PURIFICATION OF L-(+)-LACTIC ACID FROM FERMENTATION BROTH BY PERVAPORATION-ASSISTED ESTERIFICATION TECHNIQUE

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A Thesis Submitted in Partial Fulfillment of the Requirements for the

Degree of Master of Science in Biotechnology

Suranaree University of Technology

Academic Year 2010

การทำบริสุทธิ์กรด L- แลคติกจากน้ำหมักด้วยเทคนิคผสม ระหว่างระบบเพอร์วาโพเรชั่นและ เอสเทอร์ริฟิเคชั่น

นางสาวพรรวณา ขุนโนนเขวา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต

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ปีการศึกษา 2553

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พรรวณา ขุนโนนเขวา : การทำบริสุทธิ์กรด L-
แลคติกจากน้ำหมักด้วยเทคนิคผสมระหว่างระบบเพอร์วาโพเรชั่นและเอสเทอร์ริฟิเคชั่น
(PURIFICATION OF L-(+)-LACTIC
ACID FROM FERMENTATION BROTH USING PERVAPORATION-ASSISTED
ESTERIFICATION TECHNIQUE)
อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.อภิชาติ บุญทาวัน, 119 หน้า.

ปฏิกิริยาเอสเทอร์ริฟิเคชั่นของกรดแล็คติกและเอทานอลได้ถูกใช้ในการสังเคราะห์สารเอทิลแล็คเทตสำหรับใช้ในการทำบริสุทธิ์กรดแล็คติกจากน้ำหมักซึ่งใช้แป้งมันสำปะหลังเป็นแหล่งคาร์บอนเสริมด้วยสารสกัดจากยีสต์เป็นแหล่งไนโตรเจน
น้ำหมักได้ถูกผ่านกระบวนทำให้เข้มข้นโดยใช้เทคนิคอิเล็คโทรไดอะไลซิสและทำการระเหยในเบื้องต้นจนมีความเข้มข้นของปริมาณน้ำอยู่ที่ร้อยละ 25 ก่อนที่จะเข้าสู่ปฏิกิริยา
โดยเมื่อทำการเปรียบเทียบกับการทดลองควบคุมแล้วพบว่าเทคนิคผสมระหว่างระบบเพอร์วาโพเรชั่นและเอสเทอร์ริฟิเคชั่น
จะส่งผลให้ผลผลิตและอัตราการเปลี่ยนแปลงจากสารตั้งต้นไปเป็นผลิตภัณฑ์เพิ่มขึ้น
โดยเยื่อแผ่นที่ใช้ในการทดลองนี้เป็นเยื่อแผ่นที่ไม่ชอบน้ำ ผลิตขึ้นจากพอลิเมอร์ชนิดพอลิไดเมทิลไซล็อกเซน หรือ PDMS ในการทดลองนี้ได้มีการศึกษาถึงปัจจัยต่าง ๆ เช่น อุณหภูมิ ความเข้มข้นของน้ำ
และความเข้มข้นของตัวเร่งปฏิกิริยาที่มีผลต่อค่าฟลักซ์และสัมประสิทธิ์การแยก โดยจากการทดลองพบว่าอุณหภูมิของสารป้อนมีผลต่อค่าฟลักซ์และสัมประสิทธิ์การแยกมากที่สุด
ซึ่งเมื่ออุณหภูมิสูงขึ้น ค่าฟลักซ์จะเพิ่มขึ้นตามไปด้วย แต่สัมประสิทธิ์การแยกจะลดลง
โดยค่าฟลักซ์โดยรวมสูงสุดที่ได้จากการทดลองคือ 5.34 กิโลกรัมต่อตารางเมตรต่อชั่วโมง ณ อุณหภูมิ 80 องศาเซลเซียส
นอกจากนี้ยังพบว่าความเข้มข้นของน้ำในสารป้อนมีผลต่อค่าฟลักซ์และสัมประสิทธิ์การแยกเป็นอย่างมากอีกด้วย แต่ปัจจัยที่มีผลน้อยที่สุดคือความเข้มข้นของตัวเร่งปฏิกิริยา

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PANWANA KHUNNONKWAO : PURIFICATION OF L-(+)-LACTIC ACID FROM FERMENTATION BROTH USING PERVAPORATION-ASSISTED ESTERIFICATON TECHNIQUE, THESIS ADVISOR : ASST. PROF. APICHAT BOONTAWAN, Ph.D. 119 PP.

L-(+)-LACTIC ACID/ ESTERIFICATION/ PERVAPORATION

Esterification reactions of L-(+)-lactic acid with ethanol were performed to synthesize ethyl lactate with the objective to purify L-(+)-lactic acid from fermentation broth. Cassava starch was used as the main carbon source whereas Brewer's yeast extract was used as nitrogen supplement. Fermentation broth was concentrated by electrodeionization and evaporated until the water content reduced to approximately 25% prior to start the reaction. In comparison, the productivity and conversion yield of esterification reaction were significantly increased when the reaction was coupled with a pervaporation system. The membrane employed was a commercial hydrophobic membrane fabricated from modified Polydimethylsiloxane (PDMS). The effect of several process variables such as temperatures, water feed concentrations, and catalyst concentrations on separation performance were investigated. Experimental results revealed that permeate fluxes increased with increasing temperature, but separation factor decreased. The maximum permeation flux was observed at 5.34 $kg.m^{-2}.h^{-1}$ with operating temperature of 80 °C. Feed water concentration also had a profound effect on fluxes and separation factor. The effect of catalyst concentration was the least influence on separation performance.

School of Biotechnology

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ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my thesis advisor, Assistant Professor Dr. Apichat Boontawan for his invaluable help and constant encouragement throughout the course of this research. I am most grateful for his teaching and advice, not only the research methodologies but also many other methodologies in life. I would not have achieved this far and this thesis would not have been completed without all the support that I have always received from him, and my deep appreciation is also expressed to Assistant Professor Dr. Sunthorn Kanchanatawee, for all advice and guidance.

In addition, I am grateful for the teachers of School of Biotechnology and others person for suggestions and all their help.

Finally, I most gratefully acknowledge my parents and my friends for all their support throughout the period of this research.

Panwana Khunnonkwao

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LIST OF ABBREVIATIONS

°C	degree Celsius
Et al.	Et alia (and other)
SEM	scanning electron microscope
g	gram
Kg	Kilogram
L	Litre
Q	permeation flux (kg h^{-1} m^{-2})
p	pressure (kPa)
P	permeance (kg/h m^2kPa)
M_w	molecular weight
T	Temperature
w	mass fraction
x	mole fraction in the feed side
y	mole fraction in the permeate side
α_{memb}	membrane selectivity
β_{pervap}	separation factor for the pervaporation process
β_{evap}	evaporation separation factor
β_{memb}	membrance separation factor
γ	activity coefficient
A, B, C	Antoine equation constant
$\boldsymbol{r}_i$	a relative molecular volume
$\boldsymbol{q}_i$	a relative molecular surface area

CHAPTER I

INTRODUCTION

1.1 Significance of the study

Lactic acid is an important chemical that can be used in food, pharmaceutical, cosmetic, and chemical industries. Recently, there are increasing interests in the production of biodegradable polymers, such as Polylactic acid (PLA) and its copolymers from lactic acid. For their production, highly purified monomer lactic acid is needed (Vu *et al.,* 2005).

Lactic acid can be produced either from chemical synthesis or from fermentation, and the latter is prevailing. The conventional fermentation uses calcium carbonate to control the pH, and produces calcium lactate as the main product. In downstream processes, where calcium lactate is acidified with sulphuric acid during extraction process, calcium sulphate is produced as a by-product causing high chemical cost and waste generation. For purification of lactic acid, different techniques have been introduced; such as, solvent extraction, adsorption, direct distillation, electrodialysis, electrodeionization and esterification (Sun *et al.*, 2006). However, such purification procedures are difficult because of the low volatility of lactic acid (122 °C at 1661.73 Pa), with its affinity to water, and its tendency to self-polymerize. Esterification is the only downstream process, which separates other impurities from lactic acid (Joglekar *et al.*, 2006). The production process involves esterification of lactic acid with alcohols to produce lactate esters, distillation and

follows by hydrolysis of the distillated lactate ester to yield alcohol and lactic acid. This process is represented by the following reaction (Delgado *et al.*, 2007) where ethanol is used as the reactant.

$$CH_3CH(OH)COOH + C_2H_5OH \Leftrightarrow CH_3CH(OH)COOC_2H_5 + H_2O$$

Lactic acid *Ethanol* *Ethyl lactate*

Esterification reactions are characterized by thermodynamic limitations on conversion. Higher ester yields can be obtained by shifting the reaction towards products formation by hybrid processes such as reactive distillation and pervaporation-aided reactor instead of using a large excess of one of the reactants, usually the alcohol. Pervaporation has gained increasing attention in many chemical processes as an effective energy-saving separation technique. In this regard, the integration of a pervaporation process into conventional esterification process is attractive because the separation is based on the transport of the components through the membrane, which 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, pervaporation is used to continuously remove one of the reaction product to shift the equilibrium reaction to higher yields; in most cases the removed product is water (Delgado *et al.*, 2008). Alternatively, pervaporation can also be used to remove lactate ester from the reactor.

In this work, a hydrophobic membrane will be employed since it shows a high permeability to ethyl lactate compare to ethanol and water. Different operating parameters will be investigated including the effect of temperature and feed water

concentration, catalyst concentrations, fluxes on the conversion of reactants and separation factors for of the membrane tested.

1.2 Research objectives

The purposes of this study are as follows:

1. To purify L-(+)-lactic acid from fermentation broth using esterification and hydrolysis with/without pervaporation process.
2. The effect of several operating parameters; such as: process temperature, catalyst concentration, water feed concentration and separation performance of membrane.

1.3 Research hypothesis

1. Biological production of L-(+)-lactic acid is often contaminated with other organic acids.
2. Purification of L-(+)-lactic acid from fermentation broth is difficult and expensive.

1.4 Scope and limitation of the study

This work involves the purification of the L-(+)-lactic acid using pervaporation–assisted esterification and hydrolysis technique. The lactic acid purification process will be investigated using esterification and hydrolysis with/without pervaporation. Pervaporation–assisted esterification of lactic acid and ethanol will be studied in well-mixed reactors with catalysts (sulphuric acid) to study

the effect of several parameters such as process temperature, feed water concentration, catalyst concentration on the permeance flux and separation factor.

1.5 Expected Results

- High purity of L-(+)-lactic acid will be produced using the combination of pervaporation and esterification technique and optimal operating conditions will be obtained.

- This purification system can be applied for industrial scale production with improved yield of lactic acid and reduced production cost.

CHAPTER II

LITERATURE REVIEW

2.1 Lactic acid

Lactic acid (IUPAC systematic name: 2-hydroxypropanoic acid), also known as milk acid, is a as milk acid, is a chemical compound that plays a role in several biochemical processes. Lactic processes. Lactic acid was first isolated by the Swedish scientist named Carl W. Scheele in 1780 Scheele in 1780 and first commercially produced in 1881 (Vu *et al.*, 2005). Lactic acid is a acid is a carboxylic acid with a chemical formula of C3H6O3. It has a hydroxyl group adjacent to adjacent to the carboxyl group, making it an alpha hydroxyl acid (AHA). In solution, it can lose a it can lose a proton from the acidic group, producing the lactate ion, CH3CH(OH)COO−. It is CH3CH(OH)COO−. It is miscible with water or ethanol, and is hygroscopic. Lactic acid is chiral acid is chiral and has two optical isomers. One is known as L-(+)-lactic acid or (*S*)-lactic acid and lactic acid and the other, its mirror image, is D-(-)-lactic acid or (*R*)-lactic acid. L-(+)-lactic acid lactic acid is the biologically important isomer. The two optical isomer of lactic acid are show in are show in figure 1 and table 1

Table 1 shows the physiochemical properties of lactic acid (Narayanan *et al*., 2004).

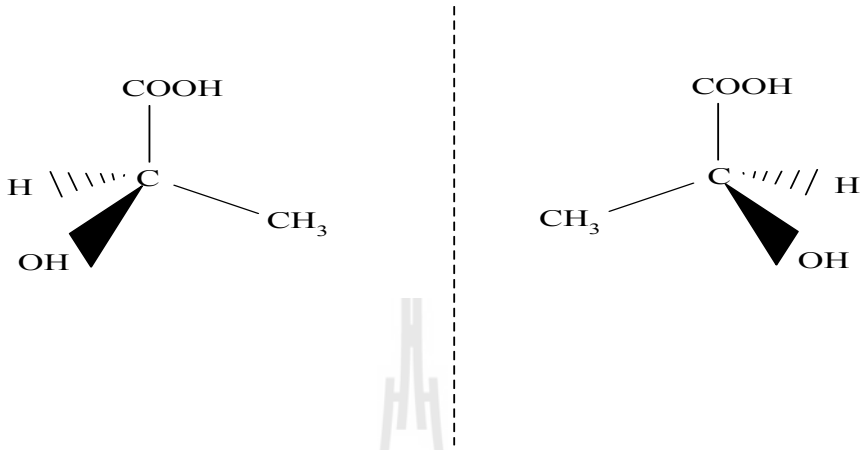


Figure 1 Chemical structure of L-(+)-lactic acid and D-(-)-lactic acid (Narayanan *et al*., 2004).

Table 1 Physiochemical properties of lactic acid (Narayanan *et al*., 2004)**.**

Properties	Value
Empirical formula	$C_3H_6O_3$
Chemical name	2-hydroxypropanoic acid
Dissolution constant (k_a)	1.37×10^{-4}
Molecular weight	90.08 g/mol
Normal boiling point, °C	122 °C (at 1.66 kPa)
Melting temperature, °C	L: 53 °C D: 53 °C

Density, d_4^{20}, g/mL	1.1

Applications for lactic acid are found in the food (additive and preservative), pharmaceutical, cosmetic, textile, polymer and leather industries. The main application of lactic acid polymer are various, such as medical application, packaging materials, biomedical materials like surgical sutures, absorbable bone plates for internal bone fixation, artificial skin, tissue scaffolds and controlled release drugs. These medical applications include its utilization with different properties in terms of tensile strength, viscosity, purity, and etc (Narayanan et al., 2004).

Polylactic acid has a potential to provide a new product platform to compete with hydrocarbon-based thermoplastics. Polylactic acid polymers have properties similar to polyethylene terephthalate (PET) and gloss, clarity and processability similar to polystyrene (PS). In addition, they provide heat stability at lower temperatures than polyolefin and can be processed by most melt fabrication techniques, especially fiber spinning. Polylactic acid is one of the few polymers whose structure and properties can be modified by polymerizing a controlled composition of the L- and D-isomers to give high molecular weight amorphous or crystalline polymers (Hujanen and Linko, 1996). Polylactic acid has a degradation time of 6 months to 2 years in the environment. In addition, esters of lactic acid, formed via combination with alcohols like methanol and ethanol, are finding increased use as environmentally benign solvents. Lactic acid esters are biodegradable, non-toxic, and have excellent solvent properties, which make them attractive candidates to replace halogenated solvents for a wide spectrum of uses. The future of the polylactic acid depends upon the technology efforts of lactic acid manufacturers to develop polylactic acid modified with other co-monomers, so as to

compete with polymers such as PET and PS, both on cost and application basis (Datta, 1995).

2.1.1 Lactic acid production by chemical reaction

The commercial process for chemical synthesis is based on lactonitrile. Hydrogen cyanide is added to acetaldehyde in the presence of a base to produce lactonitrile. This reaction occurs in liquid phase at high atmospheric pressures (4 Bars). The crude lactonitrile is recovered and purified by distillation. It is then hydrolyzed to lactic acid, either by concentrated HCl or by H_2SO_4 to produce the corresponding ammonium salt and lactic acid. Lactic acid is then esterified with methanol to produce methyl lactate, which is removed and purified by distillation and hydrolyzed by water under acid catalyst to produce lactic acid and the methanol, which can be recycled (Narayanan *et al*., 2004). This process is represented by the following reactions.

(a) Addition of Hydrogen Cyanide

$$CH_3CHO + HCN \xrightarrow{\text{Catalyst}} CH_3CHOHCN$$

Acetaldehyde Hydrogen cyanide Lactonitrile

(b) Hydrolysis by H_2SO_4

$$CH_3CHOHCN + H_2O + \tfrac{1}{2}H_2SO_4 \longrightarrow CH_3CHOHCOOH + \tfrac{1}{2}(NH_4)_2SO_4$$

Lactonitrile Sulphuric acid Lactic acid Ammonium salt

(c) Esterification

$CH_3CHOHCOOH + CH_3OH \longrightarrow CH_3CHOHCOOCH_3 + H_2O$

Lactic acid Methanol Methyl lactate

(d) Hydrolysis by H_2O

$CH_3CHOHCOOCH_3 + H_2O \longrightarrow CH_3CHOHCOOH + CH_3OH$

Methyl lactate Lactic acid Methanol

2.1.2 Lactic acid production by fermentation processes

As previously mentions, lactic acid can be produced commercially by either chemical synthesis or biotechnological production. Chemical production of lactic acid is based on the hydrolysis of lactonitile derived from acetaldehyde and hydrogen cyanide. However, the chemical production only results in a mixture of the two isomers. Biotechnological process can yield either form alone, or a mixture in different proportions of two isomers, depending on the microorganism, substrate and growth conditions. Biotechnological production is primarily carried out by bacterial fermentation of simple sugars, and bacteria species *Lactobacillus* and *Lactococcus* have received a worldwide interest in industrial processes because of their high growth rates and product yields (Abdul *et al.*, 2005). Conventional biotechnological production of lactic acid from starch materials, for instance, requires pretreatment by gelatinization and liquefaction, which is carried out at high temperatures of 90-130 °C for 15 minutes followed by enzymic saccharification to glucose and subsequent conversion of glucose to lactic acid by fermentation. Lactic acid-producing fungi,

such as *Rhizopus oryzae*, have recently received an interest in lactic acid production. The major advantage using the fungi over the bacteria is the low costs due to use of raw and/or waste materials, no requirement of specific nutrients, little pH maintenance required since most fungi can tolerate to low pH environment, and inexpensive separation of filamentous or pellet biomass from the fermentation broth (Axelsson, 1998).

2.2 Lactic acid fermentation processes

Lactic acid fermentation is known to be the end-product inhibited fermentation by an undissociated form of lactic acid. Fermentation is defined as an energy yielding process whereby organic molecules serve as both electron donors and electron acceptors. The molecule being metabolized does not have all its potential energy extracted from it. Hence, lactic acid bacteria are widely used as a low cost method for food preservation by fermentation and generally no or little heat is required during the fermentation. In fermentation, pyruvic acid molecules are turned into some waste product and a little bit of energy (only two ATP molecules per molecule of glucose is produced) (Narayanan *et al*., 2004).

2.2.1 Batch fermentation

Lactic acid production is commonly accomplished in batch fermentation. For the batch fermentation of lactic acid bacteria, the best result obtained with glucose as the substrate are 150.2 gL^{-1} for the end of fermentation and 1.34 $gL^{-1}h^{-1}$ of productivity (Yun and Ryu, 2001). In the study of the influence of various carbohydrates lactic acid fermentation by *Enterococcus faecalis*, it was found that glucose, fructose and maltose are very efficient for homofermentative production

(Yun *et al.*, 2003). Nevertheless, the major disadvantage of batch fermentation is that lactic acid concentration and volumetric productivity decreased due to inhibition of high substrate concentration which is a conventional properties of batch fermentation. On the other hand, as the time goes by the concentration of lactic acid are increased resulting in inhibition of cell growth and product formation.

2.2.2 Fed-batch fermentation

Fed-batch culture is a batch culture, and is continuously fed with substrates without removal of fermentation broth. It is generally important to batch and continuous process. There are numerous studies of fed-batch culture in lactic acid fermentation. For example, the lactic acid productions by *Pediococcus pentosaceus* was investigated in fed-batch fermentation. To reduce nutrient cost for lactic acid production, cassava starch was chosen as a nutrient source in this study. Cassava starch was treated by enzymatic hydrolysis using α-amylase (produced 35% yield of reducing sugar) before it was put in experiment. When hydrolyzed cassava starch (1-10% of starch) supplemented with in the range 10-20 g/L of spent brewer's yeast extract was used as a raw material for fermentation. Result show that, hydrolyzed cassava starch was very appropriate as cheap carbon source for growth and lactic acid production in basal medium. The experimental data under 10% hydrolyzed cassava starch supplemented with 10 g/L of commercial yeast extract and brewer's yeast extract were showed 38.3 g/L and 24.7 g/L of lactic acid, respectively after 72 h at 30^{o}C pH 6.0. In addition, this fermentation showed about 80 % yields (g lactic acid produced/g substrate utilized) with 95% of the optical purity. The nitrogen source cost for producing 1 kg commercial yeast extract can be reduced by 33 %. This finding revealed that hydrolyzed cassava starch supplemented with brewer's yeast extract is

the most promising substrate for commercial lactic acid production (Boontawan *et al, 2007*).

2.2.3 Continuous fermentation

The continuous fermentation can be produced higher productivity by varying the dilution rate, which is directly affected on the nutrient concentration (Yoo *et al.*, 1997). The developments of continuous culture techniques avoid this limitation by providing the essentially constant microbial environment. The results obtained showed that the dilution rate influences the fermentation pattern. The optimal glucose concentration on inlet feed medium was also determined for the *Lactobacillus coryniformis* fermentation. By adding a second stage or by increasing the retention time, it allows maximization of the volumetric productivity with a reasonable conversion, or to maximize for final lactic acid concentration and substrate conversion whilst still achieving reasonable volumetric productivity (Gonzalez-Vara, 1996).

2.3 Downstream processing

Fermentation-derived lactic acid can be separated by several recovery processes, such as calcium precipitation, solvent extraction, and electrodialysis (Peckham, 1944). After fermentation, the process of separating the product from the medium and converting the salt to an organic acid is complicated, involving precipitation and acidification using a mineral acid (sulfuric acid). These steps contain the major economic hurdles for organic acid production. Moreover, the processing produces large quantities of an effluent containing high concentrations of salts. For instance, in the case of calcium carbonate neutralization, one ton of gypsum by-product will be produced for every ton of lactic acid produced (Inskeep, *et al*., 1952

and Peckham, 1944), which is normally dumped in the environment as waste (Peckham, 1944).

