absorption using activated

carbon absorption or ion exchange prior to further concentrated and crystallized by evaporation. However, the dosages of $Ca(OH)_2$ or $CaCO_3$, and sulfuric acid during precipitation process are very large. In addition, another disadvantage of precipitation with $Ca(OH)_2$ or $CaCO_3$ is the formation of gypsum as a by-product, which cannot be discarded directly due to odor and color impurities. In conclusion, the precipitation with $Ca(OH)_2$ or $CaCO_3$ can be a viable process for commercial succinic acid production with low technological barriers and risks.

2.3.2 Adsorption

Adsorption has shown a good potential and some data have been gathered for the distribution properties of other carboxylic acids, including acetic, lactic, and citric acids. Adsorption with weak alkaline anion exchange adsorbents is a good method to separate succinic acid from the fermentation broth. The adsorbent NERCB 09 was effective to separate succinic acid from the model solution and fermentation broth because of its high capacity, selectivity and adsorption rate (Song and Lee, 2006). Adsorption is a promising separation method for recovery of the succinic acid because adsorbents have the advantages of low price, quick recovery and low regeneration consumption. Ion exchange adsorption has also been widely used in many organic acids separations (Li *et al*., 2009). However, adsorption is the technique that has been characterized by low separation degrees because other organic acids can also be absorbed by the adsorbent. Therefore, this step is considered only as a primary recovery of succinic from fermentation broth. Further purification steps are required in order to obtain a high purity succinic acid.

2.3.3 Electrodialysis (ED) and electrodeionization (EDI)

Electrodialysis (ED) is a well-known separation process where ionized or weakly ionized compounds in aqueous solution based on transport though ion exchange membranes in an electric field. The succinate salt-containing whole broth including cells is transported from bioreactor and subjected to electrodialysis to recover and concentrate the succinate salt in an aqueous stream. The succinate salt-containing aqueous stream is subjected to water-splitting electrodialysis to form an aqueous succinct acid solution and a based on which can be recycled to the bioreactor. The aqueous succinic acid solution is then subjected an ion exchange polish purification with first a cationic exchanger and then an anion exchange to remove positively charged impurities and to yield a highly purified form of succinic acid. The final product preferably will contain about 70 to about 95% succinic acid, up to 30%, usually between approximately 5% to 20% of acetic acid, less than 1% nitrogenous impurities and less than 10 ppm of sulfate ions or other contaminating ions. Electrodialysis is easily used to separate succinate from nonionized compounds with proper ion exchange membrane, although membranes are usually expensive and easily polluted. The succinic acid purification process composed of conventional electrodialysis followed by water-splitting electrodialysis membrane stacks, which removes most of the salt cation and produces highly pure acid stream. Although this process increased the concentration of succinic acid from 51.5% to 79.6% (w/w) and completely removed proteins and salts, the concentration of acetic acid increased from 13.2% to 19.9% (Song and Lee, 2006).

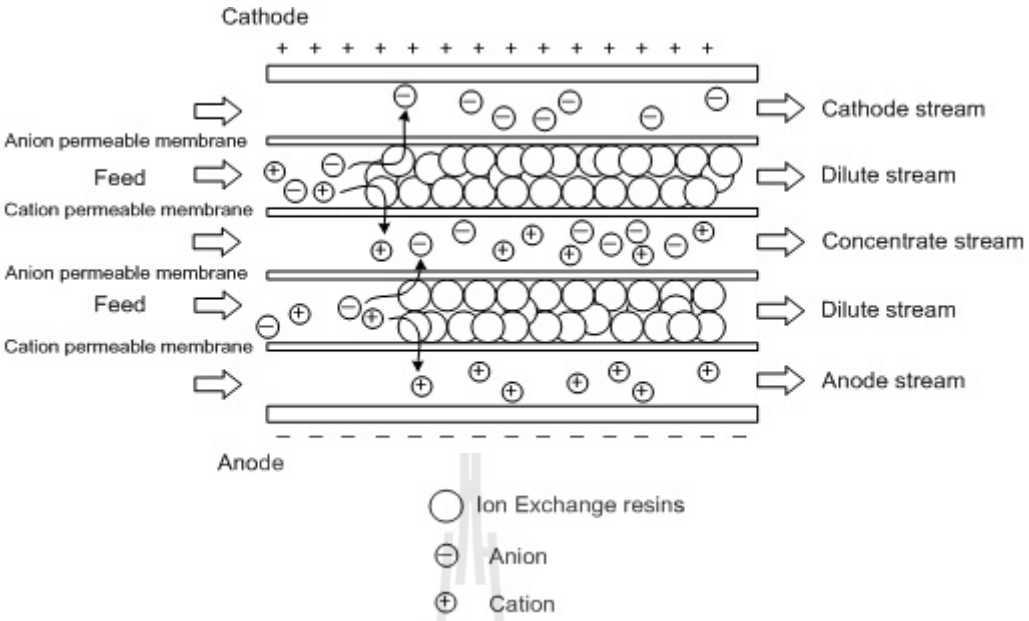

Figure 2.6 Schematic diagram of electrodeionization (EDI) process for recovery of organic acid from fermentation broth (Boontawan *et al*., 2011).

Currently, electrodeionization (EDI) is being more and more applied to produce ultrapure water. EDI is a continuous chemical-free deionization process that relies on the same fundamental principle as for mixed-bed ion exchange. Figure 2.6 shows the typical schematic diagram of EDI process. An EDI stack consists of diluted compartments, concentrated compartments and electrode compartments. The diluted compartments are filled with mixed-bed ion-exchange resins, which enhance the transport of ionic components from bulk solution toward the ion-exchange membranes under the force of a direct current. Since the concentration of ions is reduced in the diluted compartment and is increased in the concentrated compartment, the process can be used for either purification or concentration. In EDI process, ions transport occurs almost entirely through the ion-exchange resins and is not affected by the water resistivity. Due to the influence of the electric field, cations in the solution

are attracted to the cathode and anions are attracted to the anode. In this process, the mixed ion-exchange resins acts as a conducting medium. When the available ions in the diluted compartments are not sufficient for accommodating current transport through the solution, a water-splitting reaction occurs in those compartments and then relatively high concentrations of H^+ and OH^- are able to regenerate in the mixed ion-exchange resins. In conclusion, both ED and EDI fail to separate charged components as they are also migrate under direct electrical current. In addition, electricity cost to generate current is high and this process does not seems to be economical viable.

2.3.4 Solvent extractions

Solvent extractions are used for the purification, enrichment, separation and analysis of various components in mixtures. The system is based on the principle that a solute can distribute itself in a certain ratio between two immiscible solvents. The traditional product recovery method is based on precipitation of the insoluble calcium salt of carboxylic acids with $Ca(OH)_2$ or $CaCO_3$ followed by reacidification with H_2SO_4. The disadvantage of this process is handling large amounts of solid and slurry, and the production of equal amounts of calcium sulfate waste. Extraction with conventional solvents, such as ether, is impractical for the recovery of most carboxylic acids because the low activity coefficient of the acid in the aqueous phase does not allow for a substantial transfer of the acid into the solvent. One novel technique that can circumvent these drawbacks is the Pre-dispersed solvent extraction (PDSE) process that employs. PDSE by colloidal liquid aphrons (CLAs) was used for the extraction of succinic acid from aqueous solution. The loading values for succinic acid in PDSE by CLAs increased with increasing pH values in aqueous phase. This was due to increasing of the concentration of the undissociated succinic acid. The

extractability of PDSE was higher than that of conventional contacting type extraction because of the interaction between reactant and acid molecule. The stability of CLAs increased with increasing of the pH values in aqueous phase and decreasing of trioctylamine (TOA) concentration in organic phase. However, the structure of CLAs was stable at all the pH range except very low pH condition (Kim *et al*., 2006).

2.3.5 Esterification

High purity succinic acid can also be produced by esterification of crude succinic acid with alcohol to yield succinate ester. The process is followed by distillation, hydrolysis of the distillated lactate ester to yield the alcohol and succinic acid. Esterification is the only downstream process, which separates other organic acids from succinic acid. Esterification gives ester of succinic acid, and further hydrolysis of ester is necessary to get the product as pure succinic acid. Fermentation broth containing succinic acid needs to be pretreated to remove some other impurities before esterification reaction. Ethanol is a preferred reactant because it is cheap and easy to produce. Esterification of mixtures of succinic acid and other organic acids commonly produced via fermentation with ethanol is shown in Figure 2.7.

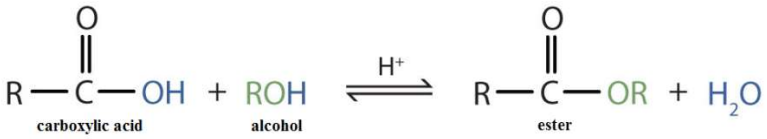

Figure 2.7 Esterification reaction of carboxylic acid with alcohol (Ball *et al*., 2011).

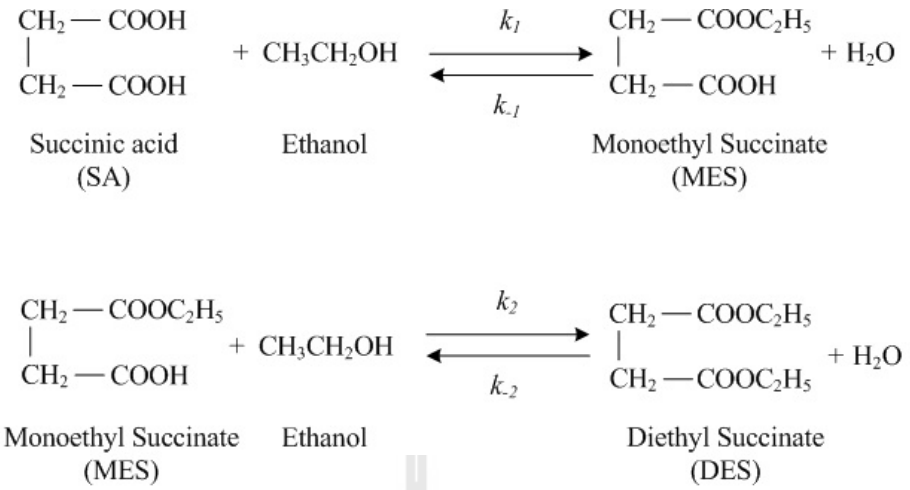

Figure 2.8 Esterification of succinic acid (SA) to monoethyl succinate (MES) and diethyl succinate (DES).

For succinic acid, it is a di-basic acid having two carboxylic acid functional groups. Succinic acid can be esterified with ethanol through a series of reactions to yield diethyl succinate (DES) and 2 moles of water are produced as by-product. A schematic reaction scheme for esterification of succinic acid with ethanol is shown in Figure 2.8. A conventional process to synthesize diethyl succinate typically would use a stream of succinic acid and ethanol which are esterified in a batch or continuous stirred-tank reactor (CSTR) using sulfuric acid as a homogeneous catalyst. Many of the difficulties associated with use of homogeneous catalysts can be eliminated through use of heterogeneous catalysts like ion exchange resins or supported clays. The heterogeneous catalyst allows easy mechanical separation of the catalyst from reaction media by decantation or filtration, reduces or eliminates corrosion problems, and facilitates continuous process operation. Succinate esters are of low toxicity and low vapor pressure and have exceptional solvent properties, making them attractive candidates as replacements for petroleum based solvents.

Esterification reactions are characterized by thermodynamic limitations on the conversion yield. Higher ester yields can be obtained by shifting the equilibrium towards products formation using hybrid processes such as reactive distillation and membrane-assisted reactors instead of using a large excess of alcohol. Membrane separation processes have gained increasing attention in many esterification processes as an effective energy-saving separation technique (Benedict *et al*., 2006). In this regard, the integration of hydrophilic membranes into conventional esterification processes is very attractive as the separation is based on the transport of the reacting components through the membrane. Mass transfer is determined by the solubility and diffusivity of the components to be separated and it is not limited by the relative volatility of the components as in distillation processes. In combination with a reactor, a membrane can be used to continuously remove one of the reaction products to shift the reaction equilibrium in order to improve yield and in most cases the removed product is water.

The kinetics of esterification reactions between succinic acid and ethanol have been extensively investigated by many researchers (Benedict *et al*., 2006, Delhomme *et al*., 2012). Since the esterification reaction of succinic acid and ethanol is a second order reversible reaction, the reaction rate of diethyl succinate (r_{DES}) can be written as:

$$r_{DES} = \frac{1}{m_{cat}} \frac{1}{v_i} \frac{dn_{ESA}}{dt} = 1.357 \times 10^6 \exp\left(\frac{-55.04}{RT}\right) \times \left(a_{SA} a_{EtOH} - \left(\frac{a_{DES} a_{H2O}}{K_{eq}}\right)\right) \quad (1)$$

Where; m_{cat} is the mass of the catalyst and v_i is the stoichiometric coefficient. The equilibrium constant K_{eq} was experimentally determined as a function of the mole fraction and the activity coefficients of the products and reactants. This

expression was obtained by correlating the kinetic data using Amberlyst 15 as a catalyst and calculating the activity coefficients with the UNIQUAC parameters obtained in the study of the phase equilibrium of the same esterification reaction. The relationship between the reaction rates of the four components can be expressed depending on their stoichiometric factors:

$$r_{DES} = r_{H_2O} = -r_{SA} = -r_{EtOH} \quad (2)$$

Where, the subscripts H_2O, SA and EtOH denote water, succinic acid and ethanol, respectively. Since the esterification reactions are investigated in batch mode, the yield of diethyl succinate can be calculated by the following equation:

$$\text{Yield of diethyl succinate (\%)} = \frac{m_{DES}}{m_{DES,cal}} \times 100 \quad (3)$$

Where, m_{DES} is the mass of diethyl succinate obtained from the experiment and $m_{DES,cal}$ is the mass of diethyl succinate calculated from the total conversion of succinic acid, respectively.

2.3.6 Membrane-assisted esterification

In recent years, there has been an increasing effort to combine downstream/upstream separation with reaction to improve process performance. Membrane separation technologies offer advantages over existing mass transfer processes. Such advantages can comprise; high selectivity, low energy consumption and moderate cost to performance ratio. In this regard, membrane technology has emerged as one of the viable separation processes. Since membranes allow selective permeation of one component from multicomponent mixture, these can help enhance

the conversion of reactants for thermodynamically or kinetically limited reactions via selective removal of one or more product species from the reaction mixture. When multiple reactions are involved, the yield or selectivity of a desired product, usually an intermediate, can be enhanced by controlled addition of one or more reactants and removal of one or more intermediates (Lipnizki *et al.,* 1999).

Vapor permeation and pervaporation are used to separate a liquid mixture by partly vaporizing it through a nonporous permselective membrane. The "feed" liquid mixture is allowed to flow along one side of the membrane and a fraction of it, the "permeate", is recovered in the vapor state on the other side of the membrane (Jalal *et al.,* 2002). The permeate is kept under vacuum by continuous pumping or is purged with a stream of carrier gas. Low vapor pressure maintained on the permeate side induces mass transport through the membrane in this process. The permeate is finally obtained in liquid state after condensation. The permeate is enriched in the more rapidly permeating component of the feed mixture, whereas the remainder of the feed that does not permeate through the membrane, the "retentate" is depleted in this component. Applications of vapor permeation and pervaporation reported in the literatures include dehydration of organic solvents, separation of aromatic/aliphatic hydrocarbon mixtures, and removal of water from solutions of organic acids and alcohols depending on the nature of selective layer of the membrane (Delgado *et al.,* 2008 and Jalal *et al.,* 2002).

2.3.6.1 Pervaporation-assisted esterification

pervaporation is a membrane separation process where one side of the membrane is in contact with the liquid feeding solution and permeation of the migrating species through the membrane matrix is induced by the application of a

vacuum pump or an inert carrier gas on the other side of the membrane (Lipnizki *et al*., 1999). As shown in Figure 2.9, 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. 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 or condenser. Separation of the fluid mixture can be successfully achieved with a selection of membranes exhibiting both high permeation rate and good selectivity. In combination with a reactor, pervaporation process can be used to continuously remove water formed during the esterification process with the main objective to shift the equilibrium of the reaction resulting in higher yield and volumetric productivity (Delgado *et al.*, 2008).

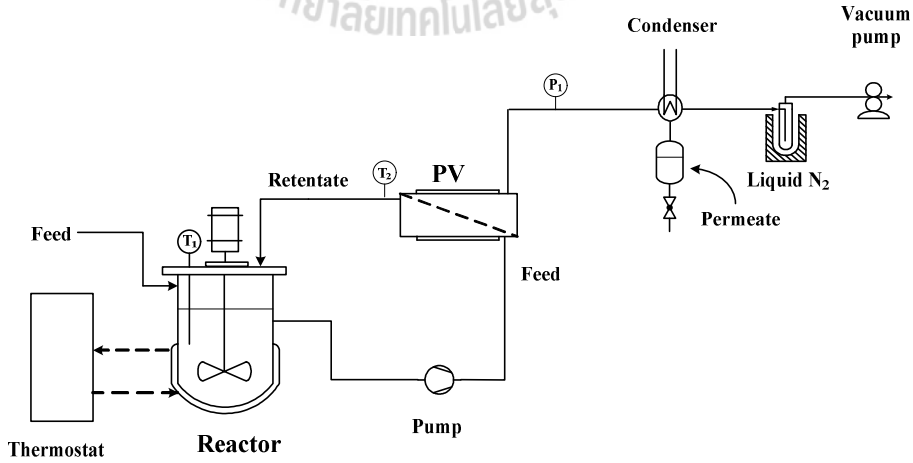

Figure 2.9 Schematic diagram of a pervaporation-assisted esterification system (Khunnonkwao, 2012).

Benedict *et al*. (2006), studied solid-catalyzed, pervaporation-assisted esterification of lactic acid and succinic acid with ethanol. Conversions in excess of the equilibrium conversion attainable in a reactor without product separation were attained by selective removal of water from the reaction mixture by pervaporation. Stripping of water pushed the equilibrium conversion very close to unity, demonstrating the efficacy of pervaporation-aided esterification. High water flux through the pervaporation membrane was obtained by maintaining high recirculation rate of the liquid and low permeate pressure. Pervaporation performance was promoted with increasing temperature. Conventional multistage distillation was adequate to separate and recover ethyl lactate and diethyl succinate from pervaporation retentate, since the alcohol–ester mixtures under consideration are not prone to azeotrope formation. Existence of mixtures of ethanol and lactic and succinic acids in single phase at above room temperature coupled with significant difference in boiling points of the two esters bonds well for simultaneous esterification of the two acids.

2.3.6.2 Vapor permeation-assisted esterification

In contrary to pervaporation, the feed side needs to be vaporized prior to enter the vapor permeation module. In addition, the vapor feed can be pressurized and superheated resulting in higher dehydration rate as shown in Figure 2.10.

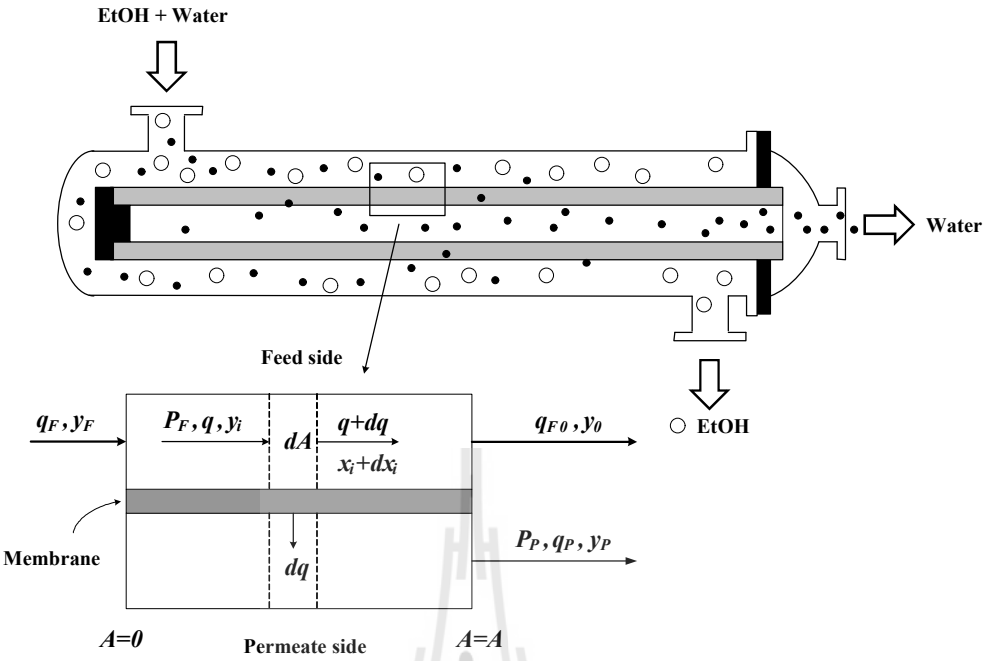

Figure 2.10 Mass transfer consideration of the VP system (Khunnonkwao *et al*., 2012).

The mass balance of water over the system can be further considered according to the amount formed and the mass transfer caused by dehydration by the membrane processes as followed:

$$\frac{dn_{H2O}}{dt} = m_{cat} r_{H2O} - A J_{H2O} \quad (4)$$

In this case, n_{H2O} is the number of moles of water, t is the time, m_{cat} is the mass of catalyst, r_{H2O} is the esterification rate of water in the reactor, A is the membrane area and J_{H2O} is the molar flux of water (mole/(m^2 h)), respectively. Based on the adsorption-diffusion model, mass flux of component i depend on the partial pressure difference across the membrane matrix as followed:

$$J_i = \frac{dq}{dA} = Q_i(y_F P_F - y_P P_P) \quad (5)$$

Where: J_i is the molar flux of the component i (mole/(s m^2)), q is the molar transfer rate (mole/s), Q_i is the permeance of the component i (mole/(s m^2 Pa)), A is the membrane area (m^2), y_F is the mole fraction in the feed side, PF is the feed pressure (Pa), y_P is the mole fraction in the permeate and PP is the permeate pressure (Pa). In addition, q_F is the molar flow rate of the inlet, q_{F0} is the molar flow rate of the retentate and y_0 is the retentate mole fraction, respectively.

The membrane performance can be described by total flux, J_{total} (kg/(m^2 h)) and separation factor (α) which can be defined as followed:

$$J_{total} = \frac{W(kg)}{A(m^2) \cdot t(h)} \tag{6}$$

Where; W is the weight of the permeate, A is the membrane area and t is the time. The separation factor (α) of the membrane was defined as:

$$\alpha = \frac{w_{i,p} w_{j,f}}{w_{i,f} w_{j,p}} \tag{7}$$

Where w_i,p and w_j,f are the weight fractions of components i and j on the permeate side, and w_i,f and w_j,p the weight fractions of components i and j on the feed side, respectively.

2.3.7 Distillation and hydrolysis

2.3.7.1 Distillation

Distillation is a method of separating mixtures based on differences in volatilities of components in a boiling liquid mixture. Distillation is a unit operation or a physical separation process and not a chemical reaction. Recently, a high efficiency small scale fractionating column was successfully developed in the

Suranaree University of Technology. Figure 2.11 shows the internal design of the distillation column and detailed descriptions can be given as; boiler, to provide the necessary vaporization for the distillation process. The boiler was constructed from a jacketed 2-L glass reactor where an oil bath was used to provide heat. Column, The column is constructed from a stainless steel with the height of 90 cm long and 6.4 cm inner diameter. The top of the column was installed with a drive shaft with a variable speed motor. Drive shaft, Drive shaft is the most important part because that used to fix the propellers.

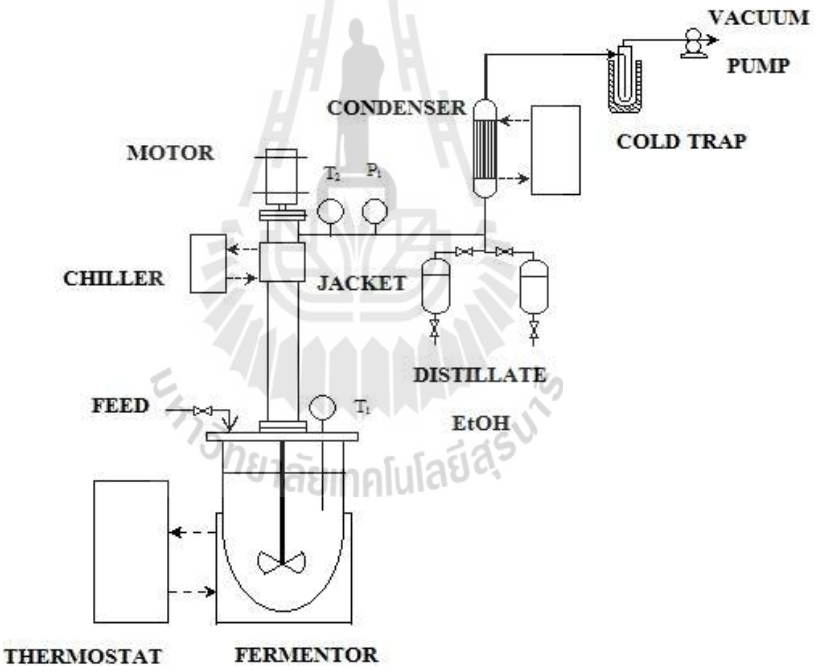

Figure 2.11 Schematic diagram of the distillation technique developed at SUT.

Various organic impurities present in the fermentation broth can play an important role in the design of purification processes. As mention previously, by-products especially organic acids present in the fermentation broth are difficult to separate because they also possess carboxylic group as well as hydrophobic R group.

For example; the electrodialysis technique is not very effective because all organic acids can be dissociated / charged and migrate under the direct electrical field and the R group of each organic acid can interact with the ligand of the adsorbent, respectively. Esterification is the most effective technique to remove formic acid, acetic acid and lactic acid by converting them into ester form. The boiling of these compounds is shown in Table 2.3. As a result, simple distillation is adequate to separate these components from diethyl succinate.

Table 2.3 Physical and Chemical properties of organic acid esters usually present in the fermentation of succinic acid.