In the meantime, traditional processes are based on precipitation steps that generate large amounts of chemical effluents. Therefore, the environmental impact and the operating costs of traditional precipitation processes can be reduced thanks to alternative technologies, such as adsorption, extraction or membrane separation, electrodialysis, electrodeionization and esterification**.** In addition, alkalis other than calcium, such as ammonium hydroxide, or sodium hydroxide (or carbonate) can also be used. In such case, it is necessary to recovered and recycled back in order to reduce the production cost (Joglekar *et al.*, 2006)**.**

2.3.1 Adsorption

Recovery of carboxylic acids from fermentation broths presents a challenging separation problem, because of the dilute, complex nature of fermentation broths. Methods of recovery that utilize separating agents, such as solid sorbents which are selective for carboxylic acids, are attractive and are reported by many researchers. The important characteristics of solid sorbents are high capacity for the acid and high selectivity for the acid as opposed to water and substrate (e.g., glucose), good regenerability, and the biocompatibility with microorganisms depending upon the process configuration (Frieling and Schugerl, 1999). If a solid sorbent can be used *in situ* or in an external recycle loop, higher overall yields can be achieved since lactic acid fermentation is subjected to end-product inhibition.

2.3.2 Solvent extraction

Lactic acid is poorly extractable by common organic solvent due to their hydrophilic nature. This is because lactic acid contains hydroxyl group in its

molecule. Therefore, reactive extraction has been considered for its recovery from aqueous solutions. Reactive extraction of the lactic acid by a suitable extractant has been found to be a promising alternative to the conventional processes. Reactive extraction uses reaction between the extractant and the material being extracted. The extractant in the organic phase reacts with lactic acid in aqueous phase, and reaction complex is solubilized into the organic phase. The lactic acid is then recovered from the organic layer by stripping. The pre-requisite of an economic recovery by extraction is a high distribution coefficient. Reactive liquid–liquid extraction has the advantage that lactic acid can be easily removed from the fermentation broth (extractive fermentation), preventing the lowering of pH. Furthermore, the lactic acid can be re-extracted whilst the extractant and diluent can also be recycled to the fermentation process. However, this implies that extractant and diluent must not be toxic to the microorganism used in fermentation. This can ideally be done for continuous fermentation. In addition, membrane extraction can overcome many drawbacks of the extractive fermentation which have plagued the process for long, and offers other numerous advantages, such as no fear of back mixing, no direct exposure of microbes to extraction reagents, thereby ensuring biocompatibility, no need for agitation, potentially high efficiency, etc. For the above reasons, membrane extraction can be considered a very promising alternative to the conventional solvent extraction for separation and purification of lactic acid. However, this technique can not separate other organic acids from lactic acid because of their solubility in organic phase (Wasewar *et al.,* 2005).

2.3.3 Electrodialysis (ED)

Electrodialysis is one of the most promising and perspective method provided by rapid development of the membrane processes, especially the membranes in the 80's and 90's. ED is an electro-membrane process in which ions are transported through ion exchange membranes from one solution to an other under the influence of direct electrical potential (Lund *et al.*, 1992). Electrodialysis fermentation (EDF) is also studied by several authors. Electrodialysis fermentation (EDF) is very promising because it can continuously remove lactic acid from the system, and maintain the pH of the broth. Most of the literatures show the feasible production of lactic acid from lactate salts in two steps: conventional ED for concentration and purification using bipolar electrodialysis for conversion of lactate salts into lactic acids with the recovery of alkali. Bipolar membrane electrodialysis also refers to water splitting electrodialysis, which can convert aqueous salt solutions into acids. A water splitting stack is similar to a conventional (mono-polar) electrodialysis stack but incorporates a third type of membrane, the bi-polar membrane, which is composed of a cation and anion membrane layers laminated together. To meet the feed requirements of bipolar membrane electrodialysis, clarification of the fermentation broth is first performed by microfiltration. After the conversion of sodium lactate into lactic acid, the product is further purified by ion exchange resins (Habova *et al.*, 2004).

2.3.4 Electrodeionization (EDI)

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.

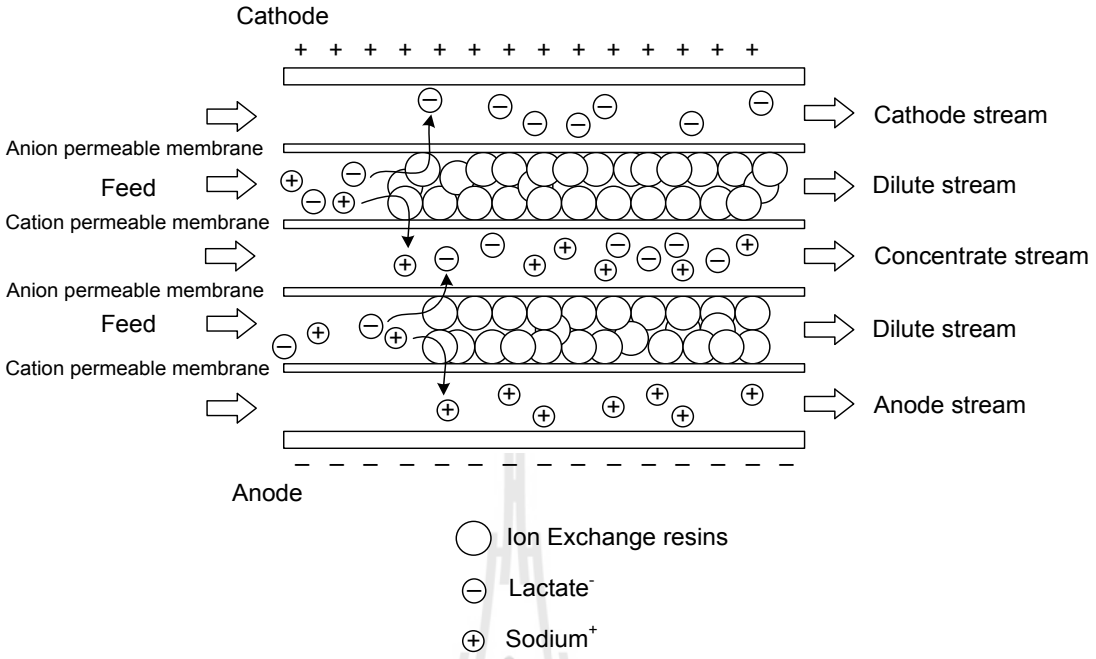


Figure 2. Schematic diagram of electrodeionization (EDI) process for recovery of lactic acid from fermentation broth (Wenten *et al.*, 2004).

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, the EDI unit with unique "electro-regeneration" can be considered as a mixed-bed ion-exchange column with continuous regeneration, and therefore is capable of complete deionization (Wenten *et al.*, 2004, Kamel and Aicha, 2006).

The amount of ions transported through the ion-exchange membrane is directly proportional to the electrical current density. The increase of the current density leads to an increase in the number of ions transferred. The electrical current, I, required to remove a number of ions is given by

$$I = zFQ\Delta C/\eta \quad (1)$$

Where; z is the valence, F is the Faraday's constant (1 Faraday = 96,500As/equiv.), Q is the flow rate, ΔC is the concentration difference between the feed and the diluate (mol/L), and η is the overall current efficiency, respectively (Wenten *et al.*, 2004).

2.3.5 Esterification

High purity lactic acid can also be produced by esterification of crude lactic acid with alcohols to yield lactate ester. The process is followed by distillation, hydrolysis of the distillated lactate ester to yield the alcohol and lactic acid (Joglekar *et al.,* 2006). Esterification is the only downstream process, which separates other organic acids from lactic acid. Esterification gives esters of lactic acid, and further

hydrolysis of esters is necessary to get the product as pure lactic acid. Simultaneous distillation with esterification–hydrolysis is called reactive distillation. Fermentation broth containing lactic acid needs to be pretreated to remove some other impurities before reactive distillation. Ethanol is a preferred reactant because it is easy to produced. Two reactions are involved in recovery of lactic acid by esterification and hydrolysis method (Delgado *et al*., 2007). They are as follows:

Esterification reaction

$$CH_3CH(OH)COOH + C_2H_5OH \Leftrightarrow CH_3CH(OH)COOC_2H_5 + H_2O$$

Lactic acid *Ethanol* *Ethyl lactate*

Hydrolysis reaction

$$CH_3CH(OH)COOC_2H_5 + H_2O \Leftrightarrow CH_3CH(OH)COOH + C_2H_5OH$$

Ethyl lactate *Lactic acid* *Ethanol*

Smith and Claborn (1940), described methods for the preparation of purified lactic esters from crude lactic acid obtained from fermentation. To obtain high yields of the pure lactic esters with alcohol containing less than four carbons, it is necessary to use a large excess of alcohol during esterification and then rapidly remove water with excess alcohol from the ester at low temperature, preferably in vacuum with aid of an efficient fractionating column. Calcium lactate is dissolved in methanol and equivalent amount of sulphuric acid is added to liberate the lactic acid to precipitate and separate calcium sulphate. Lactic acid solution is heated for 4–8 h at refluxing temperature to complete the esterification.

On the other hand, another method to purify lactic acid by preparation of methyl lactate from crude aqueous lactic acid was introduced. This method comprises of passing methanol vapor through aqueous lactic acid before condensing the effluent vapors. The condensate, a mixture of methanol, water and methyl lactate, can be distilled to recover the methyl lactate and hydrolyzed to obtain purified lactic acid (Filachione and Fisher, 1946). Schopmeyer *et al*. (1944), suggested for heating an esterification mixture of lactic acid (40–60 % (w/v) with an acidic catalyst (sulphuric acid) for esterifiation in a jacketed kettle. The vapors from the esterification kettle are continuously fed to a fractionating column, which may contain alcohol, ester and water. Continuous hydrolysis of ester in a fractionating column was done at atmospheric pressure. Alcohol produced by hydrolysis was removed from the top and returned to esterification kettle, and lactic acid along with water was drawn from the bottom. Recently, reactive distillation has drawn considerable attention because of its striking advantages, especially for equilibrium-limited reactions. Purification of lactic acid through reactive batch distillation was investigated by several investigators. The experimental setup used by the authors consisted of two columns for separation of reactants from the product and two reboilers for esterification reaction and hydrolysis reaction. The feed normally consisted of only 10–20% lactic acid by weight. Lactic acid was reacted with methanol, and methyl lactate was produced by the esterification reaction in presence of cation exchange resin (Dowex 50W). The volatile methyl lactate was distilled simultaneously with hydrolysis reaction forming lactic acid. To recover pure lactic acid through two reactions and distillation, batch distillation system consisting of two condensers, feed vessel, and reboiler was used. The yield of recovered lactic acid was as high as 95%. When impure lactic acid solution obtained

from bacterial fermentation was used as feed, highly pure lactic solution was obtained with yield of 92% in reboiler (Choi *et al*., 1999).

Recently, Sun *et al.* (2006), studied conversion of lactic acid or ammonium lactate (NH_4LA) into esters, and subsequent hydrolysis of the purified ester into lactic acid to obtain highly pure lactic acid. In this study, two reactors with a rectifying column were used to recover lactic acid from the fermentation broth. NH_4LA obtained by fermentation was used directly to produce butyl lactate by reacting with butanol for 6 hours, and the esterification yield of NH_4LA was 87.7%. In this procedure, a cation exchange resin which was modified by $SnCl_2$ replaced sulphuric acid as a catalyst, and neutral NH_4LA replaced former lactic acid as a starting material, which is not only eliminated corrosion of the reactor, but also avoided generation of calcium salts as a by-product. Then butyl lactate was rectified, and the purified butyl lactate was sequentially hydrolyzed into lactic acid in presence of the cation exchange resin in the H^+ form as a catalyst for 4 hours, and the hydrolysis yield was 89.7% with the purity of recovered lactic acid of 90%. Liberated butanol in hydrolysis process and unreacted butanol in esterification process can be recycled to the esterification, and the recovery ratio of butanol was 85.6%.

Seo *et al.* (1999), studied the feasibility of recovery of lactic acid by two batch reactive distillations using cation exchange resin (Dowex 50W) as a catalyst and glass packed column. For the recovery of lactic acid, two reactions, esterification and hydrolysis, were involved and hence, an apparatus with two distillation columns was developed and operated in a batch mode to ensure enough residence time in the reboiler and column. The effects of operating variables such as catalyst loading, molar ratio of lactic acid to methanol, feed concentration, type of alcohols and partial

condenser temperature on yield were studied. The reaction products of the esterification (methyl lactate and water) were distilled to the hydrolysis part to recover into pure lactic acid. The yield of lactic acid increased as catalyst loading in the esterification part was increased. The decrease in molar ratio of lactic acid to methanol, and lactic acid feed also improved the yield of lactic acid. Methanol as a reactant gave higher yield than any other alcohol. The yield of lactic acid was as high as 90%. Kim *et al*. (2002), carried out similar studies using Oldershaw columns and reboilers for fractionation and reactions. Concentration and temperature profiles in the reboilers and on each stage were investigated throughout the operation. Six-hour operating time was required for getting high purity lactic acid. The effect of columns on the recovery yield was investigated. The column improved the fractionation of the boilups from the reboilers. More effective fractionation in columns allowed vapor stream in columns to contain more methanol and liquid stream to contain less methanol. Thereby, methanol-concentrated recycle flow was obtained more effectively and methanol was prevented to remain in hydrolysis part. That resulted in the more effective reaction in both reboilers and improved the yield. Methanol recycle and feeding method were investigated as the factors, which could control the component boilup rate of each species and the rate of esterification reaction. The temperature of partial condenser controlled the flow rate and composition of methanol recycle stream. Semi-batch operation was compared with batch operation. Continuous feeding of methanol enhanced the recovery system performance whilst continuous feeding of lactic acid aqueous solution deteriorated the recovery compared with batch operation. In continuous esterification, the mixture of an aqueous solution of lactic acid, and an acidic catalyst of esterification is heated to produce mixture of vapors.

The use of sulphuric acid as catalyst may result in traces of acid in the product. In presence of impurities with cation exchange resin catalyst it may be difficult to maintain the operation at steady state. Therefore it may be necessary to purify the crude lactic acid from impurities like residual sugar and protein before hand.

2.4 Membrane – assisted esterification

2.4.1 Theory

The modeling of the mass transfer in membrane process is one of the fundamental aspects to understand and improve the process performance. The separation performance of a membrane separation can be described in terms of the total permeation flux through the membrane per unit of area and time and the separation factor (β) of the membrane defined as (Delgado *et al*, 2008 and Delgado *et al*, 2009):

$$\beta = \frac{w_{i,p} w_{j,f}}{w_{i,f} w_{j,p}} \tag{2}$$

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.

On the basis of the solution/diffusion model, the flux of component i through the membrane is proportional to its partial vapor pressures differences on broth sides of the membrane (Delgado *et al*, 2008,):

$$Q_i = P_i(p_{i,f}^{vapor} - y_{i,p}p_p) \quad (3)$$

Where Q_i is the permeation flux of component i, P_i is the permeance of the membrane, $p_{i,f}^{vapor}$ is the equilibrium partial vapor pressure on the feed side, $y_{i,p}$ the mole fraction in the permeate and p_p is the permeate pressure. The partial vapor pressure of each component on the feed side can be calculated from its concentration in the liquid feed:

$$p_{i,f}^{vapor} = x_{i,f}\gamma_i p_{i,f}^{s} \quad (4)$$

Where, $x_{i,f}$ is the mole fraction of component i in the feed, γ_i is the activity coefficient and $p_{i,f}^{s}$ the saturation vapor pressure at the temperature of the feed (Delgado *et al*, 2008 and Delgado *et al*, 2009).

Combining equations (3) and (4) the permeation flux of a component through the membrane can be expressed as follows:

$$Q_i = P_i(x_i\gamma_i p_{i,f}^{s} - y_{i,f}p_p) \quad (5)$$

From equation (5), the permeance of a membrane is defined as the permeation flux divided by the permeant driving force. In membrane processes, the driving force

is expressed in terms of the difference in the partial pressure of the component on the feed and on the permeate side (Delgado *et al*, 2008) :

$$P_i = \frac{Q_i}{(x_{i,f}\gamma_i p^s_{i,f} - y_{i,p}P_p)} \tag{6}$$

The membrane selectivity is defined as the ratio of the permeances. Mole-based permeances have been used in this work to define the membrane selectivity:

$$\alpha_{memb} = \frac{P_i / M_{w,i}}{P_i / M_{w,j}} \tag{7}$$

Where $M_{w,i}$ and $M_{w,j}$ are molecular weight of components *i* and *j*. The use of permeance and membrane selectivity is recommended to compare the separation performance of the membranes. These two parameters allow distinguishing the effect of the nature of the membrane and the operating conditions (Delgado *et al*, 2008 and Delgado *et al*, 2009).

Wijmans and Baker (1993) consider the overall separation factor, β_{pervap}, achieved by a pervaporation process as the product of an evaporation separation step, β_{evap}, and a membrane separation step β_{memb}:

$$\beta_{pervap} = \beta_{evap}\beta_{memb} \tag{8}$$

Although the permeating components vaporize after passing the membrane, this equation helps to understand the effect of the partial vapor pressure of the liquid phase on the separation performance. When the permeate partial pressures are much smaller than the feed partial vapor pressures, $\beta_{pervap} = \alpha_{memb}$ and equation (8) becomes:

$$\beta_{pervap} = \beta_{evap}\ \alpha_{memb} \tag{9}$$

The Antoine equation was used in this work to calculate the vapor pressure of each component:

$$log(p_i^S) = \frac{A_i - B_i}{(C_i - T(^\circ C))} \tag{10}$$

Where p_i^s the saturation vapor pressure in kPa and T the temperature in $^\circ$C. The constants, A_i, B_i, and C_i of the Antoine equation are listed in Table 2 together with the van der Waals properties r_i (a relative molecular volume) and q_i (a relative molecular surface area). In this work, the activity coefficients of the components in the liquid phase ware calculated using the UNIQUAC equation. The UNIQUAC binary interaction parameters have been already reported elsewhere (Sanz *et al*, 2007).

Table 2 Pure components parameters: van der Waals properties, r_i and q_i, and Antoine equation constants A_i, B_i and C_i (Delgado *et al*., 2008).

Compound	r_i	q_i	Antonie contants		
			A_i	B_i	C_i
Water	0.9200	1.4000	7.0436	1636.91	224.92
Ethanol	2.1055	1.9720	7.1688	1552.60	222.42
Ethyl lactate	4.4555	3.9280	7.8269	2489.7	273.15
Lactic acid	3.1648	2.8800	7.2471	1968.21	158.94

2.4.2 Membrane separation processes

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).

3.4.2.1 Pervaporation

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 3, 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).

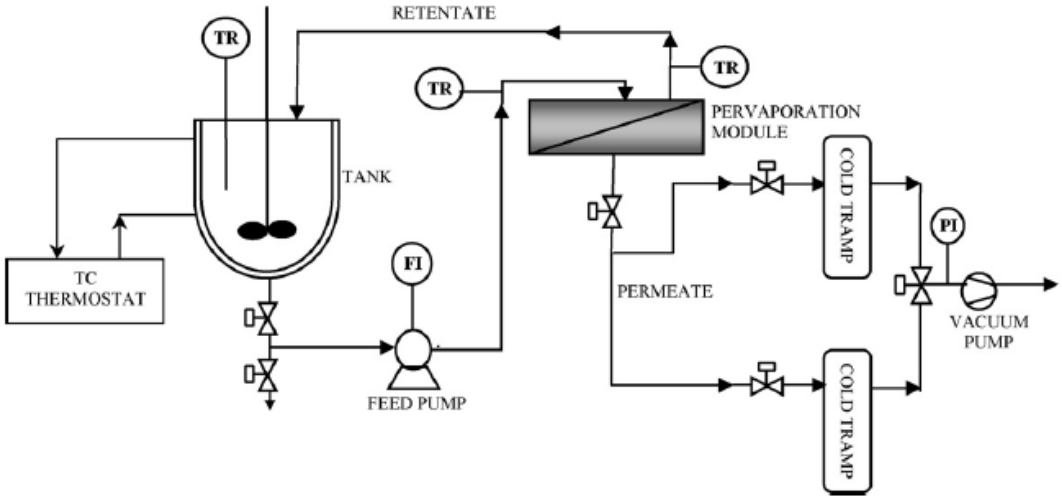


Figure 3. Schematic diagram of pervaporation system (Delgado *et al*.,2008).

Daniel *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 for simultaneous esterification of the two acids.

Delgado *et al.* (2008), studied pervaporation experiments were performed for three binary mixtures: water/ethanol, water/ethyl lactate and water/lactic acid by the membrane PERVAP® 2201. In particular, the effects of water contents in the feed and temperature were investigated. This membrane was chosen due to the higher total permeation fluxes and separation factors obtained for water/ethanol mixtures compared with the values obtained with PERVAP® 2216. The results show that the total permeation flux increases in the order ethanol = ethyl lactate < lactic acid. This tendency can be related with the higher degree of swelling of the membrane in contact with lactic acid solutions. In all cases, the actual selectivity of the membrane was found to be towards water. The total and partial permeation fluxes were found to increase with the water content in the feed and the operating temperature. Permeances also increase with increasing feed water content. Arrhenius dependence with temperature was found for the total permeation flux while little dependence was observed for the permeance on temperature. The highest values for water permeance

were obtained for liquid mixture between water and lactic acid. However, similar dependence of water permeance on feed water concentration was found for liquid mixture of water with ethanol and liquid mixture between water and lactic acid.

Delgado *et al*. (2009), examined pervaporation experiments with PERVAP® 2201 membrane were performed for the multicomponent mixture involved in the ethyl lactate synthesis: water/ethanol/ethyl lactate/lactic acid. In particular, the effect of feed composition and temperature was investigated. The total and partial permeation fluxes were found to increase with the water content in the feed and the operating temperature. However, permeation flux is not much affected by lactic acid feed concentration. Permeances also increase with increasing water feed content. Arrhenius dependence with temperature was assumed for the total permeation flux while the percentage of increase of permeance with temperature was much smaller than permeation flux. Since all the components of the mixture are highly rejected but water, this pervaporation system can be considered as a pseudo-binary mixture in the experimental conditions studied in this work. Separation factor and enrichment factor strongly depend on feed composition but this dependence is not so strong for temperature. The very high affinity towards water makes PERVAP® 2201 membrane suitable for pervaporation aided esterification of fermentation-derived organics acid such as lactic acid.