Properties	**Ethyl formate**	**Diethyl succinate**	**Ethyl lactate**	**Ethyl acetate**
Molecular formula	$C_3H_6O_2$	$C_8H_{14}O_4$	$C_3H_{10}O_3$	$C_4H_8O_2$
Molar mass (g/mole)	74.08	174.19	118.13	88.11
Physicals	Colorless liquid	Colorless liquid	Slightly yellow liquid	Colorless liquid
Density (g/cm^3), 20 ^{o}C	0.917	1.047	1.03	0.897
Melting point (^{o}C)	-80	-20	-26	-83.6
Boiling point (^{o}C)	54	218	151	77.1

A preliminary experiment was carried out for fractionation of ethanol/ethyl lactate mixtures at different mole fractions (Khunnonkwao *et al*., 2012). The installation of a reflux condenser on top of the Vigreux column played an important role for condensation of the rising ethyl lactate allowing high purity ethanol to leave the column. The experimental results of the mole fractions measured in both

the liquid and the distillate fraction were compared with the vapor liquid equilibrium (VLE) data obtained from a previous work (Benedict *et al*., 2006). It was evident that a high ethyl lactate concentration can be obtained by partial condensation of the rising ethanol/ethyl lactate vapor. In this work, up to 0.95 mole fraction of ethanol was obtained in the distillate at 0.08 mole fraction of ethanol in the liquid phase. Based on these experimental data, two distillation steps were employed to separate ethanol and ethyl lactate from the mixture. In the first step, the majority of ethanol and a small amount of ethyl lactate (approximately 1.5 wt%) were recovered in the distillate. In the subsequence step, a small portion of ethanol (approximately 5 wt%) and the majority of ethyl lactate were obtained in the distillate stream.

2.3.7.2 Hydrolysis

The purified diethyl succinate obtained from distillation can be hydrolyzed with deionized water to produce a high purity succinic acid as shown in Figure 2.12. It is a backward reaction of esterification where di-ethyl succinate reacts with 2 moles of water generating succinic acid and 2 moles of ethanol. This reaction requires proton donating substance as a catalyst such as Amberlyst 15 at the concentration of 3-5 wt%. Two hydrolysis steps can also be employed as described in the previous section. In the first step, ethanol generated from the reaction was removed by distillation with the help of a small reflux condenser. In the last step, the excessive water was removed by vacuum evaporation to produce concentrated succinic acid. Because hydrolysis is a simple process, variation of the operating parameters is not necessary.

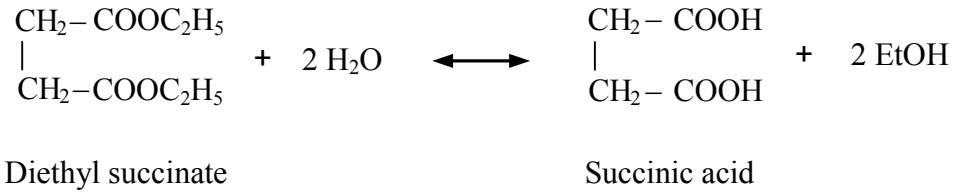

Figure 2.12 Schematic diagram for hydrolysis of diethyl succinate to succinic acid and ethanol.

CHAPTER III

MATERIALS AND METHODS

3.1 Chemicals

All chemicals were supplied by Fluka (United Kingdom). *A. succinogenes* ATCC 55618 was maintained in 10% skim milk at -70 °C. A mono channel tube ceramic nanofiltration membrane with a molecular weight cut-off 450 Da was purchased from Fraunhofer IKTS (Germany). For VP, tubular NaA zeolite membranes supplied by Mitsui Engineering & Shipbuilding (Japan) were employed for dehydration task.

3.2 Fermentation of succinic acid

Actinobacillus succinogenes ATCC 55618 seed cultivation was prepared by growing a single colony in 250 mL shake flasks at 35 °C. The composition of the pre-culture medium was as following (L^{-1}); 17.0 g tryptone, 3.0 g soy peptone, 2.5 g dextrose, 5.0 g NaCl and 2.5 g K_2HPO_4. The fermentation medium contained per liter; 85 g glucose, 25.0 g yeast extract, 3.0 g KH_2PO_4, 1.5 g K_2HPO_4, 1.0 g NaCl, 0.3 g $MgCl_2$, 0.3 g $CaCl_2$, 0.07 g $MnCl_2$, 1.0 g anti-foam agent and 50 g $MgCO_3$. Batch fermentation was carried out in a 4.0-L bioreactor (Sartorius, Germany). Temperature was controlled at 37 °C with the agitation rate 200 rpm. During the first 12 h, CO_2 was sparged at 0.2 vvm whilst pH was controlled at 6.5 by the addition of 40 wt% $MgCO_3$ solution. At the end of fermentation, pH of the broth was adjusted to 2.0 using

H_2SO_4 in order to liberate free organic acids. Cells and insoluble solids were removed by centrifugation at 8,000 rpm for 20 min followed by a cross-flow microfiltration (MF) unit. The permeate of 3.0 L was collected, and was stored at 4 °C for further study.

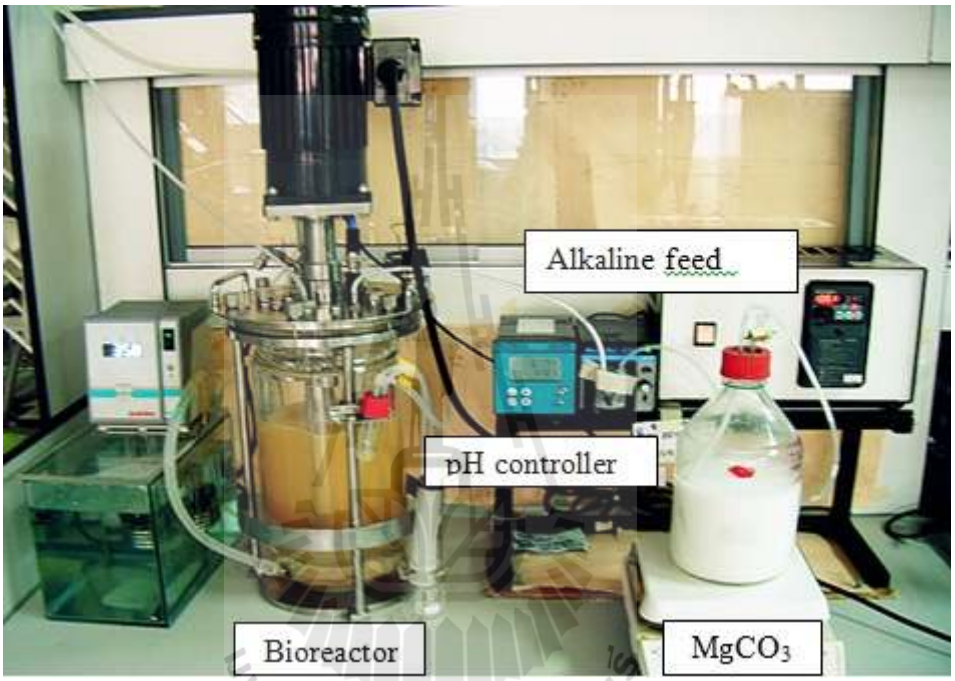

Figure 3.1 Experimental setup for batch fermentation of succinic acid using *A. succinogines* ATCC 55618.

3.3 Experimental Setup for purification processes

3.3.1 Nanofiltration (NF)

The NF experiment was carried out in a tubular membrane module as shown in Figure 3.2. It comprised of a mono-channel ceramic membrane with a stainless steel housing. The effective surface area was 55 cm^2 (inner tube diameter 0.7 cm and length 25 cm, respectively). The selective layer was TiO_2 coated on the supportive α-Al_2O_3 layer. A 3-L jacketed glass vessel was employed as a feed tank

where desired temperature was obtained by using a thermostat. A high pressure piston pump head (FMI, USA) mounted on a 1/10 hp pump drive (Masterflex, USA) was used to circulate the solution in the cross-flow mode and also to increase the liquid feed pressure with the help of a needle valve.

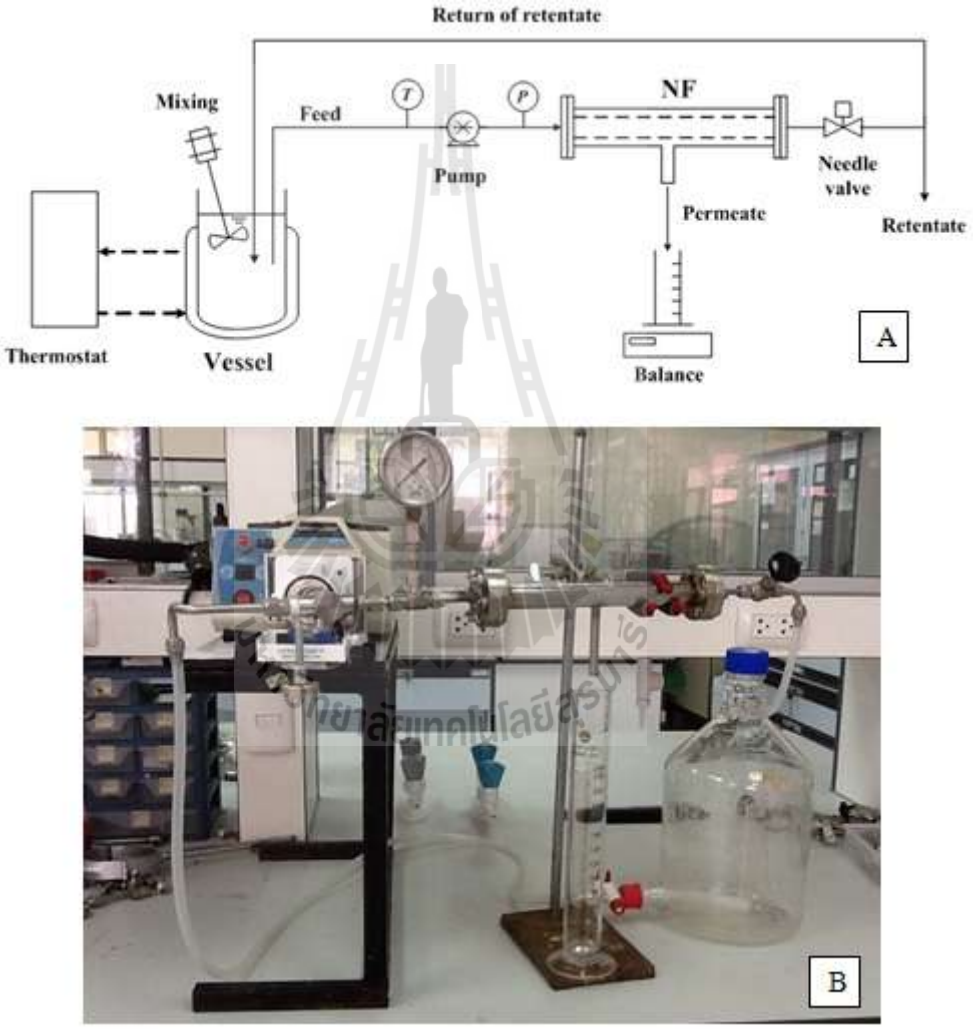

Figure 3.2 Schematic diagrams for experimental setup of the nanofiltration (A), and picture showing the real experiment (B).

NF of organic acid solutions was carried out at trans-membrane pressures in the range between 200-600 kPa and initial acid concentrations 10-70 g/L, respectively. The pH of the solution was adjusted by addition of NaOH to be in the range between 2-8. The retentate and permeate were re-circulated back into the vessel in order to avoid the time change in concentration (total recycle mode). Separation performance was examined in terms of flux and rejection. Flux of the permeate was gravimetrically measured and the values reported are the average of three experiments. The membrane used in the previous experiment was washed with water, NaOH and H_2PO_4 solutions until the initial water flux was observed. The rejection (R%) was calculated as:

$$R(\%) = \left[1 - \left(\frac{C_P}{C_R}\right)\right] \times 100 \quad (8)$$

Where C_P and C_R represent the concentration of the component in permeate and retentate stream, respectively.

Initially, NF of the clarified fermentation broth was investigated in concentration mode. The permeate was continuously removed for determination of flux, rejections, and especially protein removal efficiency. Analysis of fouling behavior due to different mechanisms was subsequently investigated by using the permeate flux measurement. The membrane resistance was estimated by the following equation based on Darcy's law (Al-Amoudi and Lovitt, 2007):

$$R_{NF} = R_m + R_f + R_C = 3600 \times \frac{TMP}{\mu J} \quad (9)$$

Where R_{NF} refers to the filtration resistance (m^{-1}), R_m is membrane hydraulic resistance, R_f is resistance due to pore blocking and adsorption and R_c is

resistance due to cake formation, J is permeate flux ($m^3/m^2.h$), TMP is the trans membrane pressure (Pa) and μ is the viscosity of the permeate (Pa.s), respectively.

For a diafiltration mode, separation of organic acids was investigated for synthetic solutions as well as the clarified fermentation broth. The feed volume was kept constant by an addition of deionized water (pH 2.0 adjusted by H_2SO_4) to make up the volume of the permeate. The NF experiment ceased when the concentration of SA in the feed tank was lower than 2.0 g/L. For further purification of SA from the fermentation broth, the permeate was concentrated by using a rotary evaporator (IKA, Sweden) until the water content reduced to approximately 20 wt%. This "crude" SA was subsequently used in the VP-assisted esterification experiment.

3.3.2 Vapor permeation-assisted esterification

After NF, the solution was evaporated using a rotary evaporator (IKA, Sweden) until the water concentration reduce to approximately 20 wt%. Then, the concentrated broth was transferred to a 2-L glass reactor followed by an addition of ethanol at the different molar ratios of succinic acid to ethanol. The mixture was allowed to reach equilibrium for 2 h before increasing the temperature of the liquid mixture to its boiling point. Fractionation was carried out by control the exit temperature of the vapor. With a suitable control condition, the distillate ethanol comprises of water concentration at 5% without diethyl succinate. The temperature of the reaction solution was kept constant by using a heating circulator (Julabo, Germany). The schematic diagram of the experimental setup for vapor permeation-esterification is shown in Figure 3.3.

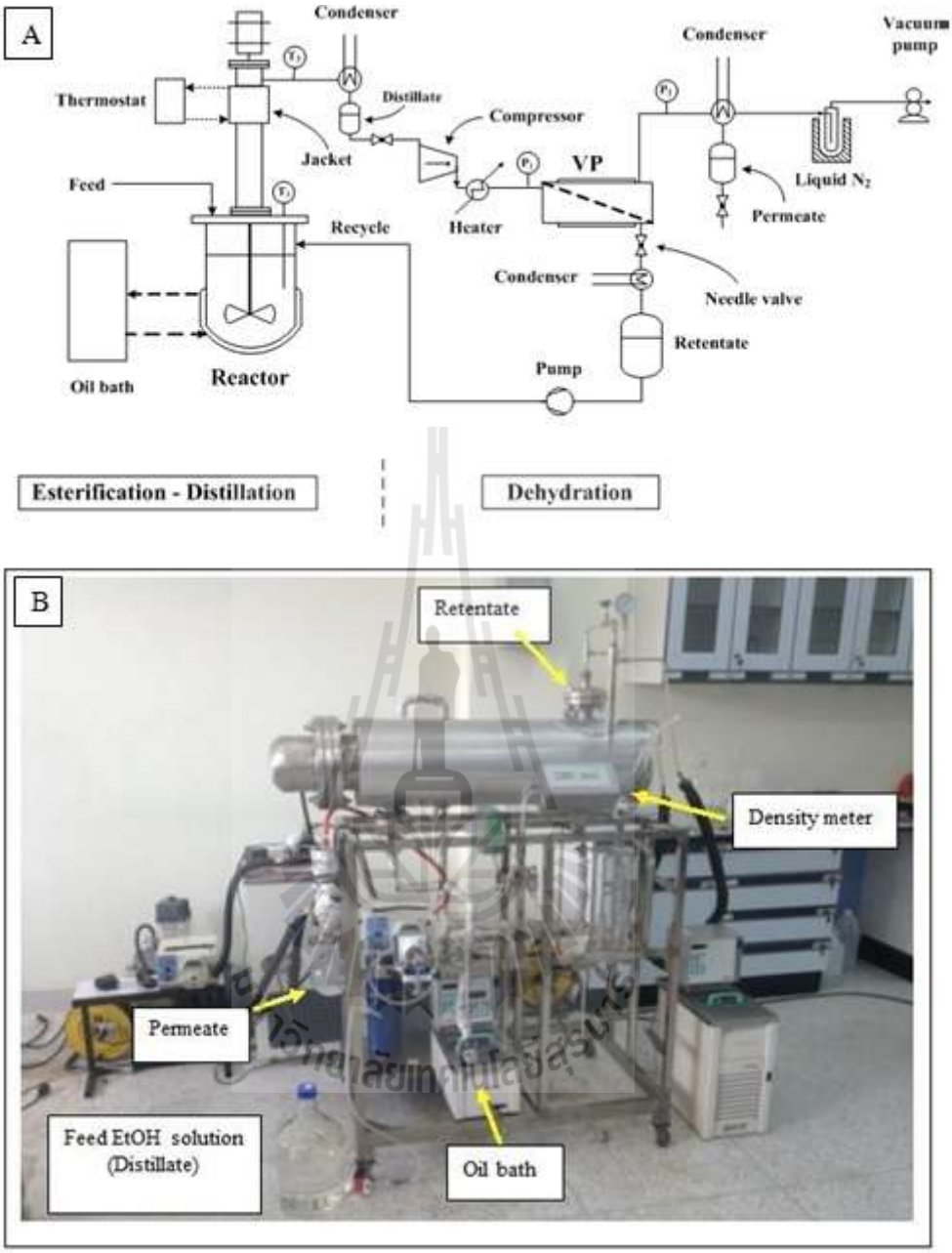

Figure 3.3 Schematic diagrams for experimental setup of the vapor permeation-assisted esterification (A) and picture showing the vapor permeation system (B).

The NaA zeolite membrane (Mitsui Engineering and Shipbuilding, Japan) with the membrane area of 2,350 cm^2 was installed in a jacketed stainless steel housing. A high pressure piston pump head (FMI, USA) mounted on a 1/10 hp pump drive (Masterflex, USA) was employed to increase the liquid feed pressure with the help of a needle valve. Prior to entering the membrane module, the pressurized liquid feed was heated to the desired inlet temperature through a shell and tube heat exchanger (Exergy LLC, USA) by using an oil bath (Julabo, Germany). On the downstream side, the permeate vapor is condensed by using two parallel glass cold traps filled with liquid nitrogen to ensure that the permeate was completely collected. The downstream pressure was maintained at approximately 3 mbar by using a vacuum pump (ChemStar®, Welch, USA).

For dehydration performance of the distillate ethanol, the total flux was gravimetrically determined at fixed time intervals by weighing the mass of the permeate collected. The values reported are the average of three experiments. The process temperature varied from 85 to 145 ° C, the feed pressure from 1.0 to 4.0 bars and the feed water concentration from 1 to 10 wt%, respectively. The distillate ethanol was dehydrated before returning to the reactor with the help of a peristaltic pump. This operation should increase the life of the membrane because zeolite membranes are highly unstable in acidic environments especially in direct contact with the acidic reactants. The esterification reaction terminated when water concentration in the reactor is lower than 0.02 wt%. In this study, two process parameters on esterification yield were investigated, including operating pressure and initial membrane area per reaction volume. The effect of operating temperature on esterification kinetic was not investigated because the reaction temperature is already at its boiling point.

3.4 Fractionation of ethanol and diethyl succinate mixture

At the end of the esterification reaction, most water was completely removed and succinic acid was completely converted to diethyl succinate. As described before, the ethanol/diethyl succinate binary mixture does not result in azeotrope formation. Therefore, the two components can be completely separated by conventional distillation. For a better fractionating result, two distillation steps was employed to separate ethanol and diethyl succinate from the mixture. In the first step, the majority of ethanol, ethyl acetate, ethyl formate, and ethyl lactate were recovered in the distillate. This can be achieved by keeping the solution temperature (T_1) at 120 °C and vapor temperature (T_2) at 79 °C. In the subsequence step, the majority of diethyl succinate was obtained in the distillate stream by reduce the vacuum pressure to 250 mBar.

The purified diethyl succinate was subsequently hydrolyzed with deionized water at the molar ratio of diethyl succinate to water at 1:10, and 3.0 wt% Amberlyst 15-E (Rohm & Hass) was used as the catalyst. Ethanol and water generated from the reaction was removed via the column where the vapor temperature (T_2) was controlled at 78 °C. The rising diethyl succinate vapor was condensed back into the hydrolysis reactor, thus maximizing the hydrolysis yield.

3.5 Analytical procedure

Cell concentration was determined by using spectrophotometer (UV–VIS Spectrometer) at wavelength 660 nm. The samples were centrifuged at 6000 rpm for 2 minutes. After removal of supernatant, phosphate buffer was added together with the same volume prior to measure the turbidity. The optical density was compared with the standard curve for Dry Cell Weight (DCW) concentration. The samples was

periodically taken from the bioreactor prior to centrifuge at 5000 rpm to separate bacterial cell from broth and the supernatant was investigated as follows; Reducing sugar was measured by Dinitrosalicylic (DNS) method according to Miller (Miller, 1959). The supernatant 0.5 mL was added with 0.5 ml of 3, 5-dinitrosalicylic acid reagent. The sample solution is boiled for 5 minutes. The absorbent measurement was carried out by using spectrophotometer at wavelength 520 nm and compare with standard curve of sugar concentration.

Ester of organic acids and ethanol concentrations were analyzed using a gas chromatograph (GC) equipped with a FID detector (SRI Instrument, USA). Helium 99.99% pure was used as carrier gas. The GC column (Carbowax®, Restek, USA) was a 30 m × 0.32 mm bonded phase fused silica capillary column. The injector and detectors were set at 250 and 300 °C. The oven was operated at programmed increasing temperature from 50 to 250 °C at the rate of 15 °C/min. The injection volume of liquid samples was 0.5 μL in splitless mode. The multiple point external standard was used for quantitative analysis. GC analysis of the samples from esterification reactions possessed an associated error of ± 3% (at 95% confident interval) based on a sample mean of 3 repeated injections. Water content of the esterification reaction was determined by using a Karl-Fischer automatic titrator (TitroLine alpha®, Schott, Germany). Quantitative analysis of organic acids, the supernatant was filtered by using 2 micron filter paper to completely remove bacterial cells. Organic acids were analyzed by HPLC (Thermo Scientific, USA), and quantification by UV detection was made at the wavelength of 210 nm. The mobile phase comprises of 1% acetonitrile + 99% 20 mM Na_2HPO_4 (pH 2) at a flow rate of 1 mL/min. The HPLC column was ZORBAX SB-Aq (4.6 mm × 150 mm). The column oven was maintained at 35 °C.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Fermentation of succinic acid by *Actinonacillus succinogenes* ATCC 55618

Figure 4.1 showed a time profile of cell growth, metabolites production, and the glucose consumption during a batch fermentation of *Actinonacillus succinogenes* ATCC 55618. Although succinic acid was the major product, the strain also produced acetic acid, formic acid and lactic acids as the by-products. Glucose concentration rapidly decreased for the first 10 h. Subsequently, the consumption rate gradually decreased and most glucose was consumed in 43 h. Forb acterial growth, a short lag phase was observed for the first 5 h followed by an exponential growth. The maximum cell concentration was obtained at 0.41 g/L. The highest succinic acid concentration was 47.2 g/L resulting in the conversion yield of 0.56 $g_{SA}/g_{glucose}$. This value is in a good agreement with literature using the same strain (Li *et al*., 2011). In addition, the final concentrations of formic acid, lactic acid and acetic acid were 12.3 g/L, 2.7 g/L and 10.5 g/L, respectively. For downstream processing of succinic acid, there are two major challenges: the first is the presence of bacterial cells and macromolecules in the aqueous broth and the second is the presence of acid by-products whose physic-chemical properties are similar to those of the succinic acid. Macromolecules and proteins cause severe NF membrane fouling, mainly as a result of adsorption on the surface as well as within the membrane pores. Development of filtration and cleaning

strategies are among the main research objectives in order to increase the lifetime, and maintain a high separation performance of the membranes.

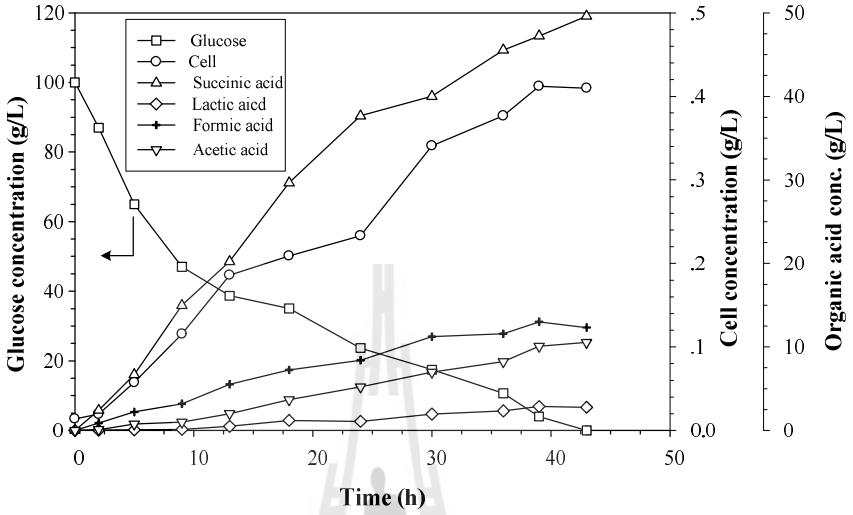

Figure 4.1 Time profile of metabolites production, cell growth, and the glucose consumption during the batch fermentation by *A. succinogenes* ATCC 55618.

4.2 Nanofiltration (NF) experiments

4.2.1 NF experiments using model solutions

The main objective of this work was to investigate the separation characteristic of organic acids using the NF. The influence of operating parameters on the separation performances using model solutions are presented in Figure 4.2. The effect of pH on rejection by the NF membrane was carried out for each organic acid solution. The experimental data on rejections are shown in Figure 4.2(A). Organic acids are dissociated according to the pH of the solution. It showed that the rejection of each organic acid is highly pH-dependent. Rejection of most studied organic acids increased significantly at pH above their dissociation constants (pKa) whilst the values significantly decreased at pH levels below their pKa (non-dissociated form).