Wasewar et al. (2009), separated lactic acid by esterification with ethanol in a pervaporation reactor. This study focused on modeling and simulation of a pervaporation reactor for esterification. The experimental data were used for the model validation and excellent agreement was found. The effect of several process variables such as temperature, initial mole ratio of reactants, ratio of the effective

membrane area to the volume of reacting mixture (S/V), catalyst concentration, and flux on conversion of reactants were studied. The conversion of lactic acid was enhanced in a pervaporation reactor as compared to a conventional reactor. The pervaporation reactor has a good potential for enhancing conversion in reversible condensation reactions (especially esterification), generating water as a product. Optimum conditions were observed at temperatures of 75 to 95 °C, for the ratio of the effective membrane area over the volume of reacting mixture (S/V) of 10 m^2/m^3, for a catalyst concentration of 30 g/L for a reactant ratio (acid/alcohol) of 2:1. The model can be used for the other esterification reactions in a pervaporation reactor.

CHAPTER III

MATERIALS AND METHODS

3.1 Apparatus

Bioreactor: B.Bruan, Germany

pH combined electrode: Mettler Toledo, Switzerland

pH meter: Sartorius, Germany

Reactor: Biofuel production from biomass research unit, School of biotechnology, Suranaree university of technology, Thailand)

Distillation: Biofuel production from biomass research unit, School of biotechnology, Suranaree university of technology, Thailand)

Membranes mudule: Biofuel production from biomass research unit, School of biotechnology, Suranaree university of technology, Thailand)

Hot plate: V.Go, USA

Thermostat: Sartorius, Germany

Gear pump: Cole-Parmer, USA

Electronic digital scale: Sartorius, Germany

High performance Liquid chromatography: Agilent technologies 1200 series, USA)

3.2 Materials and chemicals

Figure 4 shows the SEM analysis of the composite PDMS/PVDF membrane where the extremely this PDMS layer was coated on the support porous PVDF layer. The picture revealed that PDMS membrane top layer is tightly and properly cast on the top of the PVDF membrane, displaying a uniform coating thickness of approximately 2 μm. Ideally, in the fabrication of composite membranes, it is desirable to have a minimum coating thickness with as little resistance as possible. With the characteristic of large and finger-liked pore structure, the resistance of the support layer can be neglected. Comparisons of separation performances of the composite membrane were then carried out.

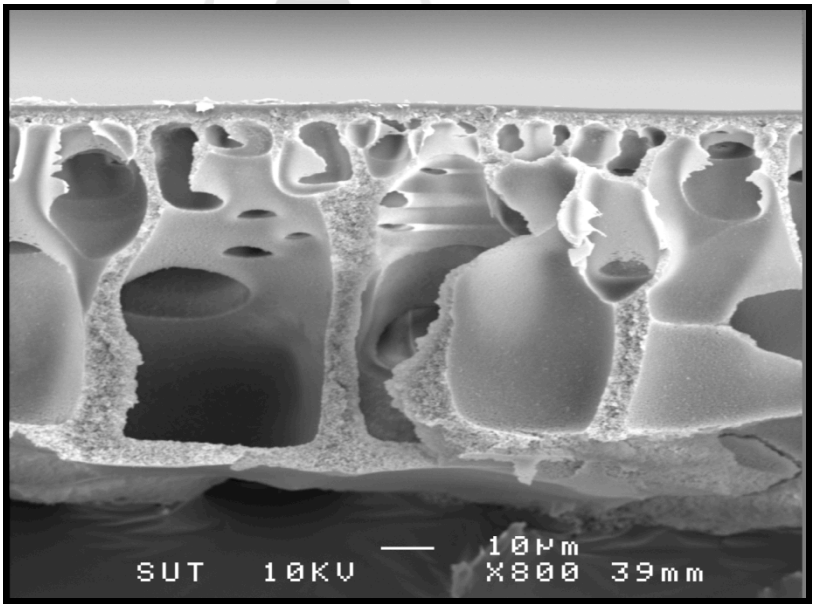


Figure 4 Scanning Electron Microscope (SEM) picture of the composite PDMS/PVDF membrane used in this study (Sulzer Chemtech GmbH, Switzerland).

Ethyl lactate wss supplied by Fluka, U.K. with a reported purity of 99%w/v. Ethanol of 99.8%w/v purity was produced in our laboratory (Biofuel Production from Biomass Research Unit, School of Biotechnology, Suranaree University of Technology, Thailand). Lactic acid powder (98%w/v) is obtained from Fluka.

3.3 Lactic acid fermentation process

3.3.1 Microorganism cultivation

Lactic acid bacteria strain of *Pediococcus pentosaceus* obtained form Pilin Boontawan thesis work used in this study. For cultivation, the following Man, Rogosa, Sharpe (MRS) comprises: 10 g/L carbon sources, 10 g/L nitrogen sources, 5 g/L sodium acetate, 2 g/L triammonium citrate, 2 g/L $Na_2HPO_4.2H_2O$, 0.1 g/L $MgSO_4.4H_2O$, 0.05 g/L $MnSO_4.4H_2O$, and 1 mL Tween 80 (Xiaodong *et al*., 1997), pH 6.0. The selected lactic acid bacteria (10% inoculums size) inoculated into the modified MRS medium (fermentation medium, the carbon source as hydrolyzed starch and nitrogen source as spout brewer's yeast extract). Regenerated culture from stock culture was transferred to the medium, and incubated in a rotary shaker at 200 rpm at 30°C for 24 hours. The regenerated culture was then streaked onto MRS agar slant, and stored at 4°C for future use. At the commencement of the experiment, culture was taken from agar slant, and regenerate into 10 mL of MRS medium. Incubation was taken place in a rotary shaker at 200 rpm at 30°C for 24 hours. Then, the inoculum was transferred into 90 mL MRS medium, and incubated using the same condition (Boontawan *et al.*, 2007 and Boontawan *et al.*, 2008).

3.3.2 Batch fermentation processes

The modified MRS medium with glucose syrup (the glucose concentration of 80 $g.L^{-1}$ were prepared in a 10 L conventional stirred tank bioreactor (B. Bruan, Germany), and autoclaved at 121°C for 15 minutes before transferring of the seed. The inoculums size (10% v/v) was from a 18-hours-old culture. An external electric control unit used to monitor and regulate condition of the broth. Temperature was set at 30 ^{o}C, and the impeller speed was maintained at 200 rpm (in order to ensure thorough mixing of the fermentation broth) under an anaerobic environment.

The pH was measured by a pH combined electrode (Mettler Toledo, Switzerland), and maintained at a set point (5.5) by the automatic addition of base (3.0 M of NH_4OH). Samples were removed aseptically at regular intervals for further analysis.

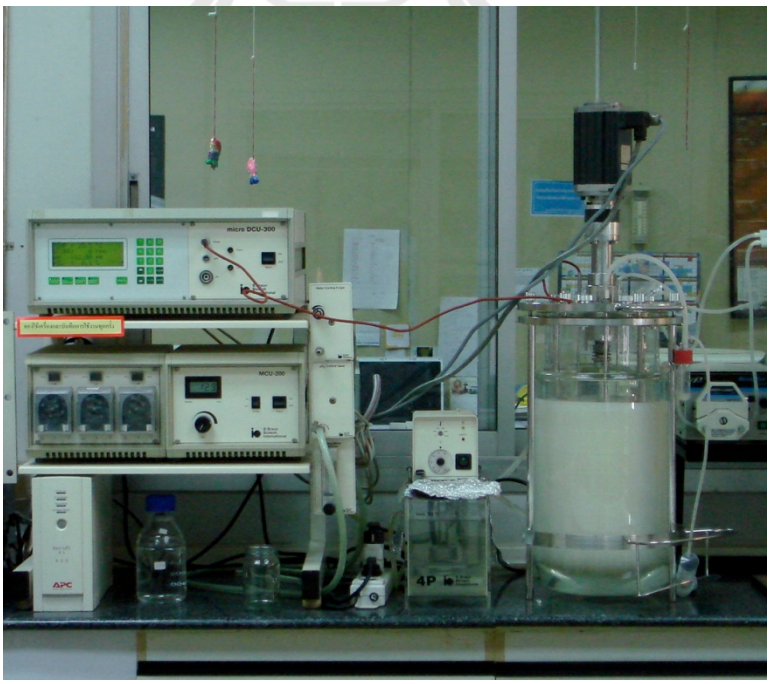


Figure 5 Equipment for fermentation processes of lactic acid production.

3.3.3 Fed-batch fermentation for lactic acid production by *Pedidococcus pentosaceus*

The modified MRS medium with glucose syrup (the glucose concentration of 80 $g.L^{-1}$ were prepared in a 10 L conventional stirred tank bioreactor (B. Bruan, Germany), and autoclaved at 121°C for 15 minutes before transferring of the seed. The inoculums size (10% v/v) was from a 18-hours-old culture. An external electric control unit used to monitor and regulate condition of the broth. Temperature was set at 30 ^{o}C, and the impeller speed was maintain at 200 rpm (in order to ensure thorough mixing of the fermentation broth) under an anaerobic environment. The fermenters were run as batch cultures for 18 hours, then operated the feeding substrate was pump into the fermenter using a peristaltic pump. In constant feed rate fed-batch fermentation, the hydrolyzed starch solution was pumped into the fermenter at a feeding rate of 10, 20 and 30 mL/h, respectively. To prevent growth in the medium it was kept refrigerated prior to use. The continuous reactors were run to steady state (minimum three retention times). When changes in hydraulic retention times were introduced, the reactor was reinoculated with a fresh inoculum to minimize the influence of contaminants.

The pH was measured by a pH combined electrode (Mettler Toledo, Switzerland), and maintained at a set point (6.0) by the automatic addition of base (3.0 M of NH_4OH). Samples were removed aseptically at regular intervals for further analysis.

3.4 EDI experiment

For EDI experiment, the experimental setup of the process used in this study was shown in Figure 6. The process for recovery of lactic acid from fermentation broth consisted of microfiltration (0.2 μm) and EDI. The microfiltration unit provides permeate containing no biomass. The microfiltration permeate was subsequently fed into the EDI apparatus. All diluted compartments were filled with mixed-bed ion-exchange resins (purolite strong acid cation-exchange, C-100E and strong base type I anion resins, A-400). Platinum and stainless steel were used for anode and cathode, respectively. The effective surface area of each membrane was 50 cm^2. The internal spacer for each compartment was 3 mm. An adjustable power supply was specially constructed, and was used to produce direct current. It could supply voltage and direct current set as 15 Volts and 1 Ampere, respectively (Boontawan *et al,* 2009). The EDI system was operated continuously. Furthermore, the lactic acid is concentrated by evaporation of fermentation broth until water content 15% w/v (temperature set as 80°C and pressure was maintained around 1 mbar). The concentration of lactic acid was measured by titration with NaOH solution (0.1 N) and HPLC (Boontawan *et al,* 2007 and Boontawan *et al*., 2008).

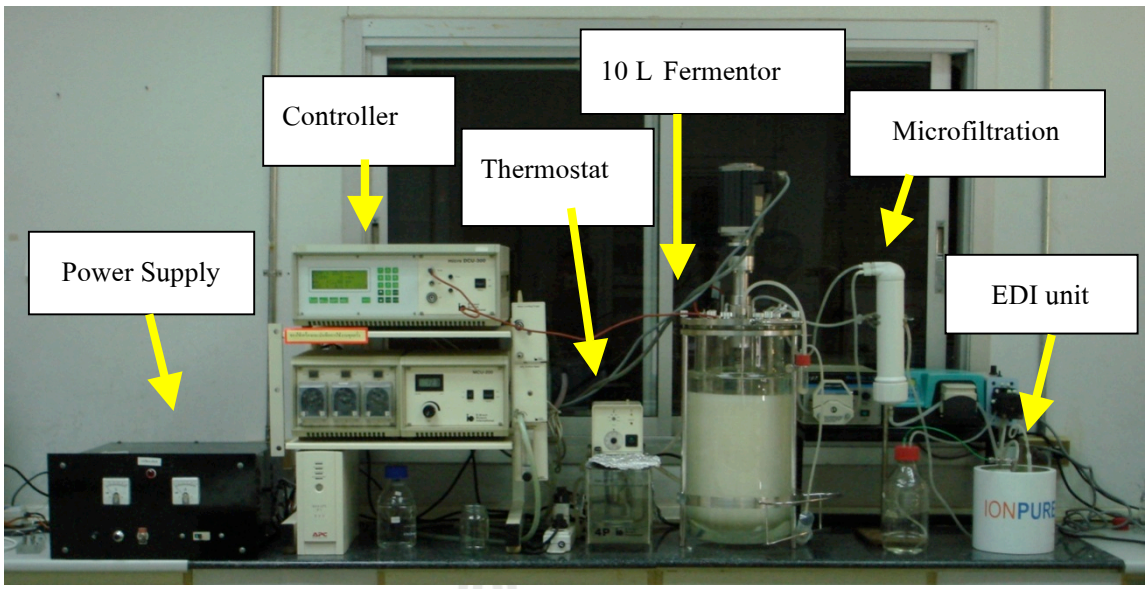


Figure 6 Experimentation set-up for separation of L-(+)-lactic acid from fermentation broth using EDI technique.

3.5 Esterification and hydrolysis

Two reactions were involved in recovering lactic acid by esterification and hydrolysis method. They were as follows:

Lactic acid + ethanol $\overset{\text{catalyst}}{\rightleftharpoons}$ ethyl lactate + water (esterification reaction)

Ethyl lactate + water $\rightleftharpoons$ lactic acid + ethanol (hydrolysis reaction)

From the two reaction equations, it can be observed that recycle of ethanol was possible. Three technical terms concerning with above two reactions were defined as follows:

Esterification yield

$$= \frac{\text{actual yield of ethyl lactate in esterification}}{\text{theoretical yield of ethyl lactate in esterification}} \times 100\%$$

Hydrolysis yield

$$= \frac{\text{actual yield of lactic acid in hydrolysis}}{\text{theoretical yield of lactic acid in hydrolysis}} \times 100\%$$

Purity of lactic acid means a percentage of lactic acid to total hydrolysis products (Sun *et al.*, 2006).

3.5.1 Esterification without pervaporation

The concentrated lactic acid from fermentation broth and ethanol were placed into a 2 L reactor equipped with a distillation column, and a condenser. Ammonium lactate and ethanol reacted to form ethyl lactate in the presence of the catalyst (sulphuric acid). The stirred reactor with a 350 rpm stirrer speed was heat to a boiling point by a heater control (keeping a temperature 80°C at a reactor)(Sun *et al.*, 2006). Owing to high solubility of ethanol in water, they were removed from the reaction for the equilibrium to shift to right by an addition of anhydrous ethanol. The catalyst concentration (H_2SO_4) for esterification was 1.5% w/v.

3.5.2 Pervaporation-assisted esterification

The experimental set-up used in this work was shown in figure 7. Pervaporation experiments were performed using a stirred tank reactor of 2 L capacity. The membrane was installed in an Aluminum permeation cell with an effective membrane area in contact with the feed mixture of 288 cm^2 (16 cm x 18 cm). The temperature of the feed liquid mixture was kept constant by using a thermostat (Sartorius, Germany). In pervaporation processes, concentration polarization was generally assumed to be of minor importance. Hence, the feed flow

rate across the membrane was chosen high enough to avoid mass transfer resistance from the bulk liquid phase to the feed membrane interface.

On the downstream side, the permeate was evaporated and was condensed by using two parallel glass cold traps filled with liquid nitrogen to ensure that all permeates were fully collected. This phase change was achieved by lowering the partial pressure on the permeate side with the help of a vacuum pump. The downstream pressure was maintained around 1 mbar. The system was allowed to reach steady state before samples are collected. To reach the steady state faster, the membrane was kept in the membrane module overnight together with the feed mixture under a slight vacuum on the permeate side to reduce the risk of building folds. The permeation flux of each component ($P = mol/m^2h$) was gravimetrically determined at fixed time intervals by weighing mass of the permeate collected. The values reported were an average of three experiments. In each run, the feed concentration was considered constant due to the small amounts of permeate in comparison to the amount in the reservoir. This was verified by analyzing feed samples at the end of each experiment. A heating element was placed around the entrance of the cold trap to avoid freezing of the permeate that would block the entrance of the permeate vessels due to extremely low vapor pressure applied.

To study the effect of operating parameters; process temperature was varied from 35 - 85°C, feed water concentration was varied from 10-40%, catalyst concentration (H_2SO_4) was varied between 0-2.5% w/v, respectively. The effect of flux on the performance of separation factor was studied by changing the operating temperature and reactant ratio.

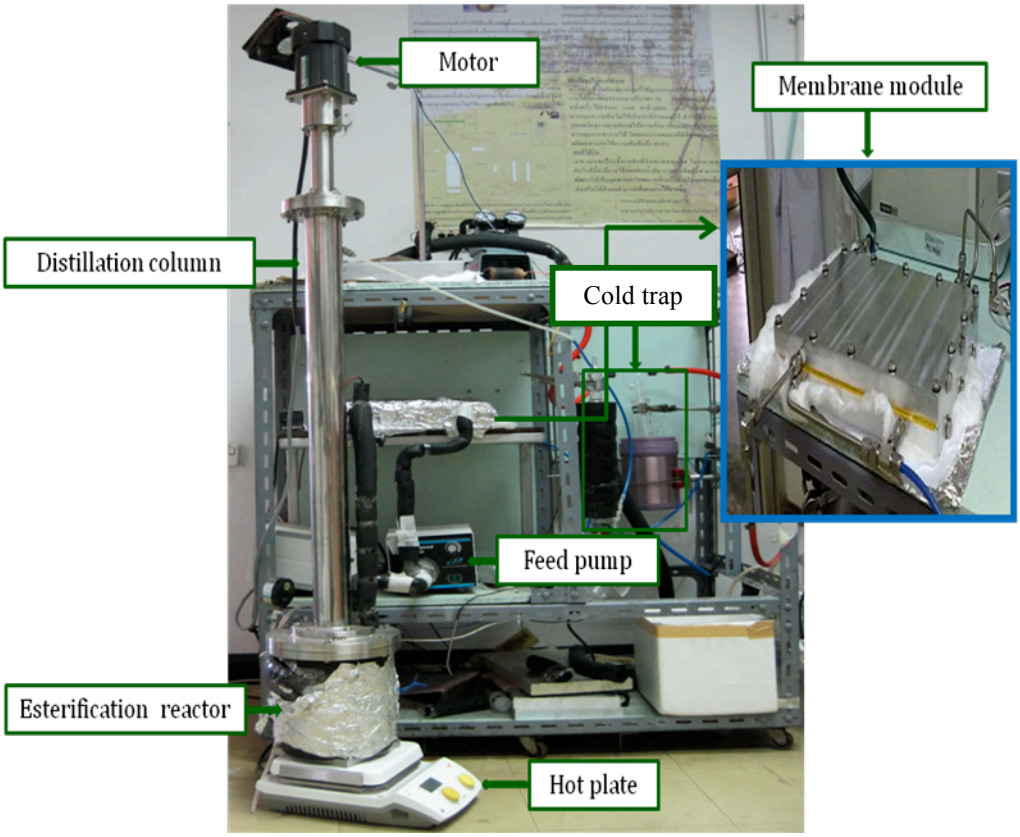


Figure 7 Experimental set-up for the pervaporation–assisted esterification.

3.5.3 Hydrolysis

The purified ethyl lactate was hydrolyzed with water to produce lactic acid in 2 L reactor equipped with a distillation column, and a condenser. The stirred reactor was operated at 350 rpm stirrer speed. The temperature of reactor was kept by a heater at 80 °C. The molar ratio of water to ethyl lactate was 15:1 (Sun *et al*, 2006).

3.6 Analytical procedures

3.6.1 Fermentation broth analysis

During fermentation, a 10 ml sample was collected once in 24 hr under aseptic conditions. After centrifugation, the supernatant was collected for analysis. Total titratable acidity (%) was estimated by titration with 0.1 N NaOH in the presence of phenolphthalein as indicator. The result was expressed as lactic acid and the yield (%) was calculated as grams of acid that were produced from reducing sugars.

$$Y_{PS} = \frac{g_{lactic}}{g_{substrate}} \text{ x } 100$$

(1) Reducing sugar present in the broth was estimated by using dinitrosalicylic acid- reagent.

(2) Growth and biomass was measured by optical density at 600 nm wavelength (OD_{600}) with spectrophotometer. Dry weight of biomass was calculated from a calibration curve between O.D. and cell dry weight.

3.6.2. Organic acids analysis

Organic acids was analyzed by HPLC (Agilent Technologies 1200 Series, USA). Quantification by UV detection was made at the wavelength of 210 nm. Samples was analyzed using a mobile phase 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. In addition, the enantiomer of lactic acid was analyzed by sumichiral OA column (4.6 mm × 150 mm). The mobile phase was 5% of 2mM copper (II) sulfate in water + 95% acetonitrile at a flow rate of 1 mL/min and the column oven was maintained at 35 °C . Quantification by UV detection was made at the wavelength of 254 nm.

3.6.3 Ethyl lactate and ethanol analysis

Ethyl lactate and ethanol concentrations were analyzed using a gas chromatograph (GC) equipped flame ionization (FID) detector (SRI Insturment, USA). Helium, 99.999% pure, was used as carrier gas. The GC column (PE-WAX) was a 30 m × 0.32 mm bonded phase fused silica capillary column. The injector and detectors were set at 200, and 300 °C, respectively. The oven was operated at programmed temperature, from 20 to 250 °C at the rate of 15 °C/min.

3.6.4 Moisture content analysis

The concentrations of water were determined by Karl-Fischer automatic titrator (Schott, Germany).

Figure 8 Experimental set-up for the Karl-Fischer automatic titrater.

CHAPTER IV

RESULTS AND DISCUSSIONS

4.1 Batch and Fed-batch Fermentation of L-(+)-lactic acid using *Pediococcus pentosaceus*

In a bioprocess development, two main streams are involved including upstream and downstream processes. Although this work focused on separation and purification of L-(+)-lactic acid from fermentation broth, the production step is also necessary. In addition, the use of homo-fermentative with high stereo-selectivity would facilitate further downstream processes. *P. pentosaceus* was successfully isolated and characterized in our laboratory. This strain possesses all desired characteristics of the above mentioned, and was used in fermentation processes of this work. Firstly, L-(+)-lactic acid fermentation in batch process was investigated. For the upstream process, 100 mL of inoculums was grown on MRS broth for 24 h and were aseptically transferred into 2 L bioreactor containing 900 mL of modified MRS broth using hydrolyzed starch as the main carbon source. The culture was grown anaerobically at 30 ^{o}C with agitation speed of 250 rpm. The time profile of reducing sugar concentration, cell concentration, and lactic acid concentration during fermentation were shown in figure 9. Formation of lactic acid resulted in lowering the pH, and undissociated form of the acid eventually leads to growth and product inhibition. Therefore, it is necessary to control the pH at above inhibitory level by a an addition of alkali solution into the bioreactor.