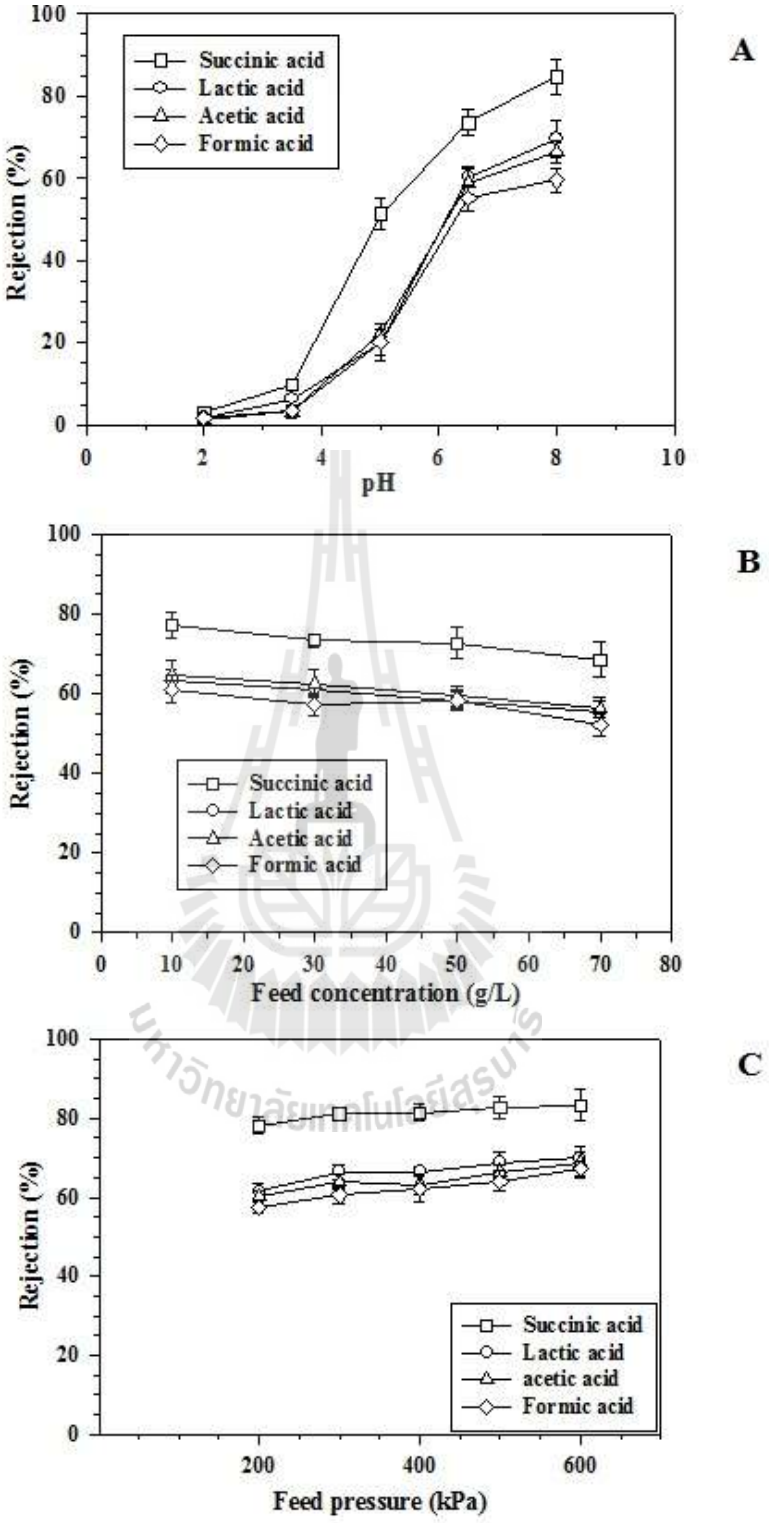

Figure 4.2 Influence of the operating parameters on the rejection of organic acidsolutions. The effect of pH (A); feed concentration (B); and operatingpressure (C). All experiments were investigated at 30.5 °C.

In addition, typical NF membranes show low rejection to monovalent ions, but exhibit high rejection to multivalent ions and larger molecules. However, the rejection of divalent ions also depends on the pH of the solution. The pKa values for formic acid, acetic acid, and lactic acid are 3.75, 4.76 and 3.08 whereas the values for succinic acid are 4.21 and 5.64, respectively. The rejections of succinic acid were obtained at 2.96, 9.76, 51.28, 73.41 and 84.66% when operated at pH 2.0, 3.5, 5.0, 6.5 and 8.0, respectively. Since the pKa values of the succinic acid are in the pH range of 4.2–5.6, an increase in the rejection observed at pH above 5.5 could be explained by the fact that the membrane has a surface charge which can significantly influence its retention characteristic. The surface charge density of a NF membrane is related to the zeta potential of the membrane surface. The zeta potential of the ceramic membranes were reported to be negative at higher pH ranging from pH 6-10 whist the value was positive at the pH below the isoelectric point (4.7) (Mullet *et al*., 1997). At lower pH the zeta potential was positive. These results indicate that the membrane used is negatively charged in the pH region in which the best results of retention are obtained. As a result, the observed increase in succinate retention can be explained by an increasing in electrostatic repulsion between dissociated form of the tested organic acid and the membrane surface. On the other hand, at pH below 4.0, the rejection of succinic acid reduced to lower than 10.0% indicating that the sieving effect played an important role on rejection. The molecular weight of succinic acid (118.09 g/mole) is relatively smaller than the MWCO 450 of the ceramic membrane. In addition, the rejection level was also influenced by the molecular weight of each organic acid. Experimental data shows that the rejection on organic acid tends to increase with increasing in the molecular size. At pH 3.5, the rejections of succinic

acid, lactic acid, acetic acid and formic acid were measured at 9.26, 6.22, 3.41and 3.27%, respectively. Formic acid possessed the lowest rejection by the membrane simply because of its small molecular size compared to the others.

The rejection experiements of each organic acid at different feed pressures and feed concentrations were performed to examine the influence of operating parameters on the rejections. Figure 4.2(B) illustrates the influence of feed concentrations on rejections by the tested membrane. The experiments were investigated at initial feed concentrations ranging from 10 to 70 g/L. Other operating conditions included feed temperature 30 °C, feed pressure 400 kPa and pH 6.5, respectively. In general, it was found that increasing in feed concentrations slightly decreased the rejections for all tested acids. The rejections for succinic acid were obtained at 77.3, 73.4, 72.8 and 68.5% for feed concentrations of 10, 30, 50 and 70 g/L, respectively. This resulted in an 11.3% reduction in rejection over the range tested in this work. For all other acids, slight decreases in rejections were observed for acetic acid, formic acid, and lactic acid at 13.8%, 14.2% and 12.9%, respectively. It was found that the effect of feed concentration did not significantly affect the retention of the NF membrane. Another important operating parameter on separation performance of NF is the feed pressure. Due to a limitation of the equipment used, the maximum feed pressure applied was 600 kPa. Figure 4.2(C) shows the influence of feed pressure on the rejection. For all tested organic acids, the feed pressure varied from 200 to 600 kPa whilst the feed concentrations, temperature and pH were maintained at 50 g/L, 30 °C and 6.5, respectively. For succinic acid, the rejections at feed pressure of 200, 300, 400, 500 and 600 kPa were obtained at 78.2, 81.1, 81.5, 82.6 and 83.3%, respectively. These experimental results suggested that the increasing

in feed pressure did not have a significant influence on succinic acid rejection. These high rejections of succinic acid (succinate) might attributed to the ion size which larger than the MWCO of the membrane due to the high degree of dissociation. The rejections of lactic acid, acetic acid and formic acid, which possess the molecular weight much small than the MWCO of the membrane, slightly increased with an increasing feed pressure. The magnitudes of rejections were obtained at 12.25%, 11.6% and 14.58% for lactic acid, acetic acid and formic acid, respectively. In conclusion, the most influential operating condition on retention performance of the NF membrane was pH of the solution. The effect of feed pressure and feed concentration seem to have a little effect to rejection characteristic. Moreover, separation of succinic acid from organic acid by-products was not effective because these acids have some similar physico-chemical properties. Firstly, they can dissociate with regard to the pH and secondly their molecular weights are not much different. In addition, the fermentation broth of succinic acid usually contains macromolecules particularly proteins and colouring molecules. The presence of proteins in fermentation broth can cause problems during purification processes especially formation of amino acids. Therefore, the advantage of NF in this work is the removal of macromolecules from the broth before subsequent purification processes. As a result, all organic acids can present in the permeate stream whilst proteins and macro molecules should remain in the retentate stream.

4.2.2 NF of fermentation broth

At the end of fermentation, the broth was filtered by using a microfiltration unit to remove bacterial cells. However, the clarified broth still contained several dissolved impurities such as proteins, polysaccharide, colouring molecules, etc. One

important criterion of the succinic acid NF process is the removal rates of proteins and other metabolites. In this case, the protein removal rate was of interest because proteins can be hydrolyzed into amino acids which generate technical problems for the purification process. If proteins are not sufficiently removed, the final product might result in yellowish color. In addition, other macromolecules with a similar size to proteins will also be removed if proteins are removed.

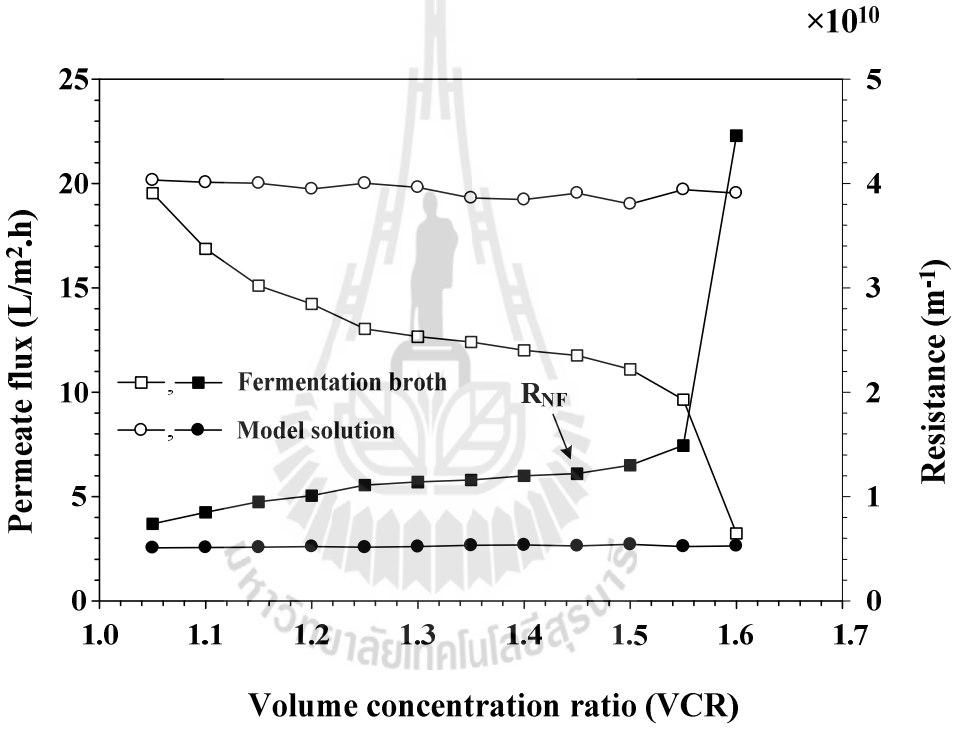

Figure 4.3 Changing in permeate flux and membrane resistance during the NF test for model solution and fermentation broth. Operating condition: feed pressure 400 kPa; pH 2.5; temperature 30.5 °C.

The separation performance of NF between model solution and the clarified fermentation broth were compared as shown in Figure 4.3. It shows the membrane flux and resistance as a function of volume concentration ratio (VCR). The value can be calculated from the initial feed volume (3.0 L) divided by the retentate

volume (Wang *et al*., 2014). NF is a pressure-driven process. Therefore, operating pressure is a very important factor affecting permeation flux. In general, the flux tends to increase with an increasing in feed pressure. Nevertheless, higher flux at the beginning induces the rapid deposition of macromolecules on the membrane surface. It is recommended that the operating pressure should be controlled so that it would not give a very high initial flux which leads to a poor overall performance of the membrane (Kang and Chang, 2005). In this experiment, the operating feed pressure 400 kPa, pH 2.5 and temperature 30.5 °C was used to characterize the flux and resistance calculation. The initial permeation rate of model solution was obtained at 20.17 $L/m^2.h$ and this steady permeability of the NF membrane varied only a small range. At the end of the experiment, the permeation rate was obtained at 19.56 $L/m^2.h$. In addition, the calculated resistances were in the range between 0.51-0.54×10^{10} m^{-1} for all 1,200 mL of collected sample. These constant membrane flux and resistance were clearly due to the absence of macromolecules deposited on the membrane surface. In contrary to the model solution, the permeation rate of realistic fermentation broth sharply dropped in the first few minutes from 19.54 to 16.87 $L/m^2.h$ followed by a continuous flux decline for the period of 1000 mL of the permeate (VCR = 1.5). The calculated resistance increased to 1.22×10^{10} m^{-1} and the value was considered as R_{NF}. Subsequently, the permeation rate sharply decreased in the final period of NF when the value reached 3.23 $L/m^2.h$ corresponding to 83.5% decrease in comparison to the initial flux. After this experiment, the membrane was heavily fouled due to deposition of protein and macromolecules on the membrane surface. In order to evaluate the fouling characteristic of the NF membrane, a direct assessment of cleaning process was carried out by water flux measurements during

two cleaning procedures; washing with distill water at 40 °C until a colorless solution was obtained (R_1), washing with 2% NaOH at 50 °C for 10 min followed by rinsing with distilled water at 40 °C for 20 min and washing with 2% phosphoric acid at 50 °C for 10 min followed by rinsing with distilled water until pH neutralization (R_2). The value of the membrane hydraulic resistance (R_m) was calculated based on the flux of deionized water using the new membrane. From Eq. (9), the initial permeation rate of deionized water was measured at 20.52 $L/m^2.h$ corresponding to the R_m of 0.70×10^{10} m^{-1}. After washing with water, the value increased to 18.60 $L/m^2.h$ and the resistance (R_1) was calculated at 0.77×10^{10} m^{-1}. As a result, the resistance due to cake formation (Rc) is the difference between R_{NF} and R_1 which is 0.45×10^{10} m^{-1}. On the other hand, the permeation rate after washing with alkaline and acid solutions increased to 20.21 $L/m^2.h$ and the resistance (R_2) was calculated at 0.71×10^{10} m^{-1}. Therefore, the resistance due to pore blocking and adsorption (R_f) is the difference between R_1 and R_2 which is 0.06×10^{10} m^{-1}. In conclusion, the fouling characterization of the NF membrane was evaluated by using a cleaning procedure. As shown in Figure 4.4 (A), the resistances R_m, R_c and R_f correspond to 58.10%, 36.84% and 5.06% of the total resistance, respectively. Figure 4.4 (B) shows the histograms of the SA, lactic acid (LA), acetic acid (ACE), formic acid (FA) and protein concentrations in feed and permeate solutions. Experimental results showed that the majority of the acids were presented in the permeate stream. Protein concentrations in the feed and permeate were 2.39 and 0.48 $g.L^{-1}$ resulting in 79.92% of the removal rate.