In this case, the pH was controlled at 6.0 by an automatic pH controller. The experimental result showed that sugar concentration constantly reduced with time which is the main characteristic of batch culture. Initial sugar concentration in which the cells experience during fermentation is very importance. Too high initial concentration results in substrate inhibition whereas too low initial concentration might result in low volumetric productivity. The bacteria assimilate carbon source, and cell concentration increased during fermentation. It was observed that *P. pentosaceus* possessed a relatively short lag phase followed by exponential phase. The growth entered its stationary phase at approximately 20 h where cell concentration reached plateau of approximately 3.0 g/L until the end of the fermentation process. During the bacterial growth, concentration of L-(+)-lactic acid (lactate) rapidly increased during the first 36 h, and then the concentration was constant followed the trend of bacterial growth. This product formation pattern indicated that lactic acid is the growth associated product. A favorable bioconversion pattern was observed in this case with a remarkable maximum concentration of lactate 49.4 g/L starting from 26.67 g/L reducing sugar and productivity was approximately 1.17 g/L.h. It yield was calculated to be as high as 1.65 $g_{product}/g_{substrate}$. The reason for this high yield was probably due to the bacteria possessed amylolytic activity which can digest oligosaccharides. However, cell growth depends on the nutrient content and the nutrient was limited in the batch process. For the upstream process, it is favorable to maximize both product yield and volumetric productivity of the fermentation process. Therefore, it is interesting to investigate a fed-batch mode where concentrated substrate solution is constantly added into the bioreactor.

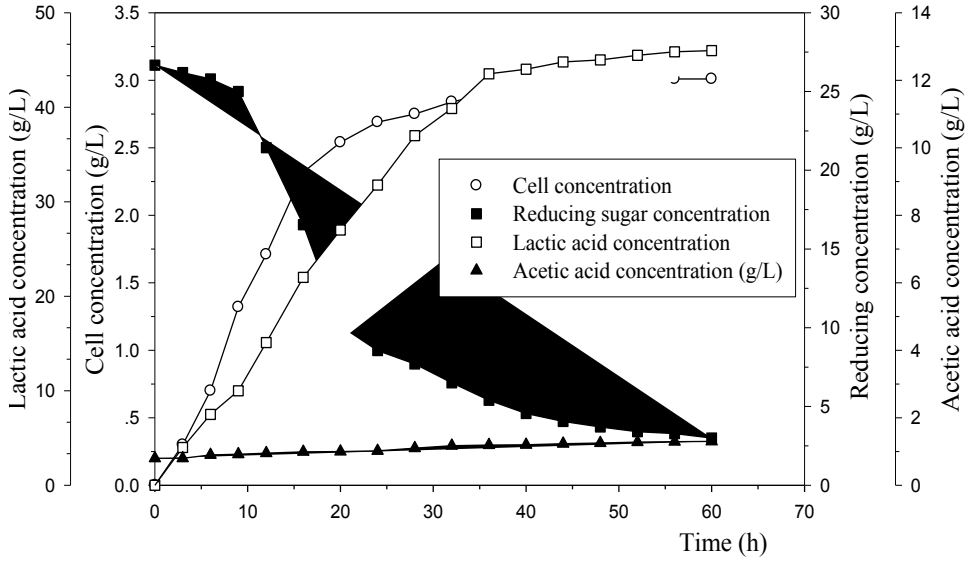


Figure 9 Time course of lactic acid production during batch fermentation by *P. pentosaceus* with controlled pH 6.0, temperature 30 ^{o}C.

Fed-batch process is a batch process fed continuously or sequentially with substrate without the removal of fermentation broth, which is generally superior to batch and continuous processing, It is especially beneficial when changing nutrient concentrations affects the productivity and biomass of the desired product (Lee *et al.*, 1999; Roukas and Kotzekidou, 1998). It was clear that substrate addition during the fermentation enhanced the biomass accumulation. There are three modes for the addition of substrate in fed batch fermentation namely intermittent feeding, constant feeding, and exponential feeding, respectively. However, process optimization of these three modes is beyond the scope of this study. Therefore, only intermittent feeding strategy was performed with the aim of increasing the final product concentration.

Fed-batch fermentation was carried out in two distinct modes. In the first mode, the *P. pentosaceus* cells were grown in batch mode by using a 2 L bioreactor with a working volume of 1.2 L (The production medium contains 26.67 g/L glucose) until the residual glucose concentration decreased to approximately 10 g/L. At this point, the growth entered early stationary phase. In the second mode, fed-batch process began with the intermittent feeding of 200 mL of feeding solution (800 g/L hydrolyzed starch supplemented with 15 g/L spent brewer's yeast extract and 2 mg/L of ferrous sulfate). This concentration was equivalent to 133 g/L reducing sugar concentration. The concentrated feeding solution was pumped into the bioreactor by using a peristaltic pump. In the experiment, samples were taken periodically before the cell concentration, residual glucose concentration, and lactate concentration were measured. As shown in figure 10, higher lactate concentration at the end of fermentation processes was obtained when compared to the process efficiency of the batch fermentation. Fermentation performance appeared to be two stages, an initial stage of the first 36 h in which cells produced lactate and utilized substrate very efficiently. This high volumetric productivity was the result of low inhibitory products and high availability of substrate, indicating that the substrate was efficiently converted into the lactate product. After an addition of the feeding solution, however, the lactate concentration slowly increased compared to the first stage. Although the inhibitory effect was minimized by the addition of an alkali solution, accumulation of lactate in the system result in increasing of osmotic pressure which might result in lowering the volumetric productivity. Substrate utilization rate was also lower at increasing lactate concentration resulting in the extended interval feeding time of the substrate solution. Nevertheless, the highest lactate concentration was obtained at

approximately 94.40 g/L, which was almost twice the lactate concentration from batch fermentation. From the figure, it was suggested that fermentation should be stopped after 90 h when the concentration of product was nearly constant. At the end of fermentation process, the broth typically contains bacterial cells, lactic acid product, residual reducing sugar, nitrogenous compounds, and some other impurities. All of these materials need to be completely removed during purification steps in order to produce pure lactic acid.

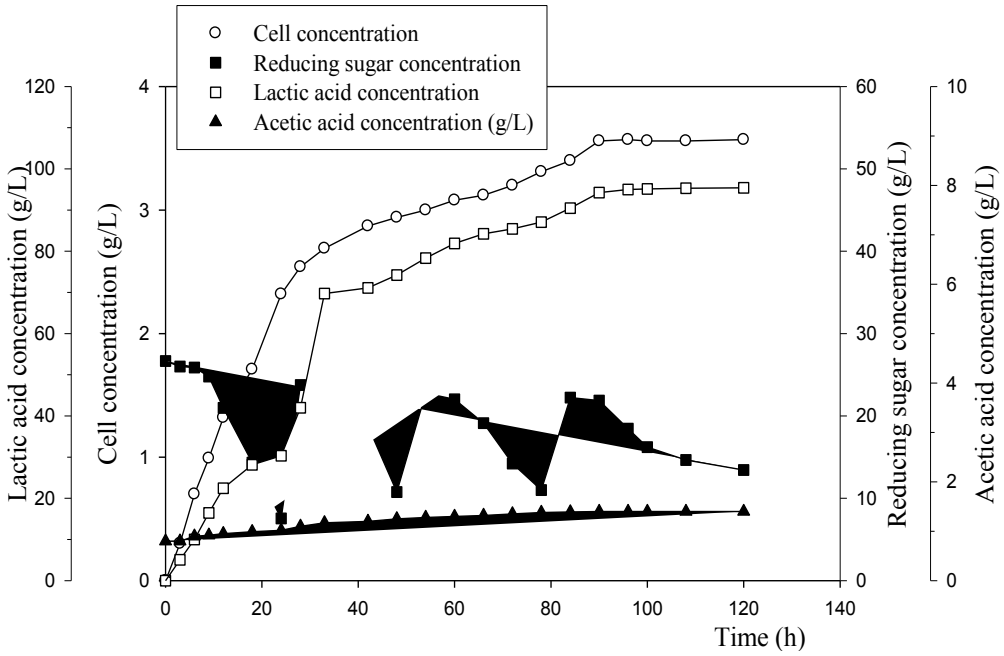


Figure 10 Time course of lactate production in fed-batch fermentation by *P. pentosaceus* with controlled pH 6.0, at 30 ^{o}C (Feeding solution was added at 24, 48, and 78 h, respectively).

4.2 Recovery of lactic acid from fermentation broth by using EDI process

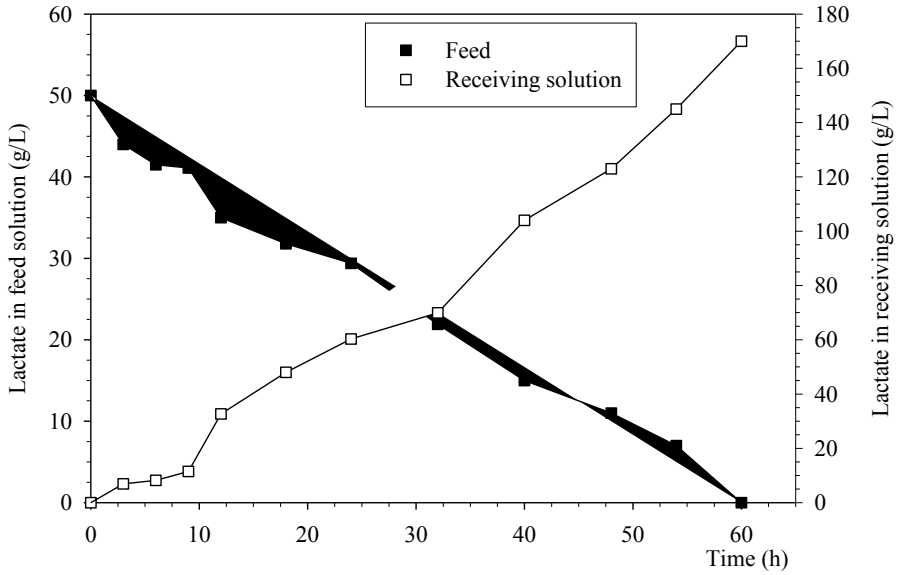
As mentioned earlier, all impurities have to be completely removed during purification processes. The early downstream steps may begin with separation of coarse materials and concentration of the lactate product. In this work, biomass was firstly removed from the fermentation broth by using microfiltration and concentration step was carried out by electrodeionization (EDI) technique. Since, lactate possesses negative charge, it can be separated from other uncharged materials by an application of direct electric current field coupling with anion and cation permeable membranes. During the experiment, voltage was kept constant at 15 Volte, 1.0 Ampere, and the process was terminated when the lactate of the feed was completely removed. Figure 11 shows the experimental results of concentration of lactate ions from clarified fermentation broth from batch and fed-batch fermentation processes. The concentrations of lactate from the feed and concentrate reservoir were analyzed by HPLC (feed solution from the bioreactor and receiving solution or concentration reservoir). The system was initially operated without direct electric current to ensure that there was no leakage between the connecting parts. Experimental results showed that there was some mass transfer of lactate across the membrane during the first 6 h, and the concentration of lactate in the concentrate reservoir increased to approximately 10 g/L before the concentration was constant. This was probably due to the natural diffusion caused by concentration difference between the feed and receiving solution. The concentration of lactate in the receiving solution rapidly increased with the introduction of direct electrical current. In general,

it was found that lactate concentration in the feed and product stream changed linearly with time.

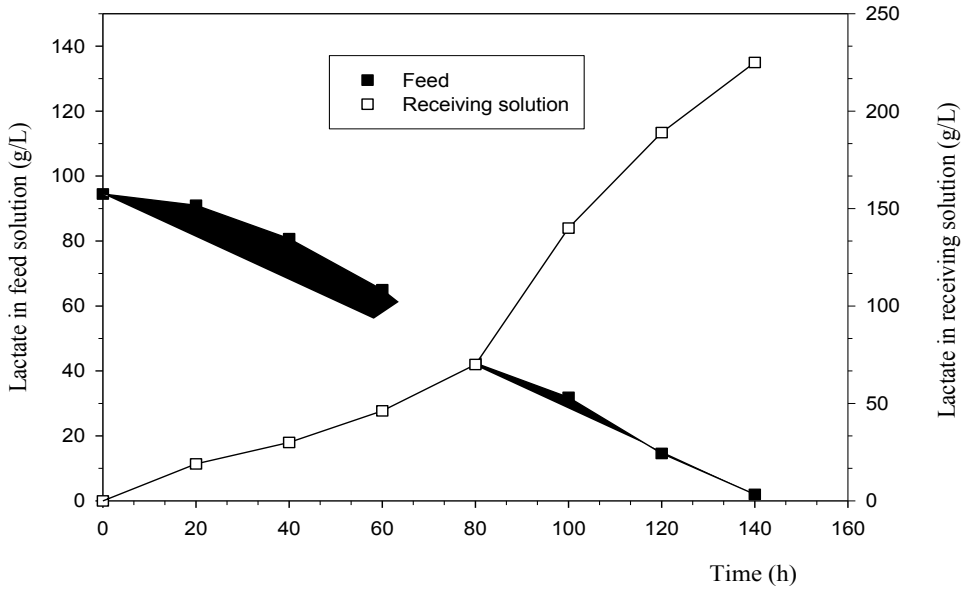
For the EDI experiment of the batch fermentation (Figure 11 A) where the initial lactate concentration was approximately 50 g/L. The volumetric mass transfer rate of lactate across the membrane module was calculated as 3.2 x 10^3 g/m^3.h, and steadily increased until the lactate concentration reached approximately 172 g/L. The volumetric productivity was approximately 2.98 g/L.h. Lactate concentration of the feed side also constantly decreased until most of lactate was transferred to the receiving solution, and the time for completing this operation was 60 h. In addition, there was no mass transfer of reducing sugar across the ion exchange membrane as observed by the DNS method. It is the fact that reducing sugar is an uncharged material, and can not migrate across the charged membrane. Moreover, the application of homo-fermentative bacteria result in the formation of only desired lactic acid. Other organic impurities produced by hetero-fermentative type will impede the separation efficiency of EDI process because they can also dissociate, and migrate across the ion exchange membrane as well. Nevertheless, the fermentation of lactic acid by this work contained a small amount of acetic acid, and also accumulated in the concentrate reservoir with the final concentration of approximately 1.40 g/L (data not shown). This organic acid impurity has to be removed by another technique which will be discussed in the subsequent section.

For the EDI experiment of fed-batch fermentation, the initial lactate concentration in the feed solution was approximately 95 g/L. The EDI performance follows the same trend as from the batch fermentation and the volumetric productivity was approximately 2.5 g/L.h. However, it was observed that operating time to

completely remove lactate ion from the feed solution was longer than batch fermentation experiment especially at the beginning of the process. The reason might probably be the application of the constant direct current applied throughout the experiment. Different current density results in different of mass transfer rate. Ideally, an EDI process can be assessed for continuous recovery of lactate from fermentation broth. However, thorough understanding of the working principle is very important to design the process system. As the ionic strength in the feed compartments of an EDI process decreases, there is a relative increasing in electrical resistance in both thin water layers bounding the mixed ion-exchange resins surface and ion exchange membrane-aqueous solution interface. Total ions concentration at these interfaces is a function of the concentration and diffusion rate of ions in the solution, the thickness of aqueous boundary layers, the relative transference numbers of ions in solution and in the resins/membranes, and the electric current (as referred in Equation $I = zFQ\Delta C/\eta$). Ions permeation through the membranes proceeds faster than in the boundary layers, therefore, the ions concentration may decrease until no ions is available for current transport through the solution, and then electrolysis occurs. Therefore, it is strongly recommended that at low lactate concentration, the EDI should be operated at constant voltage rather than constant current (Huang *et al*., 2007).



(A)



(B)

Figure 41 The concentration of lactate ion in the two phases of feed solution

and receiving solution of the EDI system (from batch fermentation (A), and from fed-batch fermentation (B).

4.3 Purification of L-(+)-lactic acid using esterification-assisted pervaporation technique

As mentioned earlier, esterification is the most powerful and effective technique to remove impurities especially organic acid contaminants. However, reaction of carboxylic group of the acid and hydroxyl group of the alcohol results in generation of the ester bond and water as the by product. The formation of water shifts the equilibrium backward, and severely lowers the esterification yield and volumetric productivity. In order to increase the esterification performance, one of the products has to be removed, either water or ethyl lactate itself. Because there are several published works concerning the separation of water with hydrophilic membranes, it is also interesting to investigate the separation of ethyl lactate from the esterification reaction by using hydrophobic membrane as well. By using this concept, ethyl lactate can be instantly removed from the reaction, and it can be further processed by using hydrolysis method to recover the pure form of lactic acid. In order to verify this concept, experimental works need to be carried out. In this section, concentrated fermentation broth obtained from EDI technique was evaporated until the water content reduced to approximately 20%w/v. This viscous solution was used as the feed stock for the whole experiments (if not stated). Esterification performances were compared between different modes.

4.3.1 Preliminary study of esterification reaction in batch mode

The objective of this experiment was to show the basic characteristic of the batch esterification reaction. The molar ratio of anhydrous ethanol to lactic acid was

3:1 and catalyst (sulphuric acid) concentration was 1.5%w/v. Figure 12 shows the concentration of acetic acid, lactic acid, ethyl lactate, ethyl acetate, and water during the batch esterification experiment. Experimental results showed that concentration of ethyl lactate was rapidly increased during the first 18 h followed by a much slower esterification rate. Ethyl lactate concentration increased to 30 g/L after 18 h, but the concentration increased to 39 only g/L at the end of the reaction (70 h). It could be explained that this lowered volumetric productivity was the effect of water formed during the reaction. This confirmed that when the water content in the system is increased, equilibrium of the reaction inversely shifts resulting in a decreasing of esterification efficiency. Ethyl acetate concentration showed a similar trend as ethyl lactate, and the maximal value was approximately 1.0 g/L after 70 h of the experiment. On the other hand, L-(+)-lactic acid and acetic acid concentration in reactor were decreased when the ester concentrations increased. However, the conversion rate of L-(+)-lactic acid to ethyl lactate was lower when the water concentration increased in the reactor. From the graph, it was observed that only 45% of the L-(+)-lactic acid was converted into ethyl lactate product. In addition, the reaction time was very long resulting in high energy input for the heating duty. As a result, further experimental work is highly recommended for removal of the excessive water from the reaction which could result in improvement of both esterification reaction yield and volumetric productivity.

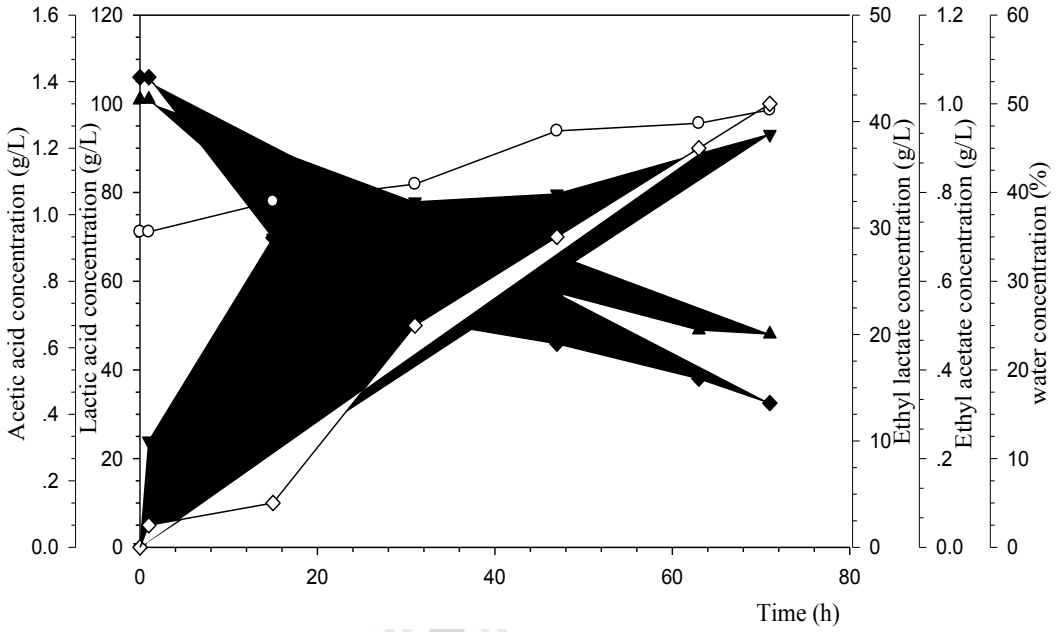


Figure 12 Time profiles of the concentration of acetic acid, lactic acid, ethyl lactate, ethyl acetate and water from fermentation broth using esterification technique (initial concentration of lactic acid was 101 g/l, the molar ratio of ethanol to lactic acid was 3:1 and catalytic concentration was 1.5%w/v) (◆ acetic acid,▲ lactic acid,▼ethyl lactate,◇ethyl acetate, and ○ water, respectively).

4.3.2 The effect of reactive distillation during esterification.

In order to obtain 100% conversion, reactive distillation is necessary to remove excessive water from the reaction system. Figure 13 shows the esterification performance during the reactive distillation with the repeated addition of anhydrous ethanol. Experimental results revealed that water concentration constantly decreased when anhydrous ethanol was excessively added into the reactor coupling with

continuous distillation of the water containing ethanol vapor. Owing to its high affinity to ethanol, water was continuously removed from the reaction. The equilibrium of the esterification reaction was then shifted forward resulting in higher conversion yield and higher esterification rate. The minimum water concentration was measured at 0.227 % at 11 h indicating that most of the lactic acid was converted to ethyl lactate. Ethyl acetate was not detected in the system because its boiling point is close to the boiling point of ethanol, and was distilled out of the system. However, a large quantity of anhydrous ethanol was used in this process. This implies that purification cost could be very high since anhydrous ethanol is very expensive. In addition, distillation of ethanol results in azeotropic mixture at 95.6 wt%, and dehydration of the remaining 4.4% of water is technically difficult.