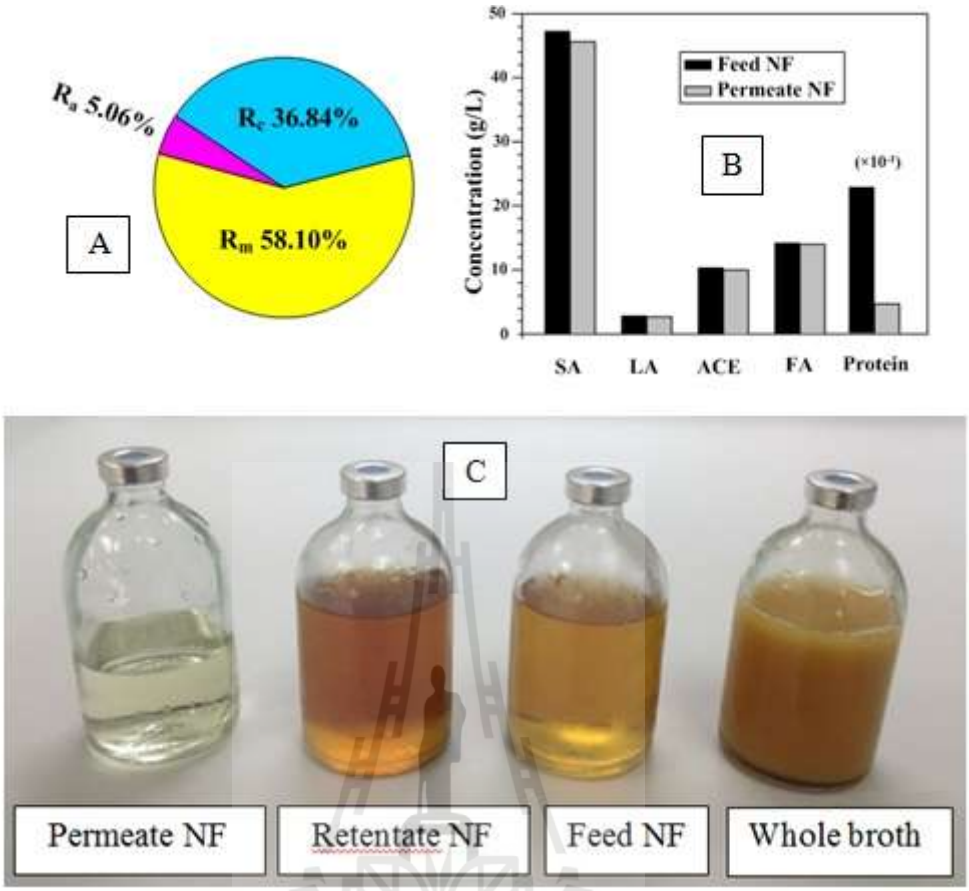

Figure 4.4 Resistance analysis of the NF using a cleaning procedure (A). Histograms showing the compositions of the feed and the permeate NF (B). Picture showing evidence of the decolouration induced by the NF process (C).

In addition, the most important advantage of NF process was the low rejection to the targeted succinic acid but high rejection to large bio-molecules resulting in the decolouration effect as shown in Figure 4.4 (C). This step facilitated further purification step since the majority of protein was removed. Moreover, the SEM images of fouled and cleaned membranes were presented in Figure 4.5. The fouled membrane was covered by a thick cake layer (Figure 4.5 (A) and Figure 4.5 (B)) whereas Figure 4.5 (C) and Figure 4.5 (D) indicated that most of foulants were successfully removed by the cleaning procedure. From this experiment, it can be

concluded that NF operated in the conventional cross-flow mode resulted in an increased loading of macromolecules and protein in the feed side. The lower flux associated with a higher VCR resulting in difficulties in operation especially a rapid flux decline, thus shortening the operating time (Wang *et al*., 2014).

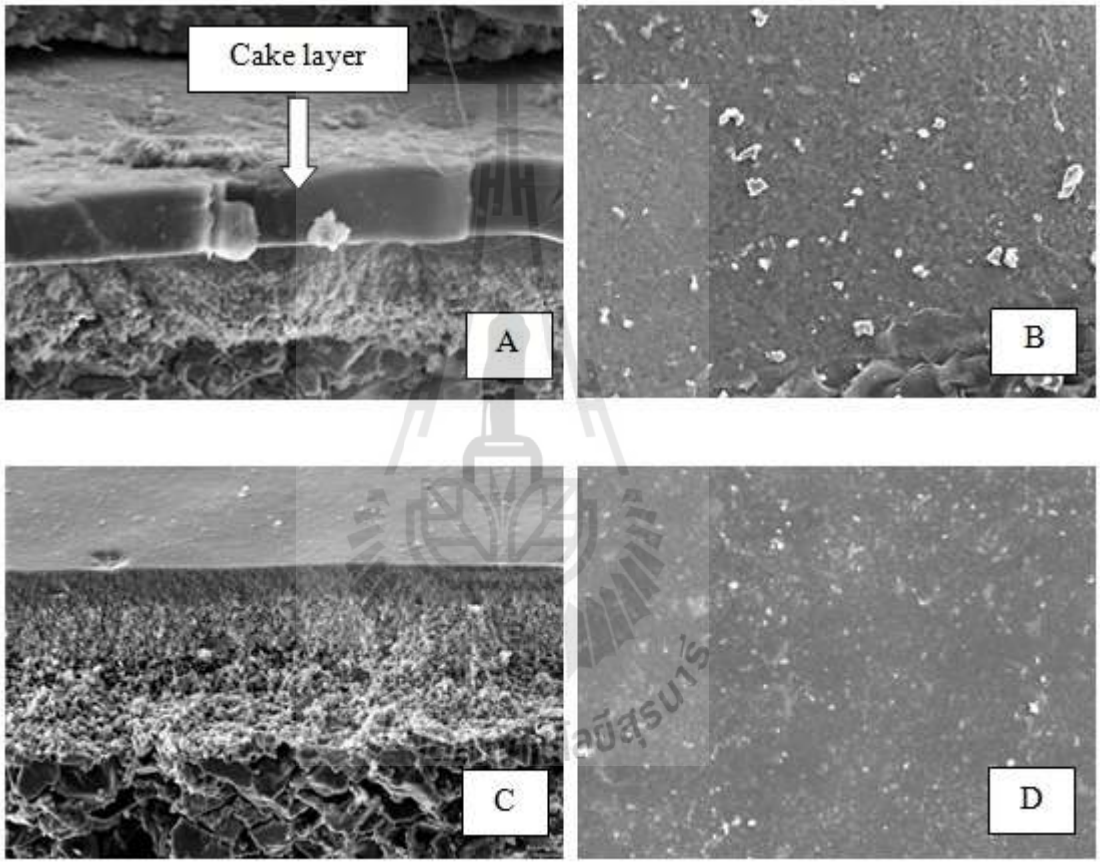

Figure 4.5 SEM images of the cross section and top surface morphologies of the ceramic NF membrane; (A) cross section of the fouled membrane showing deposition of cake layer on the membrane surface, (B) top surface of the fouled membrane, (C) cross section of the cleaned membrane and (D) top surface of the cleaned membrane.

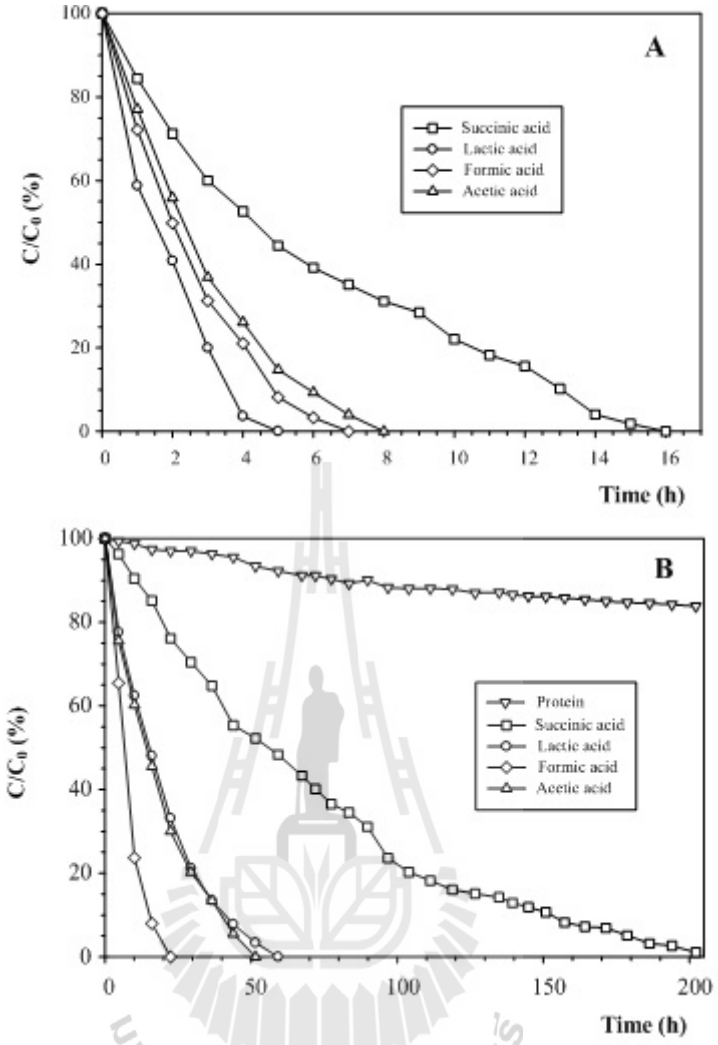

Figure 4.6 Changing in relative solutes concentration during diafiltration of model solution (A) and fermentation broth (B). Operating condition: feed pressure 400 kPa; pH 2.5; and temperature 30.5 °C.

In order to increase the separation process, NF of the fermentation broth was re-investigated in a continuous diafiltration mode. Volume of the broth was kept constant with an addition of deionized water (pH 2.5) and sampling of the feed solution was carried out periodically. Figure 4.6 shows the solutes concentration ratios of continuous diafiltration as a function of time. Experimental results for

diafiltration were compared between the model solution (Figure 4.6 (A)) and the clarified fermentation broth (Figure 4.6 (B)). In Figure 4.6 (A), the initial concentrations of succinic acid, lactic acid, acetic acid and formic acid were 50, 2.5, 10.5 and 12.5 g/L. Experimental results showed that the operation was continued for 16 h at the feed pressure of 400 kPa and 30.5 °C. The permeate flux of the solution was constant at 19.68 $L/m^2.h$. For succinic acid, the concentration decreased slower than those of the acid by-products. Formic acid showed the highest rate of reduction in concentration because it is the smallest molecule. Lactic acid concentration depleted in 4 h due to its low initial concentration of only 2.5 g/L. In contrary to the model solution, the average flux of the clarified fermentation broth was approximately 12.4 times lower than model solution. This low flux resulted in a much longer diafiltration time due to the high complexity of the fermentation broth. Depletion of formic acid, acetic acid and lactic acid was observed after 24, 50 and 65 h, respectively. For succinic acid, the time to deplete its concentration in fermentation broth was 205 h. It was indicated that there were several parameters especially fouling, that may cause a decrease in permeate flux. Improvement of the diafiltration process can be achieved such as increasing of the operating pressure and increasing the membrane area per unit volume of the fermentation broth. Although these parameters were not investigated in this work, the separation characteristic of succinic acid from fermentation broth was satisfactory with approximately 98% recovery yield of succinic acid and up to 80% protein removal. In conclusion, the characterization of fermentation broths after different purification stages is given in Table 4.1.