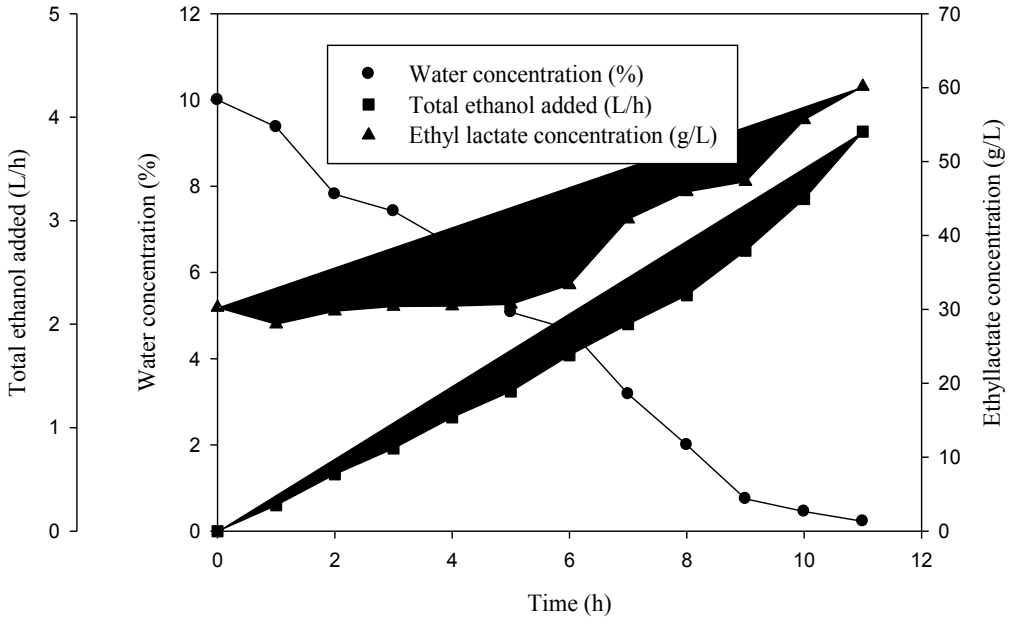


Figure 13 Time profile of water and ethyl lactate concentration during reactive distillation esterification reaction (initial water concentration 10%, catalytic concentration 1.5%w/v).

4.3.3 The effect of catalyst concentrations on ethyl lactate yield

Study of catalyst (sulphuric acid) concentration might be an alternative way to accelerate reaction rate, and esterification yield. In this experiment, the catalyst concentration was varied from 0-2.5%w/v. The other operating parameters were kept constant at temperature of 80 °C, initial L-(+)-lactic acid concentration of 30 g/L, and the molar ratio of ethanol to lactic acid was 3:1.

The effect of catalyst concentrations (%w/v) on the ethyl lactate yields are depicted in figure 14. From the graph, it was found that the effect of catalyst concentration did not have much effect on the esterification yield. During the esterification reaction, as the catalyst concentration increased, the ethyl lactate yield became higher, and the ethyl lactate yield was the highest at the catalyst concentration approximately 1.5%w/v. However, the ethyl lactate yield decreased when the catalyst concentration was above 1.5%w/v. The actual reason of this result was not clear, however, it might be as the following reason. In the reaction system where the catalyst concentration increases, the number of active species increases and the reaction velocity appears more quickly. The higher reaction velocity results in higher approach of reaction equilibrium. However, when the catalyst concentration increases more than its optimum point, some side-reactions are catalyzed which result in decreasing of the ethyl lactate yield. Therefore, the catalyst concentration of 1.5%w/v was the optimum value, and this concentration was used throughout the experiments.

Sun *et al.*, (2006) studied the effect of catalyst concentrations on ethyl lactate yield. They observed that for the ethyl lactate yield was highest at the concentration of

catalyst around 1.5%w/v and then the ethyl lactate yield was decreased when the catalyst concentration above 1.5%w/v.

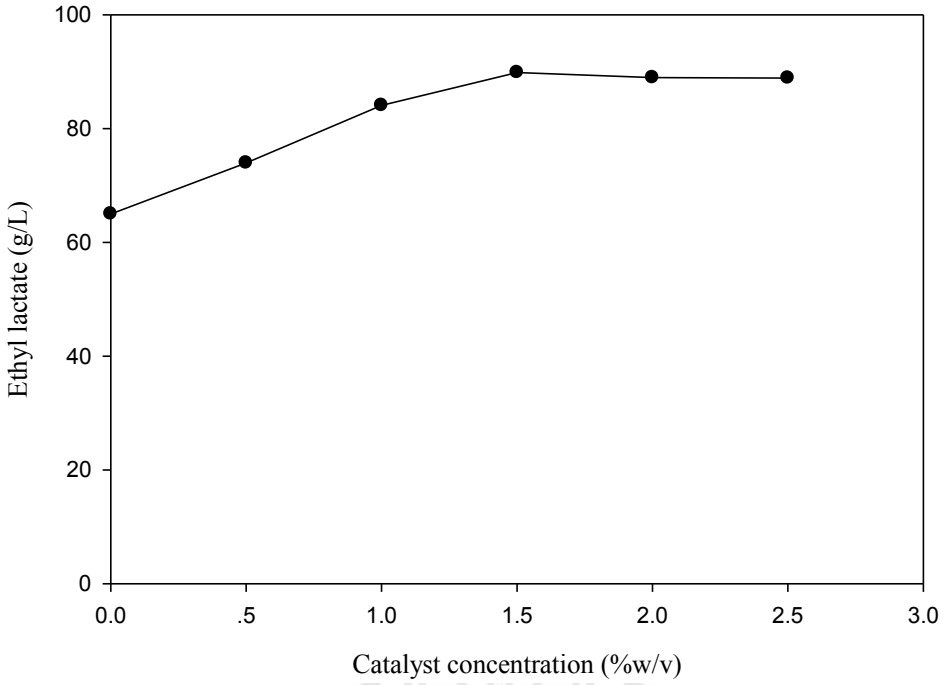


Figure 14 Effect of catalytic concentrations on ethyl lactate yield (esterification time 12 h and initial concentration of lactic acid 30%w/v).

4.3.4 The esterification-assisted pervaporation

In esterification-assisted pervaporation process, the important operating parameters are the flux across the membrane, and separation factor. Permeation fluxes of reacting species depend on the chemical property of the material employed to fabricate the top selective layer, the operating temperature, and feed composition. In this work, the commercial membrane was obtained from Sulzer Chemtech GmbH, Switzerland. Different operating conditions will be investigated for the separation performance.

4.3.4.1 Adsorption study of reacting components into the PDMS

In order to gain an insight into mass transfer characteristic of the reacting species across the polymeric membrane, determination for partition coefficient of membrane/aqueous (P_{aq}^{mem}) were carried out. In general, the degree of hydrophilicity of organic molecules is in the order of hydrocarbon < ether < ketone < ester < alcohol. When hydrophobic membrane is in contact with organic compound, it will adsorb the organic compound to varying degrees. This phenomenon results from the hydrophobic interaction between the organic solvent and the membrane phase. The polymer chain in the membrane is add to absorb organic solvent to varying degrees depending on the type of organic solvent used. In this experiment, the four reacting species were tested namely ethyl lactate, ethanol, L-(+)-lactic acid, and water. At equilibrium, the weight of the membrane then increased, and the increasing weight of individual membrane was compared. As expected for the case of water and L-(+)-lactic acid, the weight of the membrane piece was constant during the immersion indicating that there was no adsorption onto the membrane matrix. On the other hand, the weight of the membrane of ethanol and ethyl lactate were increased by 8% and 4%, respectively. This consequence implied that ethanol was absorbed better than ethyl lactate by the membrane. Since ester possesses higher degree of hydrophobicity than alcohol, ethyl lactate should be absorbed more than ethanol. However, since molecular weight of ethyl lactate is higher than ethanol, and the molecular size is also bigger. Therefore, it is more difficult to be adsorbed. As a result, this would be very

beneficial to the pervaporation process as organic compounds will be permeated at higher rate compared to water.

4.3.4.2 The effect of operating temperature and feed water concentrations on total permeation flux (Q_{total})

The effect of operating temperatures and feed water concentrations on total permeation flux is shown in figure 15. The experiments were carried out at operating temperatures between 35-85°C, feed water concentration of 10 - 40%, catalyst concentration of 1.5%w/v and membrane area was 288 cm^2, respectively. The volumetric flow rate was kept constant at 2 L/min just to ensure the homogeneity of the reaction. The experimental results revealed that the most influencing parameter which effects the total permeate flux was the operating temperature. The total permeate flux was exponentially increased with an increasing temperature. As the consequence, the driving force of all permeating species across the membrane increases because of the increasing in partial vapor pressure of each component. This increasing of the driving forces resulting in increasing of the total permeate fluxes. The highest total permeate flux was obtained at 5.345 $kg/m^2.h$ at 10% feed water concentration, and temperature of 80-85^oC, respectively. In addition, an increase in the operating temperature causes an increase in the motion of the polymer chains. As a result, diffusivity of the permeating molecules was improved. For mathematical consideration, the temperature dependence of the total permeation flux (Q_{total}) can be expressed by the Arrhenius-type relationship as followed;

$$Q_{total} = Q_0 exp\left(-\frac{E_p}{RT}\right) \quad (11)$$

Where E_p is the apparent activation energy of permeation, Q_0 the pre-exponential Arrhenius factor (kg/m^2.h), R is gas constant (kJ/mol K), and T the absolute temperature (K), respectively. The activation energy can be calculated from the slope of the logarithm of overall permeation fluxes versus the inverse absolute temperature at different feed compositions as shown in figure 16. The apparent activation energy of the total permeation of 42.65 kJ/mol was found from the average value of the slopes at different water concentrations in the feed (range of variation: 42.62-42.67 kJ/mol).

In addition, total permeate flux increased when water concentration of the reaction solution decrease. It is the fact that lowered concentration of water results in increasing the hydrophobicity of the feeding solution. This phenomenon could also be explained by the swelling of the hydrophobic membrane pore in the presence of ethyl lactate and ethanol. The polymer chains of the membrane matrix stretch at different degree depending on the operating parameters including organic types, organic concentrations, and temperature. For the effect of different organic species, this phenomenon is also called the plasticizing effect. This effect is represented by interaction parameters, when component *i* equal to component *j*. Positive interaction parameters correspond to a positive plasticizing effect. For component *i* equal to component *j*, the interaction parameters represent the influence of the components on mass transport of each other. A positive value here means a positive effect of the component on the permeation flux of another.

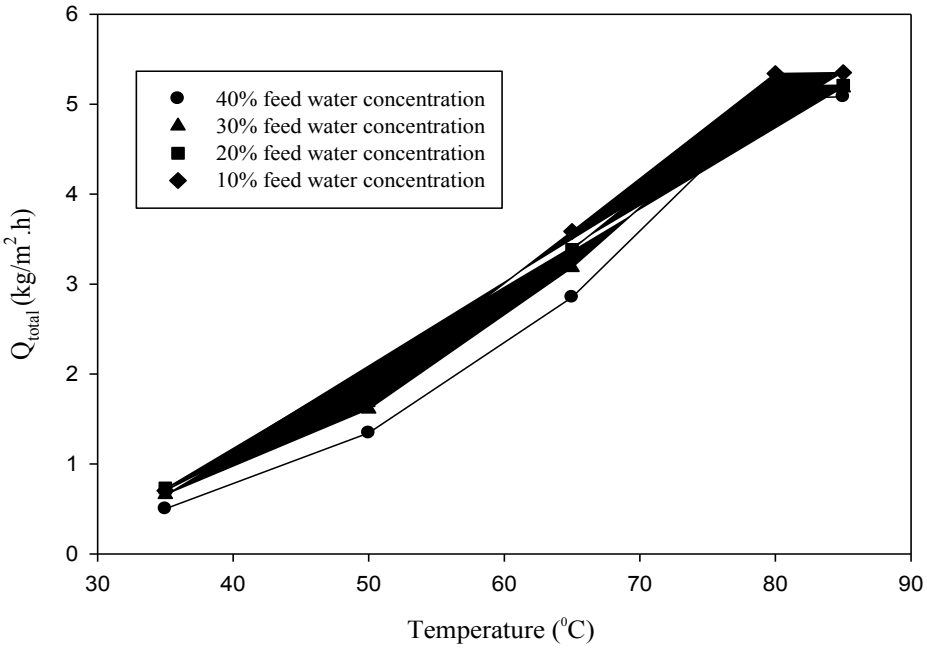


Figure 15 Influence of operating temperatures on total permeate fluxes at different feed water concentrations.

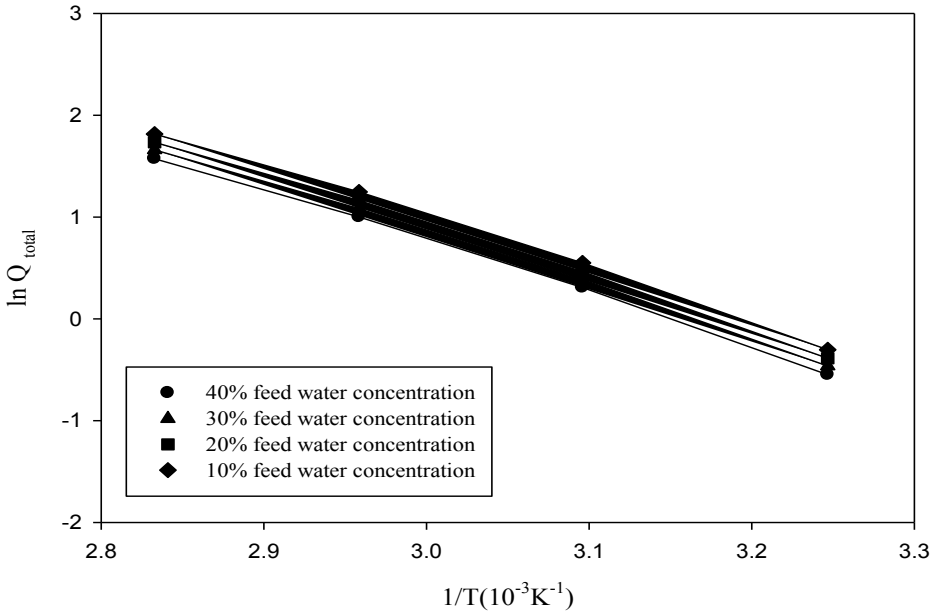


Figure 16 Temperature dependence of total permeation flux at various feed water concentrations using Arrhenius-type relationship.

4.3.4.3 The effect of operating temperature and feed water concentration on permeance of ethyl lactate ($P_{ethyl\ lactae}$)

The simulation results for effect of reaction temperatures and feed water concentrations on ethyl lactate permeance are shown in figure 17. The temperature was varied from 35-85°C. The feed water concentration was also varied from 10-40% with a constant value of catalyst concentration (1.5%w/v), and membrane area of 288 cm^2. It can be observed from figure 18 that ethyl lactate permeance increased with an increasing in temperature. The highest ethyl lactate permeance was obtained at 18.85 $mol/m^2.h.kPa$ at 80-85°C. Therefore, the optimum temperature for this operation was 80 °C. An increase in temperature induced not only an acceleration of esterification yield but also acceleration in pervaporation flux. Asuming Arrhenius dependence on temperature for the permeane of ethyl lactate an average apparent activation energy of permeation of 41.16 kJ/mol was obtained (figure 18) (rang of variation: 39.36-42.51 kJ/mol).

Ethyl lactate permeance was increased at higher temperature, so the permeance parameter for ethyl lactate was the function of temperature. As a result, ethyl lactate permeance was increased with an increase in temperature.

In addition, ethyl lactate permeances of the four investigated temperatures are presented in figure 17 as a function of water concentration in the feed. Ethyl lactate permeance increases when feed water concentration decrease because of a higher swelling degree of the hydrophobic membrane pore. However, degree of pore swelling measurement was beyond the scope of this study. It should be kept in mind that the permeation driving force for ethyl lactate, i.e., feed partial ethyl lactate vapor

pressure minus the partial permeate pressure, is smaller than the permeation driving force for the ethanol.

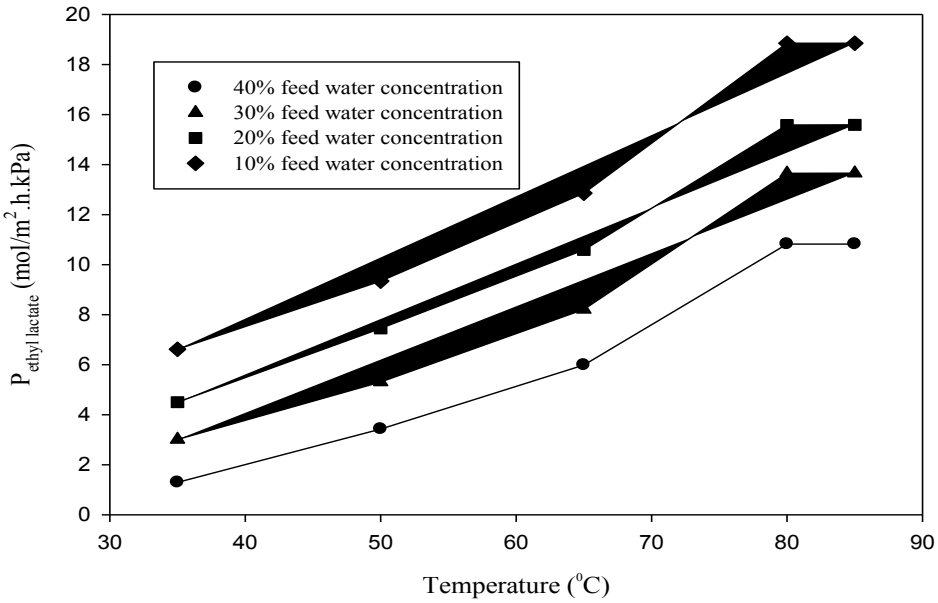


Figure 17 Influence of operating temperatures on ethyl lactate permeances at different feed water concentrations.

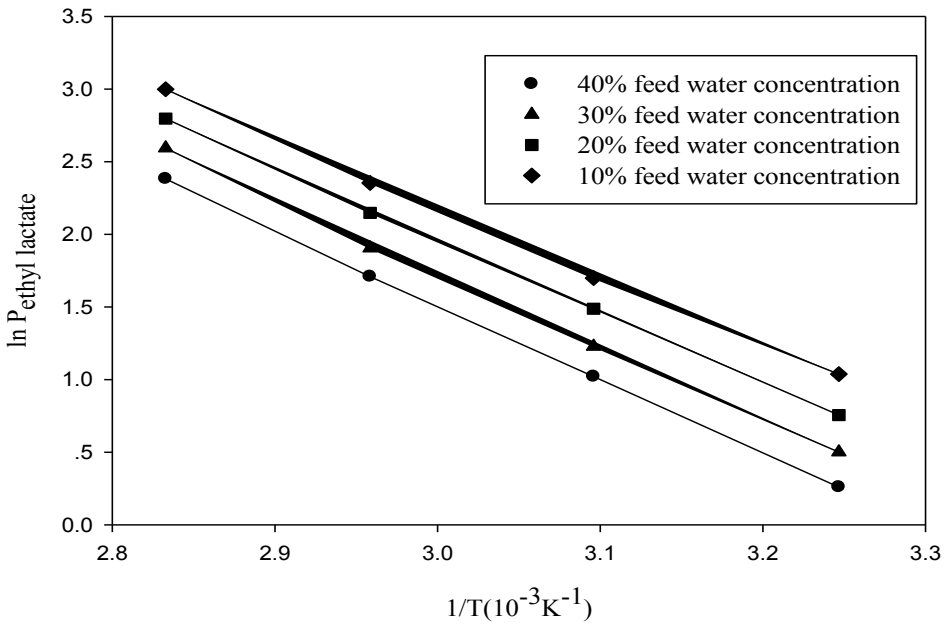


Figure 18 Temperature dependence of ethyl lactate permeance at various feed water concentrations using Arrhenius-type relationship.

4.3.4.4 The effect of operating temperature and feed water concentration on permeance of ethanol ($P_{ethanol}$)

The ethanol permeance is presented in figure 19 as a function of temperature at the four different feed water concentrations. As expected, ethanol permeance increases with temperature and decrease with increasing water concentration in the feed. In figure 18, higher values of ethanol permeances were observed compared to the permeance of ethyl lactate in the pervaporation process. The higher value of ethanol premeance is represented by the higher in permeability than ethyl lactate. The permeation of ethanol through the membrane is more complex than ethyl lactate because of the high number of interactions between the liquid feed components. Arrhenius-type dependence with temperature can be assumed obtaining and average apparent activation energy of 16.92 kJ/mol (variation range: 16.73-17.02 kJ/mol) (figure 20).

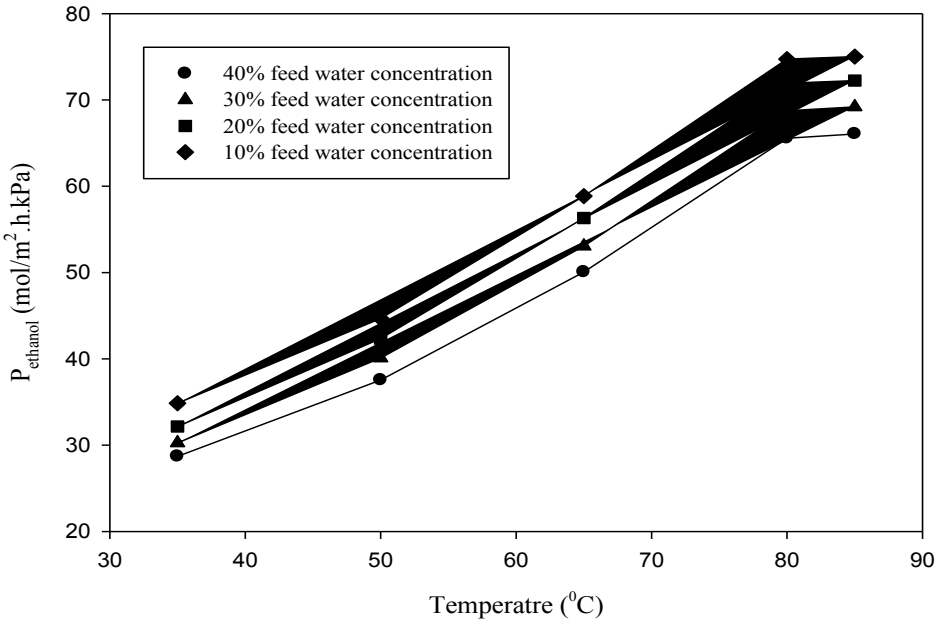


Figure 19 Influence of operating temperatures on ethanol permeance at different feed water concentrations.

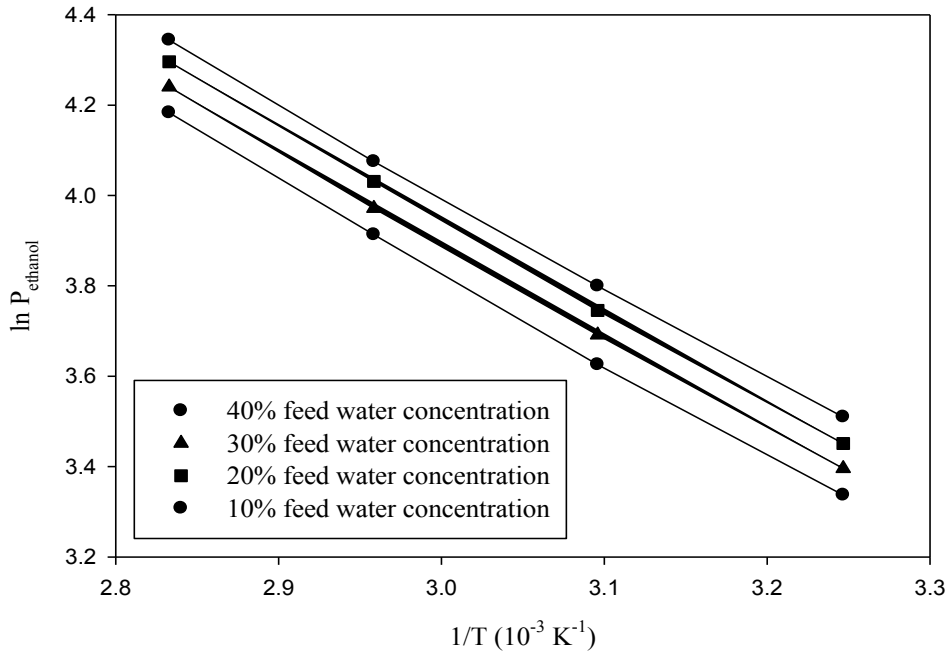


Figure 20 Temperature dependence of ethanol permeance at various feed water concentrations using Arrhenius-type relationship.

4.3.4.5 The effect of operating temperature and feed water concentration on permeance of water (P_{water})

Water permeance has been plotted in figure 21 versus the feed water content. Following the same trend of the other permeating species, it can be observed that permeances increased with temperature and feed water concentration. The values of water permeances were lower than ethanol permenaces, because of the extremely high hydrophilicity property of the water. An apparent activation energy of water permeance of 10.98 kJ/mol was found from the average value of the slopes at different water concentrations in the feed (range of variation: 10.88-11.13 kJ/mol) (figure 22).

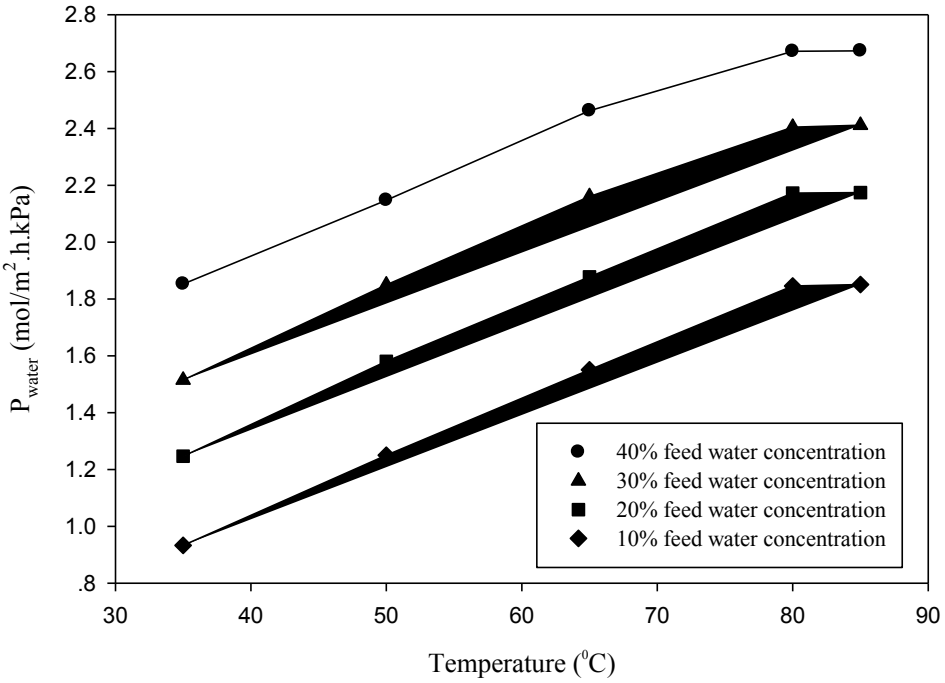


Figure 21 Influence of operating temperatures on water permeances at different feed water concentrations.

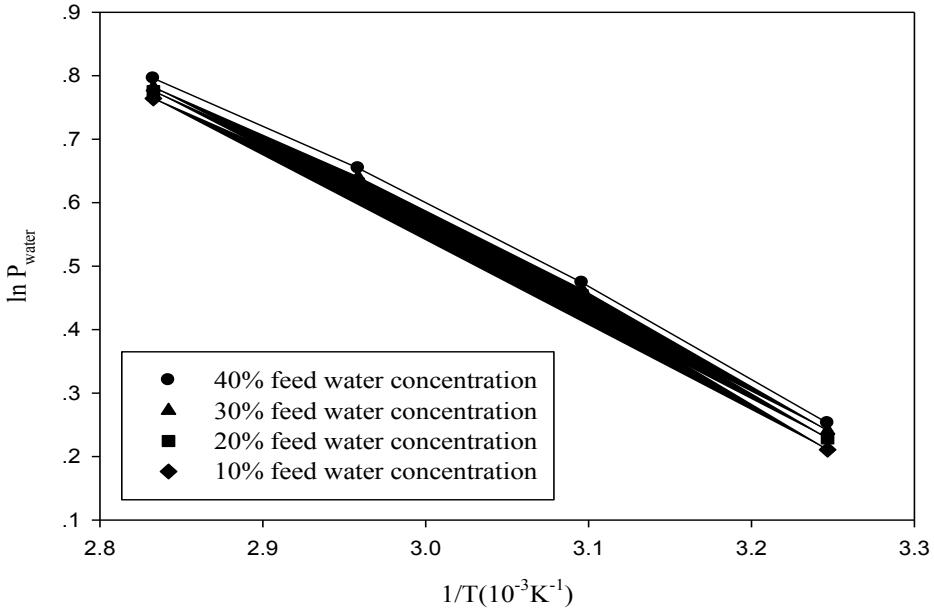


Figure 22 Temperature dependence of water permeance at various feed water concentrations using Arrhenius-type relationship.

4.3.4.6 The effect of operating temperature and feed water concentrations on separation factors

In figure 23, separation factors of the four different feed compositions have been plotted as a function of operating temperatures. In general, it was showed that separation factor exhibited an inverse trend compared with permeation fluxes. As a result, separation factors decreased at higher temperature of the feed solution. Therefore, process optimization to obtain the most suitable condition for this esterification-assisted pervaporation technique. Separation factor is strongly dependent on not only the temperature, but also on the feed water concentrations. In addition, the higher swelling degree of the membrane pore in contact with organic compounds facilitates the permeability of the different components leading to decrease in the separation factor. In addition, the separation factor increased with decreasing feed water composition of the same operating temperature. The reason for this different behavior from separation factor could be that while selectivity is mainly dominated by the membrane transport properties, separation factor takes into account the membrane transport properties and thermophysical properties of the feed mixture. Ethyl lactate concentration in feed increases with decreases feed water concentration, consequently the ethyl lactate permeance increases with decreasing water concentration in the feed. Separation factors of different feed water concentrations at 35 ^{o}C were obtained in the range between 2.7-3.4, and this characteristic was obtained for the other operating temperatures.

Regarding to the effect of temperature, it can be seen that temperature has a greater influential effect on separation factor compared to the effect of feed water concentration. In this system, separation factors dramatically decreased at elevated

temperature. The separation factors were obtained in the range between 1.3-3.3 depending on the temperatures and feed water concentrations. At 40% feed water concentration and temperature from 35 to 85 ^{o}C; for example, separation factor decreased from 3.3 to 1.7 which correspond to approximately 50% of the separation efficiency. However, separation factor is not the issue of interest because the permeation flux of ethyl lactate is the most important. This is because the required flux is related to the required membrane area and directly involves the investment cost.

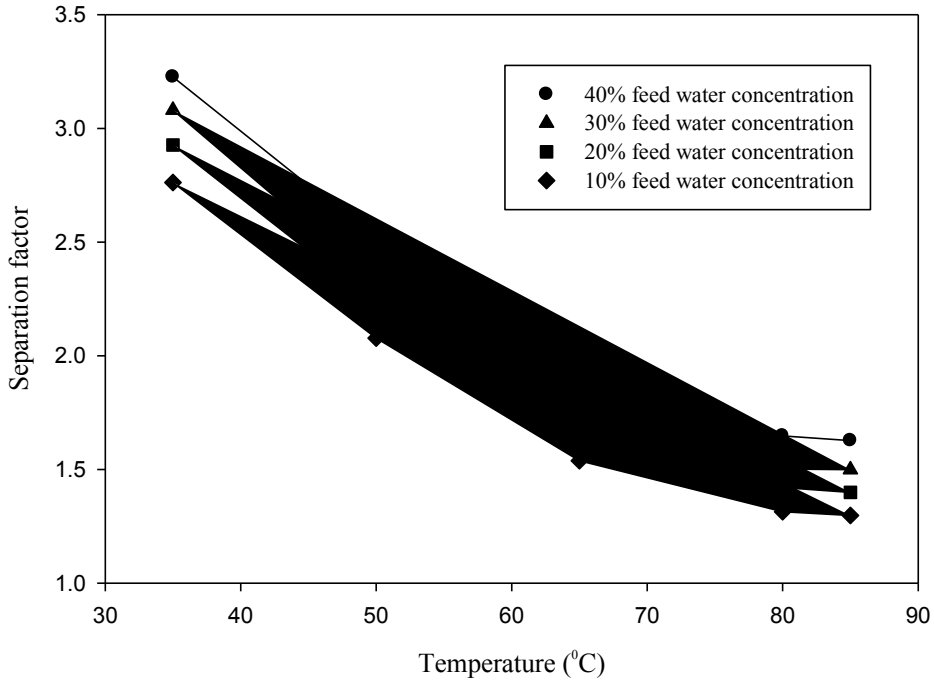


Figure 23 Influence of operating temperatures on separation factor at different feed water concentrations.

4.4 The hydrolysis of ethyl lactate

The lactic acid yield of hydrolysis process is shown in figure 23, under the operating temperature of 80°C and the molar ratio of water to ethyl lactate was 15:1. The hydrolysis reactor was coupled with a distillation column. In hydrolysis reaction, the ethyl lactate will react with water to produce lactic acid and ethanol. Therefore, ethanol and water in the reaction can be removed by the distillation column. Hydrolysis is a simple process, and variation of operating parameter is not necessary. The results showed that the maximal value of lactic acid was obtained at 91%w/v L-(+)-lactic acid. The productivity of L-(+)-lactic acid was approximately 179.83 g/L.h.

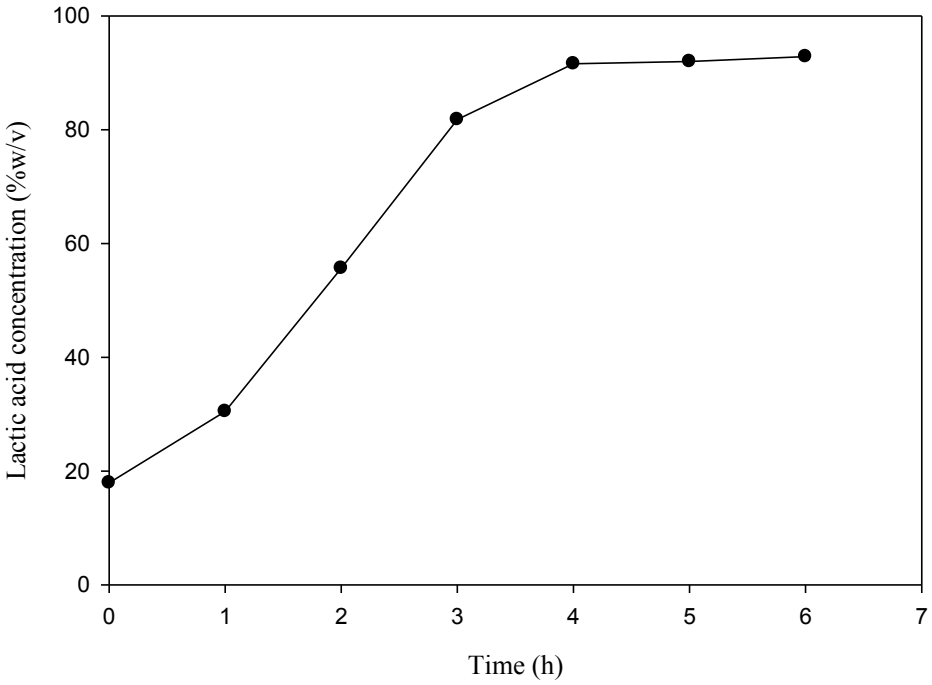


Figure 24 Time profiles of the lactic acid concentration on hydrolysis process (temperature was 80°C, the molar ratio of water to ethyl lactate was 15:1).

Figure 25 shows the images of samples taken during purification steps. The left was the evaporated fermentation broth which contained several organic acid impurities including a small amount of acetic acid. The middle was the permeate from the esterification-assisted pervaporation technique showing a clear and colorless characteristic. The right was the purified L-(+)-lactic acid with its light yellow color. This color was the result of high acid concentration which also made the solution very viscous. After this purification process, the L-(+)-lactic acid is suitable to be further investigation for the polymerization step with the main goal of bioplastic production. For figure 26 (A) shows the HPLC chromatogram of fermentration broth. This result showed the fermentation broth mainly contains impurities such as sugar(glucose) and other organic acid especially acetic acid. Therefore, this impurities have to be remove during purification process. In addition, HPLC chromatogram of enantiomer separation confirmed the homofermentative characteristic of the *P. pentosaceus* (figure 26 (B)). Acetic acid was not detected because it was removed from the system during distillation in the form of ethyl acetate. Moreover, only 2 peaks of D-(-)- and L-(+)-lactic acid were detected with the concentration ratio of 5 to 95 %, respectively. This result showed a very high optical purity of L-(+)-lactic acid was produced.

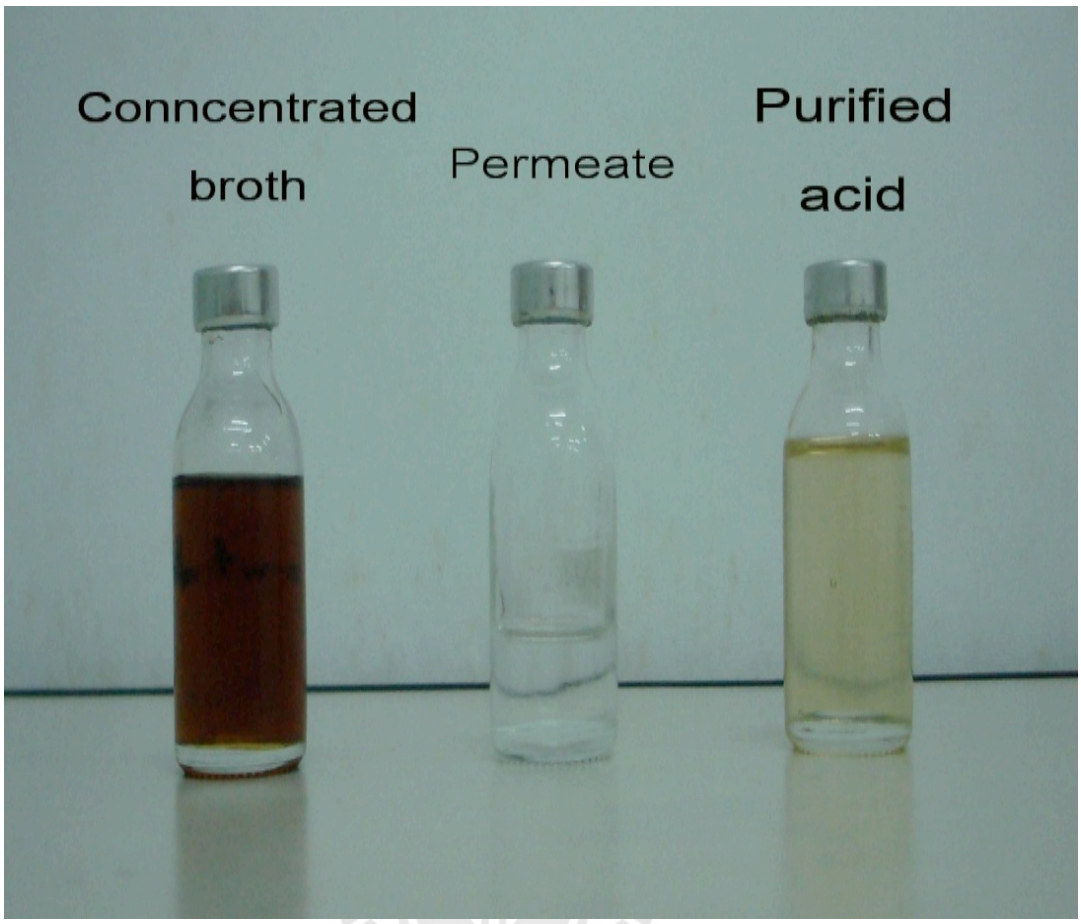
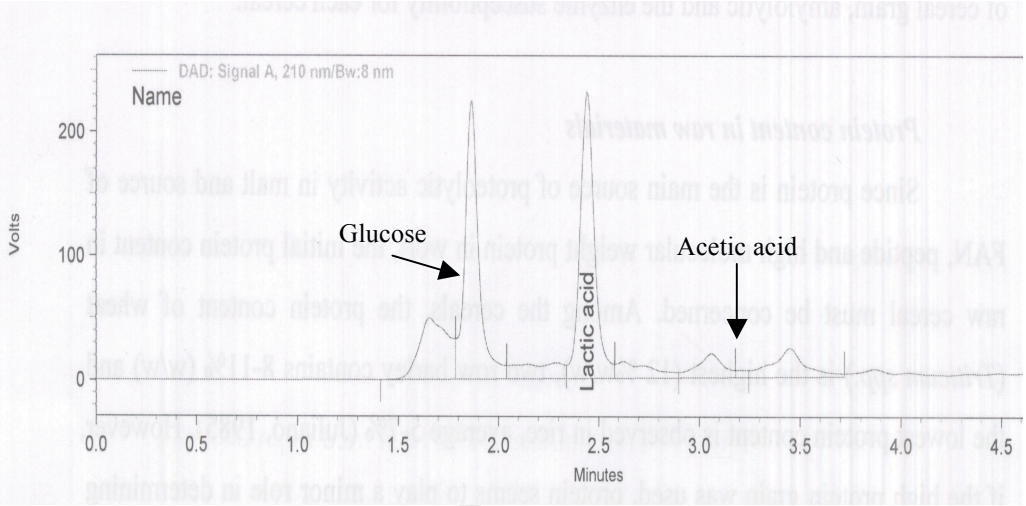
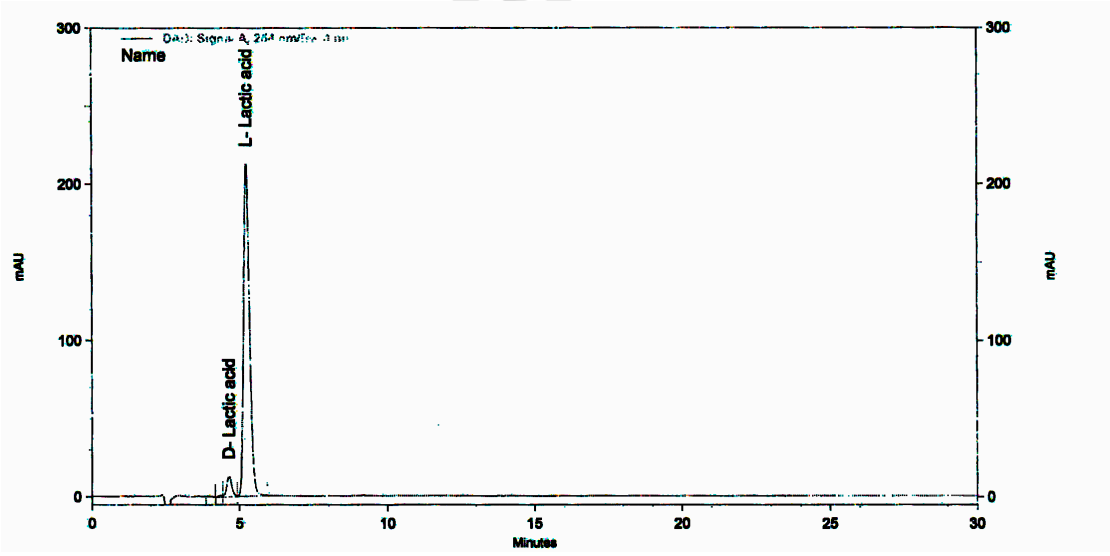


Figure 25 Image of samples taken during purification processes of lactic acid showing the concentrated fermentation broth (Left), permeate (Middle), and purified L-(+)-lactic acid after hydrolysis (Right).



(A)



(B)

Figure 26 HPLC chromatogram of fermentation broth (A) and chiral separation shows more than 95% optical purity of purified L-(+)-lactic acid (B).

4.5 Comparative productivity of downstream processing for lactic acid

Table 3 shows the productivity of lactic acid downstream processing. This result shows that the productivity of pervaporation – assisted esterification higher more than another technique. Therefore, the pervaporation – assisted esterification Pervaporation-assisted esterification process can improve productivity of esterification reaction.