Table 4.1 Composition of fermentation broths after different filtration processes.

Components	Whole broth	MF permeate	NF permeate
Biomass (g/L)	0.41	0.00	0.00
Proteins (g/L)	2.46	2.39	0.48
Succinic acid (g/L)	47.20	47.20	45.80
Lactic acid (g/L)	2.70	2.70	2.68
Acetic acid (g/L)	10.51	10.50	10.44
Formic acid (g/L)	12.32	12.32	12.29
Na^+ (mg/L)	347.81	346.87	344.23
Mg^{2+} (mg/L)	2,388.54	2,327.54	40.24
Cl^- (mg/L)	924.66	925.53	928.43
PO_4^{3-} (mg/L)	285.98	286.42	2.06

4.3 Esterification of SA and ethanol using model solution

4.3.1 Effect of reactant molar ratio

The objective of this work was to find the optimal concentrations of succinic acid, ethanol and water for the esterification reaction. Initial mass ratios of ethanol to succinic acid varied from 1.60 to 3.44 corresponding to 4.09 to 8.82 in molar ratio basis. Since the solubility of succinic acid is relatively low in ethanol solution (Jiang *et al*., 2013), it is necessary to increase water concentration in order to completely dissolve the solid succinic acid prior to start the reaction. Nevertheless, the low solubility of succinic acid in ethanol is beneficial to the purification process proposed in this work, since ethanol solution containing some water can be used to dissolve the solid succinic acid instead of using the expensive anhydrous ethanol.

However, the initial water concentration in the solution plays an important role in the conversion of succinic acid to the diethyl succinate product. A low initial water concentration results in an incomplete dissolution of the solid succinic acid whilst high initial water concentration leads to a low conversion yield and productivity.

Table 4.2 The amount of reactants at the beginning and equilibrium during esterification reactions of succinic acid and ethanol. All experiments were conducted at 70 °C, pH 2.5. The product yield ($Y_{DES/SA}$) refers to the amount of diethyl succinate at equilibrium divided by the amount of succinic acid at the beginning (in molar basis).

No.	At the beginning (g)			At equilibrium (g)					$Y_{DES/SA}$ mole (%)
	SA	EtOH	H_2O	SA	DES	MES	EtOH	H_2O	
1	40.0	63.75	11.25	3.93	44.88	7.06	37.83	21.39	76.02
2	40.0	63.75	41.25	27.34	16.99	1.47	54.31	44.94	28.77
3	40.0	74.57	11.25	3.47	46.45	6.99	57.66	16.91	78.67
4	40.0	90.22	11.25	3.27	46.89	6.88	73.18	16.94	79.41
5	40.0	113.4	11.25	2.20	48.56	6.82	95.82	17.11	82.24
6	40.0	137.65	11.25	0.87	50.52	6.86	119.40	17.32	85.56

SA = succinic acid, EtOH = ethanol, DES = diethyl succinate, MES = monoethyl succinate

Table 4.2 shows the experimental results of products and reactant quantities presented during the esterification reaction between succinic acid, ethanol, and water. The first two substances were mixed at 70 °C followed by an addition of water until the solid succinic acid was fully dissolved (a clear solution was observed).

In experiment No. 1 and 2, the effect of initial water concentration was investigated at the weight fraction of 0.10 to 0.28 corresponding to 0.27 to 0.57 in molar ratio. In experiment No. 2, the excessive amount of water resulted in the molar recovery yield of diethyl succinate only 28.77%.

The time course of succinate species, water and ethanol concentrations of this experiment is shown in Figure 4.7. The initial productivity of diethyl succinate was obtained at 1.39 g/L.h. Subsequently, the value gradually decreased, and reach plateau after 250 min of operation. The maximum diethyl succinate concentration was obtained at 117.2 g/L whilst the remaining concentration of succinic acid was 188.52 g/L corresponding to 68.34% of the initial value. Formation of diethyl succinate and monoethyl succinate in the system resulting in an increasing in water concentration from 284.5 to 309.9 g/L. This low conversion of succinic acid was clearly the result of thermodynamic limitation caused by an increasing in water concentration. In contrary to experiment No. 2, the equilibrium conversion of succinic acid to diethyl succinate increased from 28.77 to 76.02% when the mole fraction of water decreased from 0.57 to 0.26 as observed in experiment No. 1. Only 9.83% of succinic acid remained unreacted in the system. From these two experiments, it can be concluded that the initial water concentration should be kept low but not lower than the solubility of the solid succinic acid. In experiments No. 3-6, the influence of the different reactant ratios of ethanol and succinic acid were examined. It was found that an increasing in ethanol:succinic acid ratio lead to a higher diethyl succinate yield. The highest diethyl succinate yield was obtained at 85.56 mole% when the ethanol: succinic acid ratio was 8.82. By using a large excess of ethanol, the reaction yield of the diethyl succinate increased. However, this approach affects the investment cost since a larger

size of reactor is required affecting the investment cost. In addition, recovery of the diluted diethyl succinate product will become more technically and economically difficult.

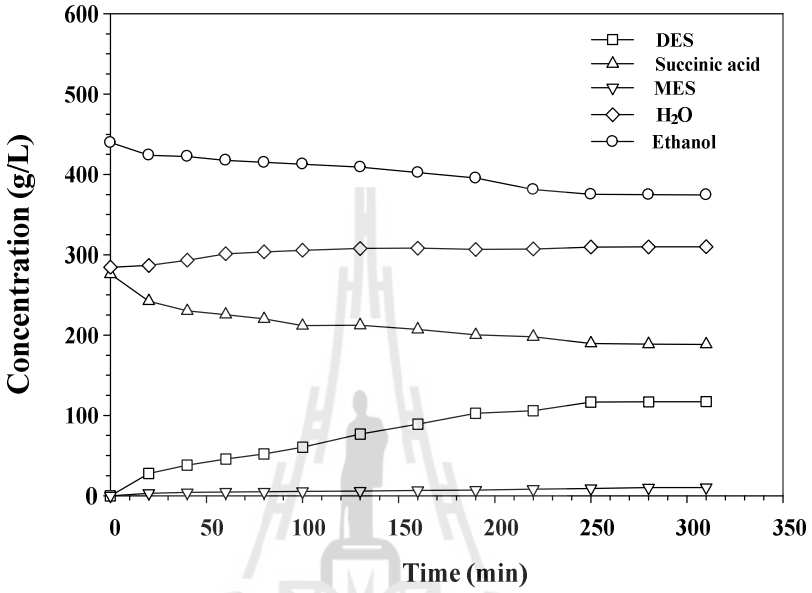

Figure 4.7 Concentration profiles of succinic acid (—△—), diethyl succinate (—□—), monoethyl succinate (—▽—), ethanol (—○—), and H_2O (—◇—) during esterification reaction of SA and ethanol (experiment No. 2). Initial weight of succinic acid:ethanol:water = 40:63.75:41.75, temperature = 70 °C, pH = 2.5.

4.3.2 Effect of temperature

Figure 4.8 shows the time course of product and reactant concentrations during the esterification reaction of succinic acid and ethanol. The experiments were performed in a temperature range between 65 and 95ºC. The initial concentration ratio of succinic acid:ethanol:H_2O for all experiments was 3.5:5.5:1.0. In general, it can be seen that the reaction rate increases with an increasing reaction temperature.

Volumetric productivity of diethyl succinate at 65 °C was obtained at 2.59 g/L.h for the first 80 min, and the value gradually decreased until reached plateau after 300 min of operation. The highest diethyl succinate concentration was obtained at 390 g/L. In addition, the high volumetric productivity of diethyl succinate at both 80 and 95 °C were measured at approximately 11.13 g/L.h. The equilibrium time for the first run was reached after 90 min whilst it took 60 min to reach equilibrium for the latter. Although the effect of operating temperature plays an important role on volumetric productivity of the diethyl succinate product, the equilibrium conversion was nearly equal in the range of temperatures considered in this work. At equilibrium, the conversion yields of succinic acid at 65, 80 and 95 °C were obtained at 90.18, 90.32 and 90.81%, respectively. This thermodynamic limitation on conversion of succinic acid to diethyl succinate was the typical characteristic of the esterification reaction. In order to increase the productivity and shift the reaction towards product formation, it is necessary to operate the reaction at a high temperature (i.e. at its boiling point) as well as remove water from the system.

4.4 VP-assisted esterification of succinic acid and ethanol

4.4.1 Dehydration performance of the ceramic membrane

In order to avoid the direct contact of the ceramic membrane with the acidic reactants, the esterification reaction was operated at its boiling point and the distillate ethanol was dehydrated using vapor permeation (VP) prior to re-circulate back into the reactor. The detailed experimental results for dehydration performance of the NaA zeolite membrane at different operating conditions were previously reported

(Khunnonkwao *et al*., 2012). In this work, the dehydration performance of ethanol solution by the VP membrane is shown in Table 4.3.

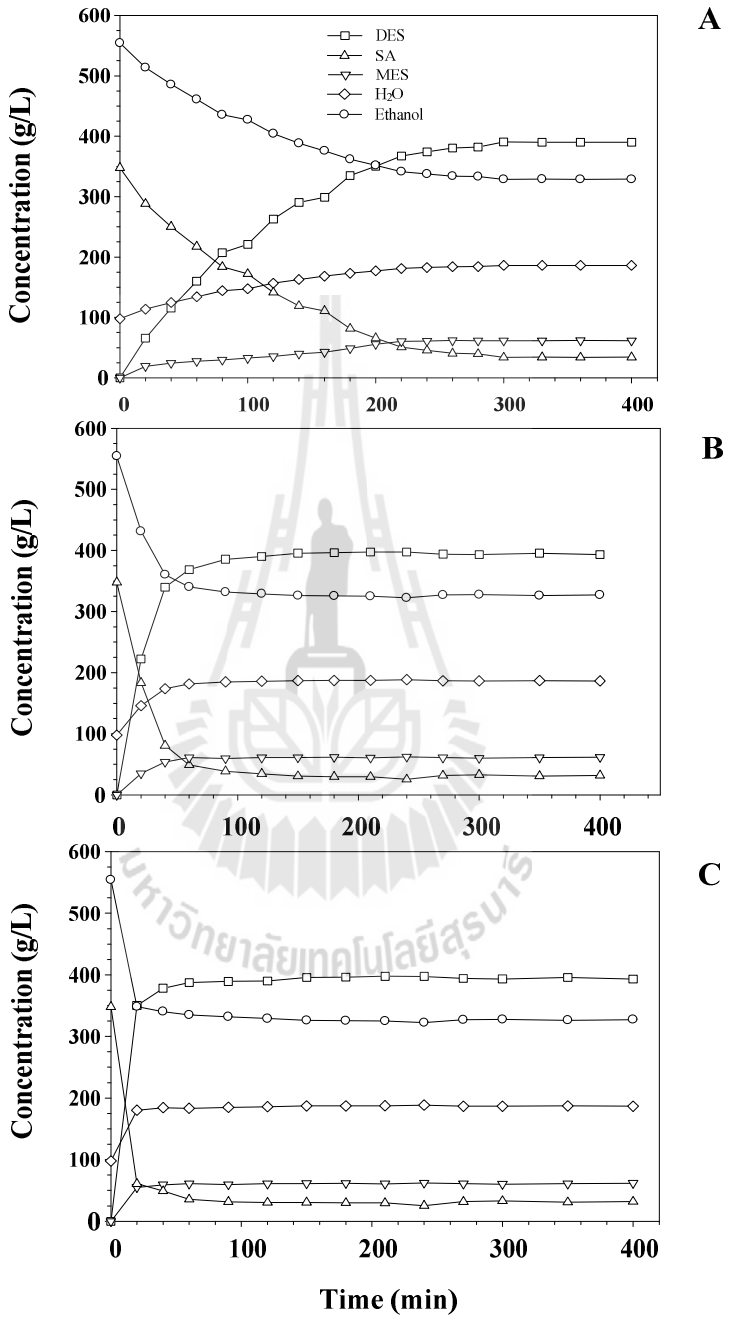

Figure 4.8 Experimental concentration profiles for esterification between SA and ethanol at operating temperatures of 65 °C (A), 80 °C (B) and 95 °C (C). All experiments were carried out at initial concentration ratio of succinic acid: ethanol: H_2O = 3.5:5.5:1.0, pH = 2.5, agitation rate = 500 rpm.

Table 4.3 Vapor permeation of ethanol solutions at different operating conditions using NaA membrane. For all experiments, the feed flow rate was 0.70 kg/h.