Table 3 Productivity of downstream processing for lactic acid

Downstream process technique	Literature for downstream process	Productivity (g/L.h)
Esterification using rectifying column	Sun *et al.* (2006)	118.75
Esterification using zeolite A vapour permeation membrane	Jalal *et al.* (2002)	125.00
Esterification using zeolite T vapour permeation membrane	Kazuhiro *et al.* (2002)	131.58
Esterification using pervaporation reactor	Wasewar *et al.* (2009)	140.00
Pervaporation - assisted esterification using hydrophobic membeane	This research	179.83

In this case, the pH was controlled at 6.0 by an automatic pH controller. The experimental result showed that sugar concentration constantly reduced with time which is the main characteristic of batch culture. Initial sugar concentration in which the cells experience during fermentation is very importance. Too high initial concentration results in substrate inhibition whereas too low initial concentration might result in low volumetric productivity. The bacteria assimilate carbon source, and cell concentration increased during fermentation. It was observed that *P. pentosaceus* possessed a relatively short lag phase followed by exponential phase. The growth entered its stationary phase at approximately 20 h where cell concentration reached plateau of approximately 3.0 g/L until the end of the fermentation process. During the bacterial growth, concentration of L-(+)-lactic acid (lactate) rapidly increased during the first 36 h, and then the concentration was constant followed the trend of bacterial growth. This product formation pattern indicated that lactic acid is the growth associated product. A favorable bioconversion pattern was observed in this case with a remarkable maximum concentration of lactate 49.4 g/L starting from 26.67 g/L reducing sugar and productivity was approximately 1.17 g/L.h. It yield was calculated to be as high as 1.65 $g_{product}/g_{substrate}$. The reason for this high yield was probably due to the bacteria possessed amylolytic activity which can digest oligosaccharides. However, cell growth depends on the nutrient content and the nutrient was limited in the batch process. For the upstream process, it is favorable to maximize both product yield and volumetric productivity of the fermentation process. Therefore, it is interesting to investigate a fed-batch mode where concentrated substrate solution is constantly added into the bioreactor.

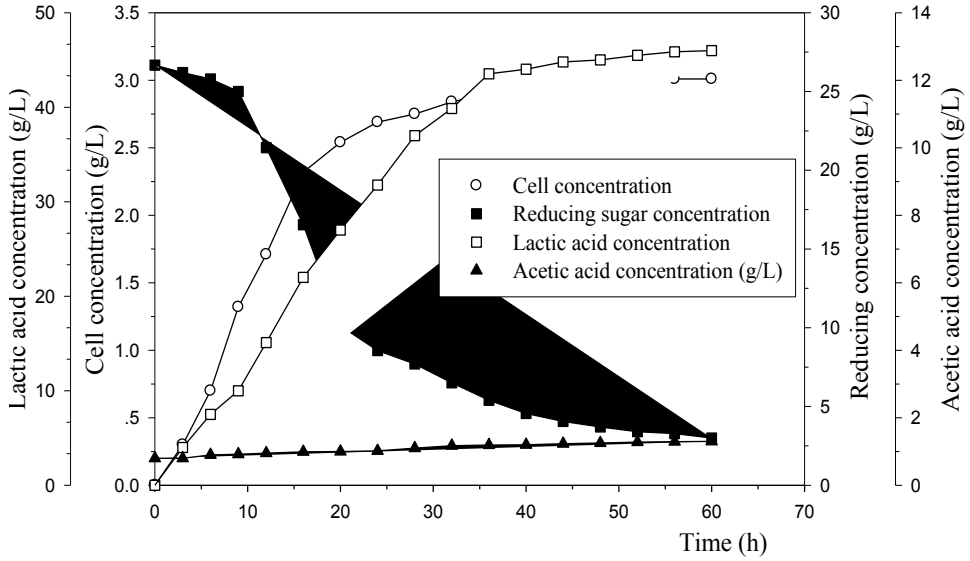


Figure 9 Time course of lactic acid production during batch fermentation by *P. pentosaceus* with controlled pH 6.0, temperature 30 ^{o}C.

Fed-batch process is a batch process fed continuously or sequentially with substrate without the removal of fermentation broth, which is generally superior to batch and continuous processing, It is especially beneficial when changing nutrient concentrations affects the productivity and biomass of the desired product (Lee *et al.*, 1999; Roukas and Kotzekidou, 1998). It was clear that substrate addition during the fermentation enhanced the biomass accumulation. There are three modes for the addition of substrate in fed batch fermentation namely intermittent feeding, constant feeding, and exponential feeding, respectively. However, process optimization of these three modes is beyond the scope of this study. Therefore, only intermittent feeding strategy was performed with the aim of increasing the final product concentration.

Fed-batch fermentation was carried out in two distinct modes. In the first mode, the *P. pentosaceus* cells were grown in batch mode by using a 2 L bioreactor with a working volume of 1.2 L (The production medium contains 26.67 g/L glucose) until the residual glucose concentration decreased to approximately 10 g/L. At this point, the growth entered early stationary phase. In the second mode, fed-batch process began with the intermittent feeding of 200 mL of feeding solution (800 g/L hydrolyzed starch supplemented with 15 g/L spent brewer's yeast extract and 2 mg/L of ferrous sulfate). This concentration was equivalent to 133 g/L reducing sugar concentration. The concentrated feeding solution was pumped into the bioreactor by using a peristaltic pump. In the experiment, samples were taken periodically before the cell concentration, residual glucose concentration, and lactate concentration were measured. As shown in figure 10, higher lactate concentration at the end of fermentation processes was obtained when compared to the process efficiency of the batch fermentation. Fermentation performance appeared to be two stages, an initial stage of the first 36 h in which cells produced lactate and utilized substrate very efficiently. This high volumetric productivity was the result of low inhibitory products and high availability of substrate, indicating that the substrate was efficiently converted into the lactate product. After an addition of the feeding solution, however, the lactate concentration slowly increased compared to the first stage. Although the inhibitory effect was minimized by the addition of an alkali solution, accumulation of lactate in the system result in increasing of osmotic pressure which might result in lowering the volumetric productivity. Substrate utilization rate was also lower at increasing lactate concentration resulting in the extended interval feeding time of the substrate solution. Nevertheless, the highest lactate concentration was obtained at

approximately 94.40 g/L, which was almost twice the lactate concentration from batch fermentation. From the figure, it was suggested that fermentation should be stopped after 90 h when the concentration of product was nearly constant. At the end of fermentation process, the broth typically contains bacterial cells, lactic acid product, residual reducing sugar, nitrogenous compounds, and some other impurities. All of these materials need to be completely removed during purification steps in order to produce pure lactic acid.

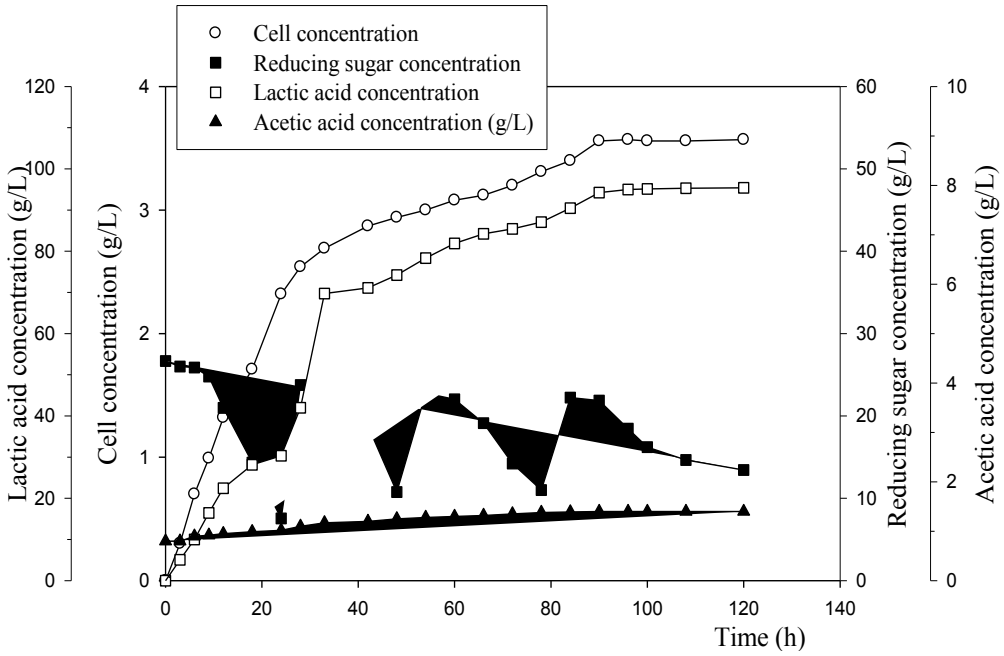


Figure 10 Time course of lactate production in fed-batch fermentation by *P. pentosaceus* with controlled pH 6.0, at 30 ^{o}C (Feeding solution was added at 24, 48, and 78 h, respectively).

4.2 Recovery of lactic acid from fermentation broth by using EDI process

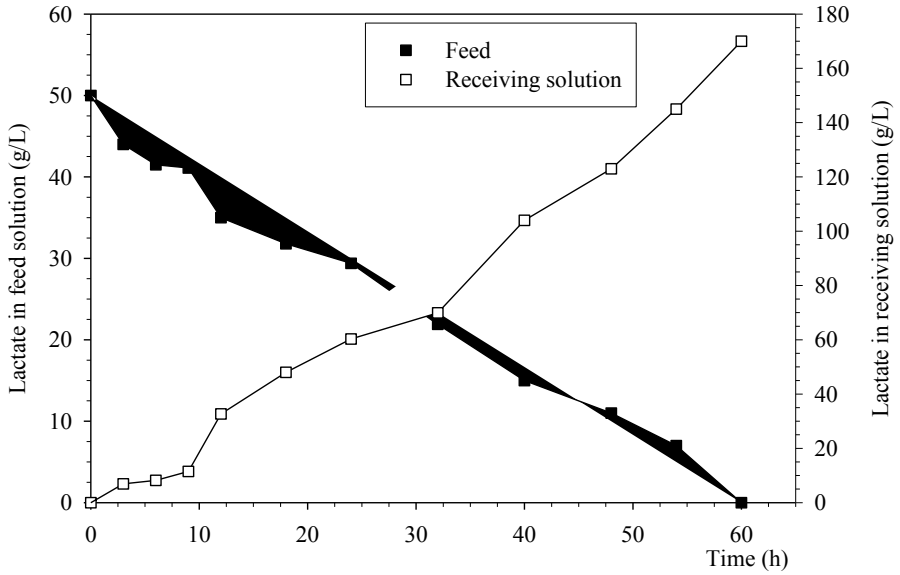
As mentioned earlier, all impurities have to be completely removed during purification processes. The early downstream steps may begin with separation of coarse materials and concentration of the lactate product. In this work, biomass was firstly removed from the fermentation broth by using microfiltration and concentration step was carried out by electrodeionization (EDI) technique. Since, lactate possesses negative charge, it can be separated from other uncharged materials by an application of direct electric current field coupling with anion and cation permeable membranes. During the experiment, voltage was kept constant at 15 Volte, 1.0 Ampere, and the process was terminated when the lactate of the feed was completely removed. Figure 11 shows the experimental results of concentration of lactate ions from clarified fermentation broth from batch and fed-batch fermentation processes. The concentrations of lactate from the feed and concentrate reservoir were analyzed by HPLC (feed solution from the bioreactor and receiving solution or concentration reservoir). The system was initially operated without direct electric current to ensure that there was no leakage between the connecting parts. Experimental results showed that there was some mass transfer of lactate across the membrane during the first 6 h, and the concentration of lactate in the concentrate reservoir increased to approximately 10 g/L before the concentration was constant. This was probably due to the natural diffusion caused by concentration difference between the feed and receiving solution. The concentration of lactate in the receiving solution rapidly increased with the introduction of direct electrical current. In general,

it was found that lactate concentration in the feed and product stream changed linearly with time.

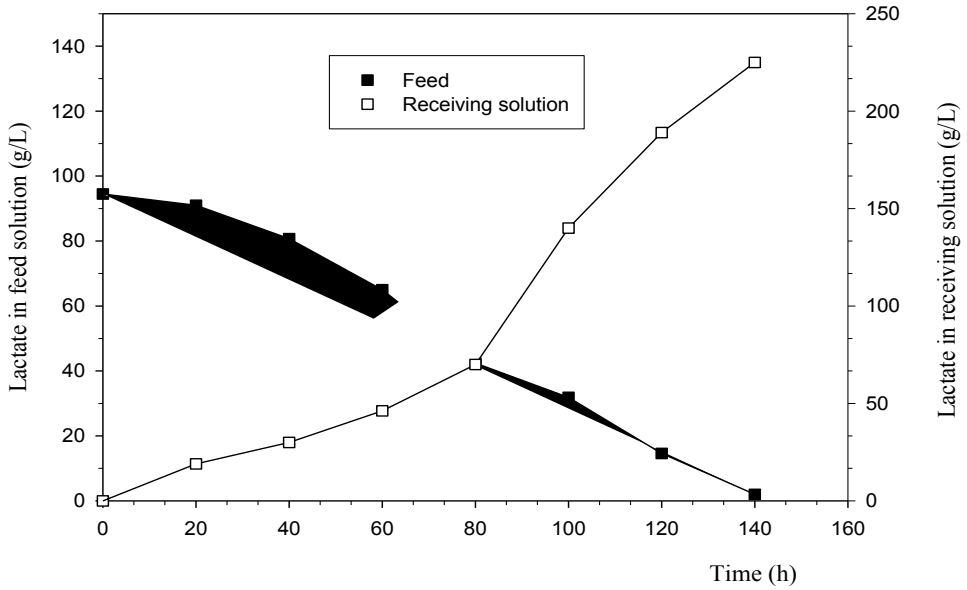
For the EDI experiment of the batch fermentation (Figure 11 A) where the initial lactate concentration was approximately 50 g/L. The volumetric mass transfer rate of lactate across the membrane module was calculated as 3.2 x 10^3 g/m^3.h, and steadily increased until the lactate concentration reached approximately 172 g/L. The volumetric productivity was approximately 2.98 g/L.h. Lactate concentration of the feed side also constantly decreased until most of lactate was transferred to the receiving solution, and the time for completing this operation was 60 h. In addition, there was no mass transfer of reducing sugar across the ion exchange membrane as observed by the DNS method. It is the fact that reducing sugar is an uncharged material, and can not migrate across the charged membrane. Moreover, the application of homo-fermentative bacteria result in the formation of only desired lactic acid. Other organic impurities produced by hetero-fermentative type will impede the separation efficiency of EDI process because they can also dissociate, and migrate across the ion exchange membrane as well. Nevertheless, the fermentation of lactic acid by this work contained a small amount of acetic acid, and also accumulated in the concentrate reservoir with the final concentration of approximately 1.40 g/L (data not shown). This organic acid impurity has to be removed by another technique which will be discussed in the subsequent section.

For the EDI experiment of fed-batch fermentation, the initial lactate concentration in the feed solution was approximately 95 g/L. The EDI performance follows the same trend as from the batch fermentation and the volumetric productivity was approximately 2.5 g/L.h. However, it was observed that operating time to

completely remove lactate ion from the feed solution was longer than batch fermentation experiment especially at the beginning of the process. The reason might probably be the application of the constant direct current applied throughout the experiment. Different current density results in different of mass transfer rate. Ideally, an EDI process can be assessed for continuous recovery of lactate from fermentation broth. However, thorough understanding of the working principle is very important to design the process system. As the ionic strength in the feed compartments of an EDI process decreases, there is a relative increasing in electrical resistance in both thin water layers bounding the mixed ion-exchange resins surface and ion exchange membrane-aqueous solution interface. Total ions concentration at these interfaces is a function of the concentration and diffusion rate of ions in the solution, the thickness of aqueous boundary layers, the relative transference numbers of ions in solution and in the resins/membranes, and the electric current (as referred in Equation $I = zFQ\Delta C/\eta$). Ions permeation through the membranes proceeds faster than in the boundary layers, therefore, the ions concentration may decrease until no ions is available for current transport through the solution, and then electrolysis occurs. Therefore, it is strongly recommended that at low lactate concentration, the EDI should be operated at constant voltage rather than constant current (Huang *et al*., 2007).



(A)



(B)

Figure 51 The concentration of lactate ion in the two phases of feed solution

and receiving solution of the EDI system (from batch fermentation (A), and from fed-batch fermentation (B).

4.3 Purification of L-(+)-lactic acid using esterification-assisted pervaporation technique

As mentioned earlier, esterification is the most powerful and effective technique to remove impurities especially organic acid contaminants. However, reaction of carboxylic group of the acid and hydroxyl group of the alcohol results in generation of the ester bond and water as the by product. The formation of water shifts the equilibrium backward, and severely lowers the esterification yield and volumetric productivity. In order to increase the esterification performance, one of the products has to be removed, either water or ethyl lactate itself. Because there are several published works concerning the separation of water with hydrophilic membranes, it is also interesting to investigate the separation of ethyl lactate from the esterification reaction by using hydrophobic membrane as well. By using this concept, ethyl lactate can be instantly removed from the reaction, and it can be further processed by using hydrolysis method to recover the pure form of lactic acid. In order to verify this concept, experimental works need to be carried out. In this section, concentrated fermentation broth obtained from EDI technique was evaporated until the water content reduced to approximately 20%w/v. This viscous solution was used as the feed stock for the whole experiments (if not stated). Esterification performances were compared between different modes.

4.3.1 Preliminary study of esterification reaction in batch mode

The objective of this experiment was to show the basic characteristic of the batch esterification reaction. The molar ratio of anhydrous ethanol to lactic acid was

3:1 and catalyst (sulphuric acid) concentration was 1.5%w/v. Figure 12 shows the concentration of acetic acid, lactic acid, ethyl lactate, ethyl acetate, and water during the batch esterification experiment. Experimental results showed that concentration of ethyl lactate was rapidly increased during the first 18 h followed by a much slower esterification rate. Ethyl lactate concentration increased to 30 g/L after 18 h, but the concentration increased to 39 only g/L at the end of the reaction (70 h). It could be explained that this lowered volumetric productivity was the effect of water formed during the reaction. This confirmed that when the water content in the system is increased, equilibrium of the reaction inversely shifts resulting in a decreasing of esterification efficiency. Ethyl acetate concentration showed a similar trend as ethyl lactate, and the maximal value was approximately 1.0 g/L after 70 h of the experiment. On the other hand, L-(+)-lactic acid and acetic acid concentration in reactor were decreased when the ester concentrations increased. However, the conversion rate of L-(+)-lactic acid to ethyl lactate was lower when the water concentration increased in the reactor. From the graph, it was observed that only 45% of the L-(+)-lactic acid was converted into ethyl lactate product. In addition, the reaction time was very long resulting in high energy input for the heating duty. As a result, further experimental work is highly recommended for removal of the excessive water from the reaction which could result in improvement of both esterification reaction yield and volumetric productivity.

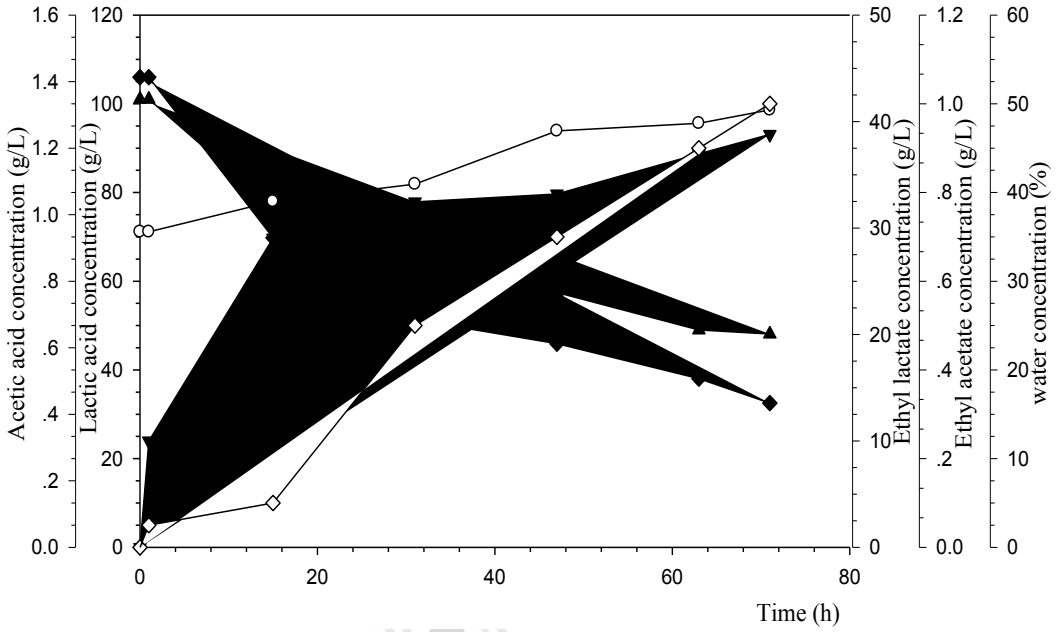


Figure 12 Time profiles of the concentration of acetic acid, lactic acid, ethyl lactate, ethyl acetate and water from fermentation broth using esterification technique (initial concentration of lactic acid was 101 g/l, the molar ratio of ethanol to lactic acid was 3:1 and catalytic concentration was 1.5%w/v) (◆ acetic acid,▲ lactic acid,▼ethyl lactate,◇ethyl acetate, and ○ water, respectively).

4.3.2 The effect of reactive distillation during esterification.

In order to obtain 100% conversion, reactive distillation is necessary to remove excessive water from the reaction system. Figure 13 shows the esterification performance during the reactive distillation with the repeated addition of anhydrous ethanol. Experimental results revealed that water concentration constantly decreased when anhydrous ethanol was excessively added into the reactor coupling with

continuous distillation of the water containing ethanol vapor. Owing to its high affinity to ethanol, water was continuously removed from the reaction. The equilibrium of the esterification reaction was then shifted forward resulting in higher conversion yield and higher esterification rate. The minimum water concentration was measured at 0.227 % at 11 h indicating that most of the lactic acid was converted to ethyl lactate. Ethyl acetate was not detected in the system because its boiling point is close to the boiling point of ethanol, and was distilled out of the system. However, a large quantity of anhydrous ethanol was used in this process. This implies that purification cost could be very high since anhydrous ethanol is very expensive. In addition, distillation of ethanol results in azeotropic mixture at 95.6 wt%, and dehydration of the remaining 4.4% of water is technically difficult.