No.	Temp. (°C)	Pressure (kPa)	Water conc. in feed (wt%)	Water conc. in permeate (wt%)	Total flux ($kg/m^2.h$)	α
1	100	100	5.0	99.64	2.04	2735
2	115	100	5.0	99.41	2.17	2356
3	130	100	5.0	99.13	2.44	2165
4	145	100	5.0	98.94	2.85	1773
5	145	100	5.0	98.94	2.85	1773
6	145	200	5.0	98.91	3.98	1724
7	145	300	5.0	98.71	4.82	1454
8	145	400	5.0	98.05	5.11	955
9	145	400	2.5	96.64	4.21	1122
10	145	400	5.0	98.05	5.10	955
11	145	400	7.5	98.73	6.95	959
12	145	400	10.0	99.02	11.13	909

Two important operating conditions, feed temperature and feed pressure were investigated for the permeation flux and separation factor. The influence of feed temperature (No. 1-4) was examined at initial water concentration of 5 wt% and feed pressure of 100 kPa. Experimental results show that the total flux increases with an increasing temperature. The higher dehydration performance can be attributed to the elevated temperature resulting in an increased driving force. The permeate fluxes exponentially increased to a different extent with an increasing feed temperature. Based on the flux at 100 °C, the value increased 6.3%, 19.61% and 39.71% when the temperature increased to 115, 130 and 145 °C, respectively. The ethanol flux

remained close to zero for all investigated temperatures. On the other hand, the separation factor is inversely proportional to the permeation flux. The value decreased from 2735 to 1773 when the temperature increased from 100 to 145 °C. However, it was found that high water concentrations of more than 98.9 wt% were obtained in the permeate side resulting in high separation factors up to 2735. This high separation factor was attributed to the molecular sieve effect of the zeolite NaA in the selective layer (Sato *et al*., 2008). Experiments No. 5-8 show the relationship between total permeation flux and feed pressure at the constant feed temperature of 145 °C. Since VP is also a pressure driven membrane process, it is shown that the total flux increased with increasing feed pressure. It was observed that a linear relationship between water flux and feed pressure occurred for feed pressures of up to 300 kPa before the linear relation changed to a sub-linear relation at higher feed pressures of 300-400 kPa. With these operating pressures, the permeation fluxes were obtained at 2.85, 3.98, 4.82 and 5.11 $kg/m^2.h$, respectively. These results indicated that the permeation rates of water were not always constant at all the investigated pressures. In addition, the initial feed water concentrations were varied between 2.5-10 wt% in order to obtain a high total flux and high separation factor (No. 9-12). The feed temperature and operating pressure were kept constant at 145 °C and 400 kPa, respectively. Experimental data showed that the permeate flux increased with an increasing feed water concentration. The higher water flux was clearly attributed to the higher water partial pressure resulting in an increased driving force. The highest total permeation flux obtained at 10 wt% feed water concentration was 11.13 $kg/m^2.h$, which was more than 2.5 times higher than the value at 2.5 wt% feed water concentration.

4.4.2. VP-assisted esterification of the NF-treated fermentation broth

In this study, the combination of esterification reaction with dehydration using vapor permeation technique was studied in order to increase the yield and productivity of diethyl succinate. A previous work confirmed that ester yield strongly depended on the dehydration rate (Khunnonkwao *et al.,* 2012). This operation could be achieved by increase the membrane area per initial volume (A/V_o) of the reaction. In addition, it is also one of the most important variables in the design of the membrane area required for a given dehydration task. The time course for succinic acid, ethanol, diethyl succinate, monoethyl succinate and water concentrations during the esterification reaction of NF-treated fermentation broth and ethanol is presented in Figure 4.9. The operating conditions were maintained as follows; feed temperature of VP system 145 °C, feed pressure 400 kPa, pH 2.5 and initial molar ratio of succinic acid:ethanol:H_2O 3:12:5.5, respectively. As a result, a high value of A/V_o at 157 m^{-1} was achieved.

It was expected that the higher A/V_o ratio resulted in a higher dehydration rate, and a higher ester yield. At the beginning, the esterification reaction was allowed to reach equilibrium for 2 h. Subsequently, temperature of the reaction was increased to its boiling point. With the help of the high efficiency distillation column, the fractionated ethanol solution was composed predominantly of ethanol and water with only a trace amount of diethyl succinate and monoethyl succinate (0.09 g/L and 0.03 g/L). Before VP, the profile of water concentration in the liquid phase followed the typical characteristic of esterification reaction. The value initially increased and subsequently decreases when the VP was introduced. 4 hours after the VP started, the water concentration significantly reduced from 188.32 g/L to approximately

35.54 g/L. After this point, the value gradually reduced until the concentration reached 1.98 g/L within 9 h of operation. The low dehydration rate for the last 5 h of esterification reaction was attributed to the low driving force caused by a low water concentration in the distillated ethanol.

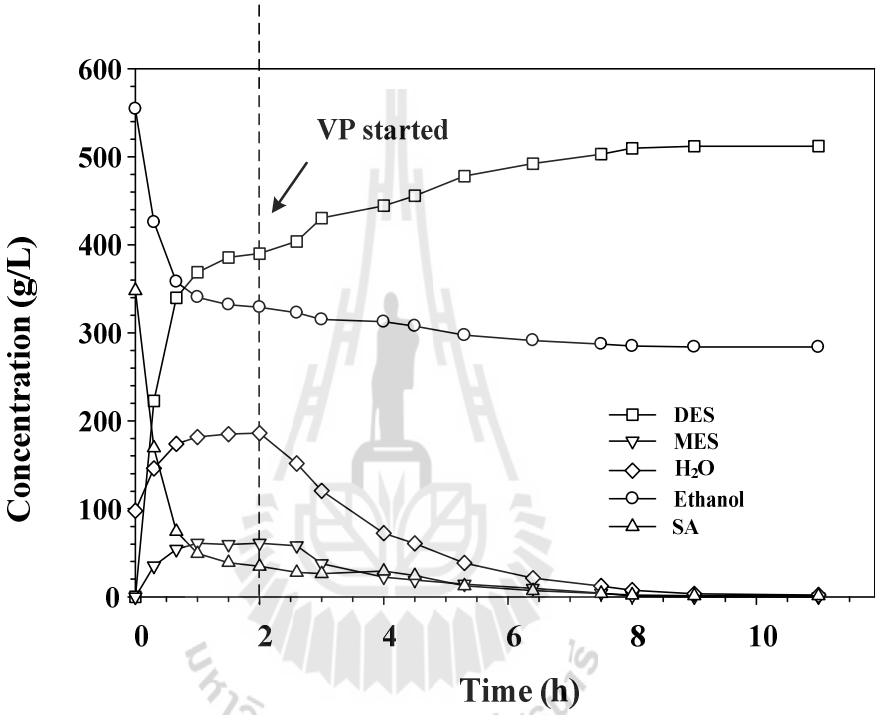

Figure 4.9 Concentration profiles of the reaction components for VP-assisted esterification by using NF-treated fermentation broth. The experiment was carried out at the following conditions: feed temperature of VP system 145 °C, A/V_o ratio 470 m^{-1}, initial molar ratio of succinic acid:ethanol:water = 3:12:5.5, pH = 2.5, and feed pressure of VP = 400 kPa.

4.4.3 Fractionation and hydrolysis

At the end of the esterification reaction, most water was removed whilst all succinic acid and monoethyl succinate were converted to diethyl succinate. Because the organic acid by-products were also esterified with ethanol, the system presented ethyl formate, ethyl lactate, and ethyl acetate at the concentration of 48.8 g/L, 28.2 g/L and 55.3 g/L, respectively. Since the boiling points of ethyl formate, ethyl acetate, ethyl lactate, and diethyl succinate are 54.0, 77.1, 151.0 and 218.0 °C, a simple distillation can be used to completely separate diethyl succinate from ethanol and other esters (Benedict *et al*., 2006). In this work, a 45 cm-long Vigreux column was equipped with a small condenser placed on top of the column in order to precisely control the temperature of the vapor leaving the column. This operation can effectively control the purity of the leaving vapor as higher boiling point compounds will be condensed back into the reactor. In this work, two distillation steps were employed and the operating conditions are shown in Table 4.4. In the first step, most of ethanol, ethyl formate, ethyl acetate and ethyl lactate were removed as the distillate by controlling the liquid temperature of 120 °C and vacuum pressure of 250 mBar. Most diethyl succinate was recovered in the final step by lowering the vacuum pressure to 20 mBar at 150 °C. Finally, the fractionated diethyl succinate was subjected to hydrolysis with deionized water using 3 wt% Amberlyst 15-E as a catalyst. The operating temperature was maintained at 110 °C and the initial molar ratio of water to DES was 15:1. During the hydrolysis reaction, diethyl succinate reacted with water to produce 1 mole of succinic acid and 2 moles of ethanol. Two purification steps were employed. In the first step, ethanol generated from the reaction was removed by distillation with the help of a Vigreux column. In the last step, the

excessive water was removed by vacuum evaporation to produce concentrated succinic acid. Because hydrolysis is a simple process, variation of the operating parameters is not necessary. Finally, a high purity succinic acid was obtained after hydrolysis of the purified diethyl succinate with deionized water as shown in Figure 4.10.

Table 4.4 Process parameters for fractionation and hydrolysis of DES. T_1 is the liquid temperature and T_2 is the vapor temperature, respectively.

Operation	**Temperature (°C)**		**Vacuum pressure (mBar)**	**Distillate**
	T1	**T2**		
Fractionation				
- 1st step	120	79	250	- 74 % EtOH, 10% Ethyl formate, 6% Ethyl acetate, 2% Ethyl lactate, 8% DES
- 2nd step	150	125	20	- 100% DES
Hydrolysis				
- 1st step	110	78.2	Atmospheric	- 94.2 % EtOH
- 2nd step	75	57.1	250	- ~100 % H_2O

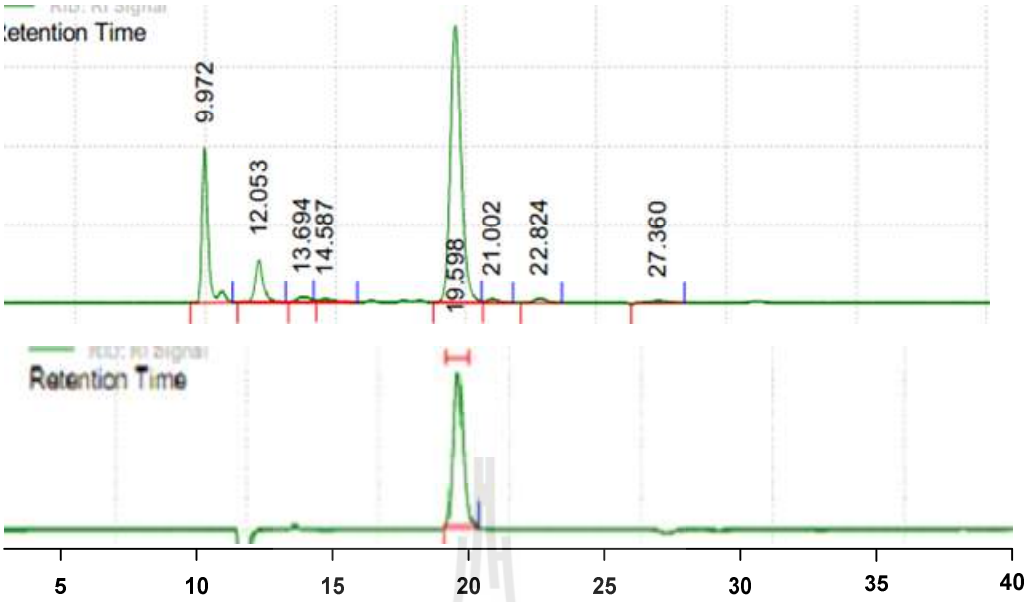

Figure 4.10 Chromatograms of the fermentation broth (top) and purified SA after hydrolysis (bottom). The retention times for formic acid, lactic acid, acetic acid, and succinic aicd are 9.97, 12.05, 13.69, and 19.60 min, respectively. Hydrolysis conditions: 3 wt% Amberlyst 15-E (Rhom & Haas), 15:1 molar ratio of water to DES at 110 °C.

CHAPTER V

CONCLUSIONS

Two membrane-based processes were successfully employed for the separation and purification of fermentation-derived succinic acid. Nanofiltration has a high potential as one of the purification step for the recovery of organic acids from the fermentation broth. Among operating conditions, the pH of the solution played a major role on the retention of organic acid salts. The removal of proteins and colouring molecules is the major usefulness of the system. The deposition of these molecules on the membrane surface was subjected to fouling. Membrane remediation was achieved after a series of cleaning process. Diafiltration of nanofiltration was successfully introduced for a complete removal of organic acids from the fermentation broth. Separation of organic acids was achieved based on esterification and vacuum fractionation methods. Dehydration of ethanol solutions was evaluated as a function of operating conditions by using a zeolite membrane. The results for VP-assisted esterification revealed an enhanced yield of diethyl succinate. A high A/V_o ratio of 470 m^{-1} increased the product yield and the reaction completed in 9 h after VP started. After fractionation and hydrolysis, a high purity SA was obtained.

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BIOGRAPHY

Miss Sumalee Srisuno was born on August 13, 1988 in Roi-Et province. She obtained her Bachelor of Science degree in Environmental Science from Environmental Science Department, Faculty of Science, Khonkaen University in 2010. After that, she decided to further study master degree in the field of biotechnology. During study, she received financial support from the Suranaree University of Technology. After she finished coursework, she worked in the project title of "Purification of Succinic Acid from Fermentation Broth using Vapor Permeation- Esterification", in Bioplastic Production from Biomass Research Unit, School of Biotechnology, Institute of Agricultural Technology, Suranaree University of Technology, Thailand. During this time she had practiced skills in the field of fermentation technology, distillation, and adsorption technique, and she had an experience oral presentation in the title of "Purification of Succinic acid from Synthetic Solutions using Esterification coupled with Reactive Distillation Technique" at the 1st SUT International colloquium, 2013.

Publications Nanofiltration coupled with vapor permeation-assisted esterification as an effective purification step for fermentation-derived succinic acid.