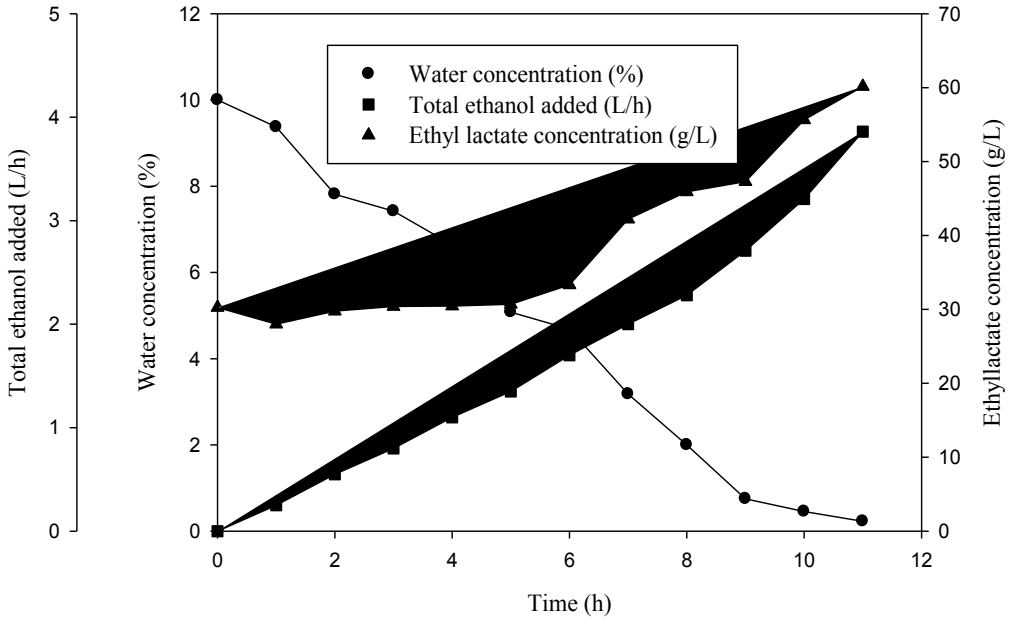


Figure 13 Time profile of water and ethyl lactate concentration during reactive distillation esterification reaction (initial water concentration 10%, catalytic concentration 1.5%w/v).

4.3.3 The effect of catalyst concentrations on ethyl lactate yield

Study of catalyst (sulphuric acid) concentration might be an alternative way to accelerate reaction rate, and esterification yield. In this experiment, the catalyst concentration was varied from 0-2.5%w/v. The other operating parameters were kept constant at temperature of 80 °C, initial L-(+)-lactic acid concentration of 30 g/L, and the molar ratio of ethanol to lactic acid was 3:1.

The effect of catalyst concentrations (%w/v) on the ethyl lactate yields are depicted in figure 14. From the graph, it was found that the effect of catalyst concentration did not have much effect on the esterification yield. During the esterification reaction, as the catalyst concentration increased, the ethyl lactate yield became higher, and the ethyl lactate yield was the highest at the catalyst concentration approximately 1.5%w/v. However, the ethyl lactate yield decreased when the catalyst concentration was above 1.5%w/v. The actual reason of this result was not clear, however, it might be as the following reason. In the reaction system where the catalyst concentration increases, the number of active species increases and the reaction velocity appears more quickly. The higher reaction velocity results in higher approach of reaction equilibrium. However, when the catalyst concentration increases more than its optimum point, some side-reactions are catalyzed which result in decreasing of the ethyl lactate yield. Therefore, the catalyst concentration of 1.5%w/v was the optimum value, and this concentration was used throughout the experiments.

Sun *et al.*, (2006) studied the effect of catalyst concentrations on ethyl lactate yield. They observed that for the ethyl lactate yield was highest at the concentration of

catalyst around 1.5%w/v and then the ethyl lactate yield was decreased when the catalyst concentration above 1.5%w/v.

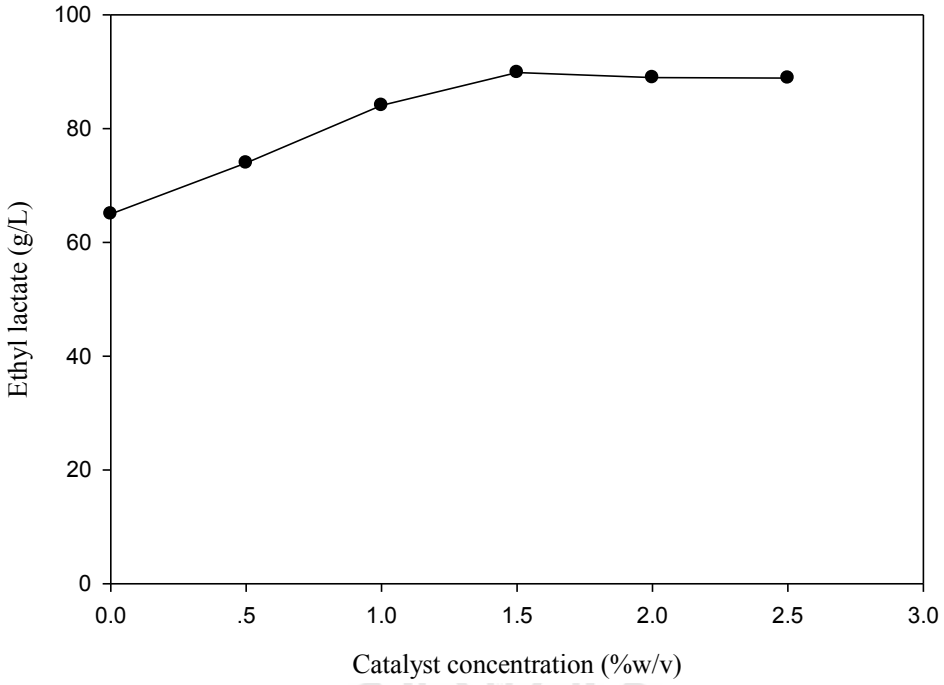


Figure 14 Effect of catalytic concentrations on ethyl lactate yield (esterification time 12 h and initial concentration of lactic acid 30%w/v).

4.3.4 The esterification-assisted pervaporation

In esterification-assisted pervaporation process, the important operating parameters are the flux across the membrane, and separation factor. Permeation fluxes of reacting species depend on the chemical property of the material employed to fabricate the top selective layer, the operating temperature, and feed composition. In this work, the commercial membrane was obtained from Sulzer Chemtech GmbH, Switzerland. Different operating conditions will be investigated for the separation performance.

4.3.4.1 Adsorption study of reacting components into the PDMS

In order to gain an insight into mass transfer characteristic of the reacting species across the polymeric membrane, determination for partition coefficient of membrane/aqueous (P_{aq}^{mem}) were carried out. In general, the degree of hydrophilicity of organic molecules is in the order of hydrocarbon < ether < ketone < ester < alcohol. When hydrophobic membrane is in contact with organic compound, it will adsorb the organic compound to varying degrees. This phenomenon results from the hydrophobic interaction between the organic solvent and the membrane phase. The polymer chain in the membrane is add to absorb organic solvent to varying degrees depending on the type of organic solvent used. In this experiment, the four reacting species were tested namely ethyl lactate, ethanol, L-(+)-lactic acid, and water. At equilibrium, the weight of the membrane then increased, and the increasing weight of individual membrane was compared. As expected for the case of water and L-(+)-lactic acid, the weight of the membrane piece was constant during the immersion indicating that there was no adsorption onto the membrane matrix. On the other hand, the weight of the membrane of ethanol and ethyl lactate were increased by 8% and 4%, respectively. This consequence implied that ethanol was absorbed better than ethyl lactate by the membrane. Since ester possesses higher degree of hydrophobicity than alcohol, ethyl lactate should be absorbed more than ethanol. However, since molecular weight of ethyl lactate is higher than ethanol, and the molecular size is also bigger. Therefore, it is more difficult to be adsorbed. As a result, this would be very

beneficial to the pervaporation process as organic compounds will be permeated at higher rate compared to water.

4.3.4.2 The effect of operating temperature and feed water concentrations on total permeation flux (Q_{total})

The effect of operating temperatures and feed water concentrations on total permeation flux is shown in figure 15. The experiments were carried out at operating temperatures between 35-85°C, feed water concentration of 10 - 40%, catalyst concentration of 1.5%w/v and membrane area was 288 cm^2, respectively. The volumetric flow rate was kept constant at 2 L/min just to ensure the homogeneity of the reaction. The experimental results revealed that the most influencing parameter which effects the total permeate flux was the operating temperature. The total permeate flux was exponentially increased with an increasing temperature. As the consequence, the driving force of all permeating species across the membrane increases because of the increasing in partial vapor pressure of each component. This increasing of the driving forces resulting in increasing of the total permeate fluxes. The highest total permeate flux was obtained at 5.345 $kg/m^2.h$ at 10% feed water concentration, and temperature of 80-85^oC, respectively. In addition, an increase in the operating temperature causes an increase in the motion of the polymer chains. As a result, diffusivity of the permeating molecules was improved. For mathematical consideration, the temperature dependence of the total permeation flux (Q_{total}) can be expressed by the Arrhenius-type relationship as followed;

$$Q_{total} = Q_0 exp\left(-\frac{E_p}{RT}\right) \quad (11)$$

Where E_p is the apparent activation energy of permeation, Q_0 the pre-exponential Arrhenius factor (kg/m^2.h), R is gas constant (kJ/mol K), and T the absolute temperature (K), respectively. The activation energy can be calculated from the slope of the logarithm of overall permeation fluxes versus the inverse absolute temperature at different feed compositions as shown in figure 16. The apparent activation energy of the total permeation of 42.65 kJ/mol was found from the average value of the slopes at different water concentrations in the feed (range of variation: 42.62-42.67 kJ/mol).

In addition, total permeate flux increased when water concentration of the reaction solution decrease. It is the fact that lowered concentration of water results in increasing the hydrophobicity of the feeding solution. This phenomenon could also be explained by the swelling of the hydrophobic membrane pore in the presence of ethyl lactate and ethanol. The polymer chains of the membrane matrix stretch at different degree depending on the operating parameters including organic types, organic concentrations, and temperature. For the effect of different organic species, this phenomenon is also called the plasticizing effect. This effect is represented by interaction parameters, when component *i* equal to component *j*. Positive interaction parameters correspond to a positive plasticizing effect. For component *i* equal to component *j*, the interaction parameters represent the influence of the components on mass transport of each other. A positive value here means a positive effect of the component on the permeation flux of another.

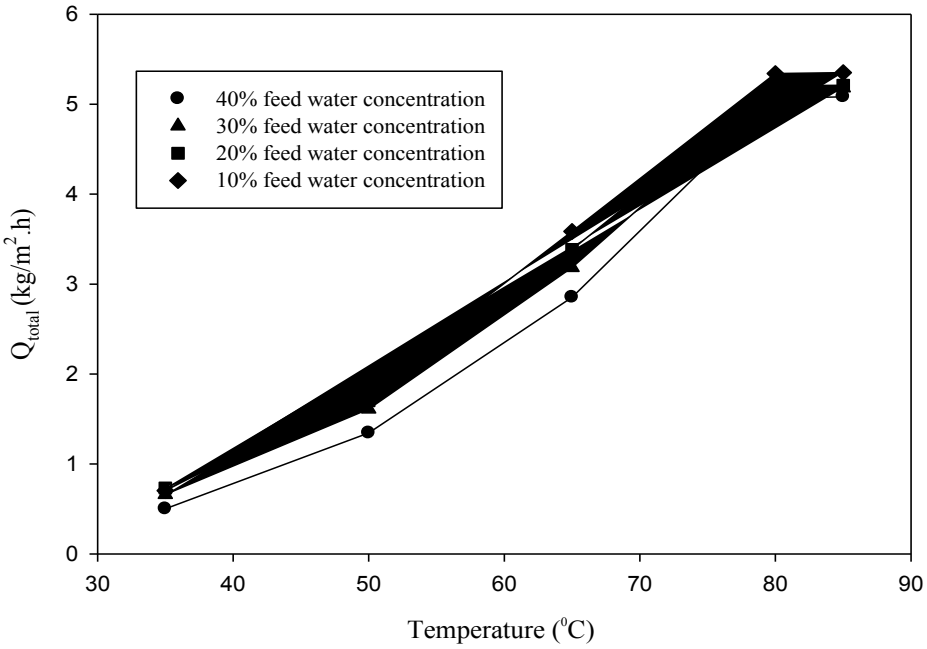


Figure 15 Influence of operating temperatures on total permeate fluxes at different feed water concentrations.

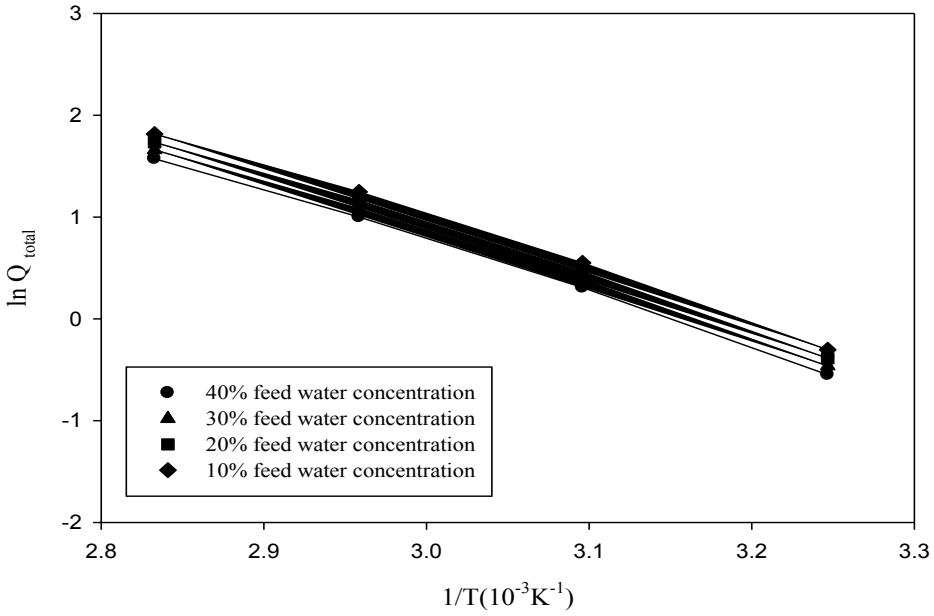


Figure 16 Temperature dependence of total permeation flux at various feed water concentrations using Arrhenius-type relationship.

4.3.4.3 The effect of operating temperature and feed water concentration on permeance of ethyl lactate ($P_{ethyl\ lactae}$)

The simulation results for effect of reaction temperatures and feed water concentrations on ethyl lactate permeance are shown in figure 17. The temperature was varied from 35-85°C. The feed water concentration was also varied from 10-40% with a constant value of catalyst concentration (1.5%w/v), and membrane area of 288 cm^2. It can be observed from figure 18 that ethyl lactate permeance increased with an increasing in temperature. The highest ethyl lactate permeance was obtained at 18.85 $mol/m^2.h.kPa$ at 80-85°C. Therefore, the optimum temperature for this operation was 80 °C. An increase in temperature induced not only an acceleration of esterification yield but also acceleration in pervaporation flux. Asuming Arrhenius dependence on temperature for the permeane of ethyl lactate an average apparent activation energy of permeation of 41.16 kJ/mol was obtained (figure 18) (rang of variation: 39.36-42.51 kJ/mol).

Ethyl lactate permeance was increased at higher temperature, so the permeance parameter for ethyl lactate was the function of temperature. As a result, ethyl lactate permeance was increased with an increase in temperature.

In addition, ethyl lactate permeances of the four investigated temperatures are presented in figure 17 as a function of water concentration in the feed. Ethyl lactate permeance increases when feed water concentration decrease because of a higher swelling degree of the hydrophobic membrane pore. However, degree of pore swelling measurement was beyond the scope of this study. It should be kept in mind that the permeation driving force for ethyl lactate, i.e., feed partial ethyl lactate vapor

pressure minus the partial permeate pressure, is smaller than the permeation driving force for the ethanol.

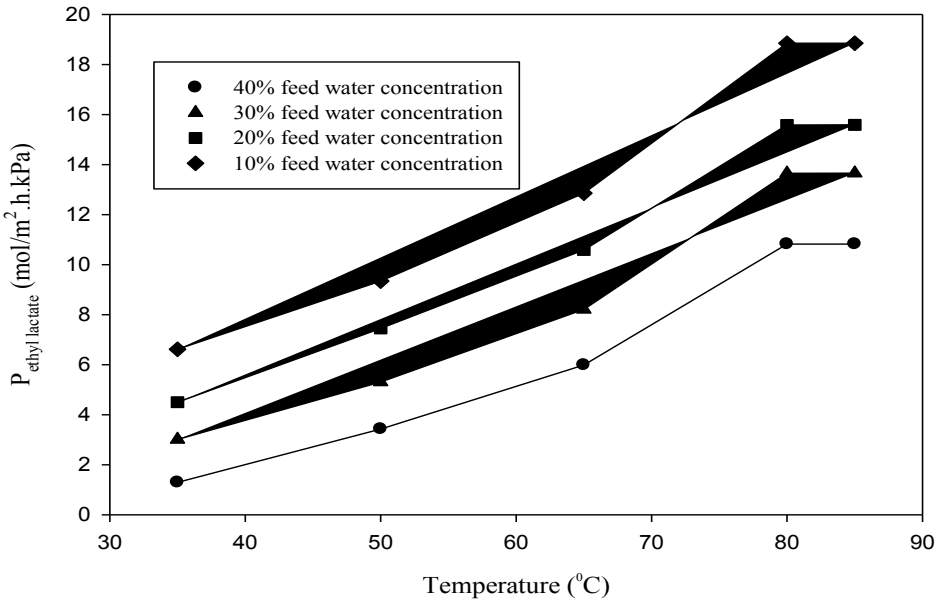


Figure 17 Influence of operating temperatures on ethyl lactate permeances at different feed water concentrations.

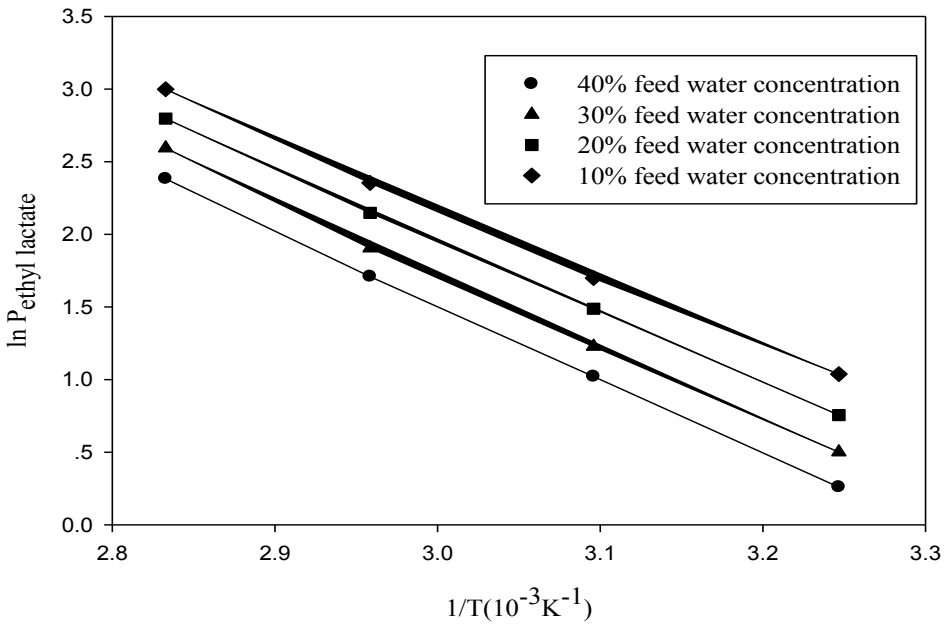


Figure 18 Temperature dependence of ethyl lactate permeance at various feed water concentrations using Arrhenius-type relationship.

4.3.4.4 The effect of operating temperature and feed water concentration on permeance of ethanol ($P_{ethanol}$)

The ethanol permeance is presented in figure 19 as a function of temperature at the four different feed water concentrations. As expected, ethanol permeance increases with temperature and decrease with increasing water concentration in the feed. In figure 18, higher values of ethanol permeances were observed compared to the permeance of ethyl lactate in the pervaporation process. The higher value of ethanol premeance is represented by the higher in permeability than ethyl lactate. The permeation of ethanol through the membrane is more complex than ethyl lactate because of the high number of interactions between the liquid feed components. Arrhenius-type dependence with temperature can be assumed obtaining and average apparent activation energy of 16.92 kJ/mol (variation range: 16.73-17.02 kJ/mol) (figure 20).

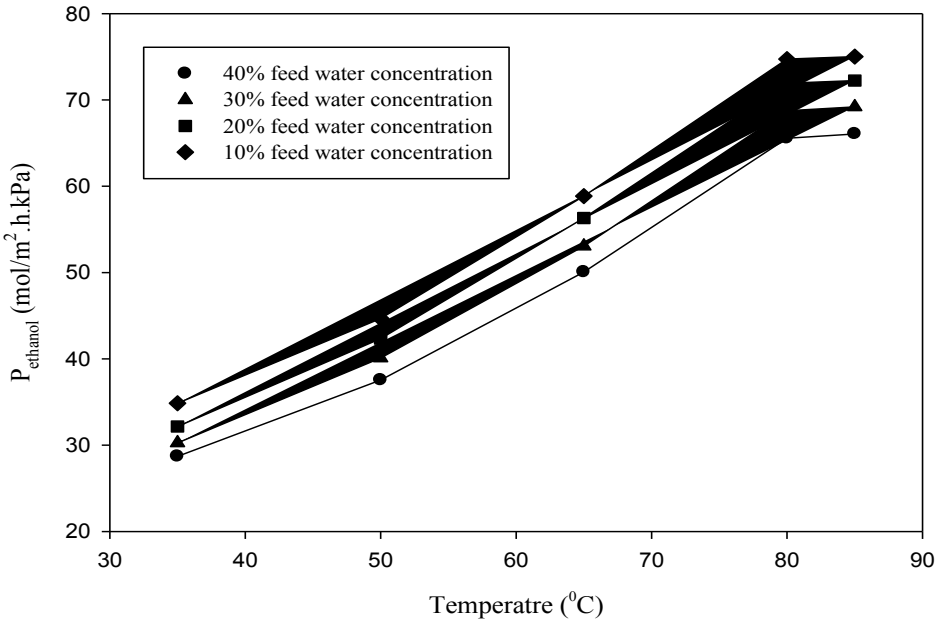


Figure 19 Influence of operating temperatures on ethanol permeance at different feed water concentrations.

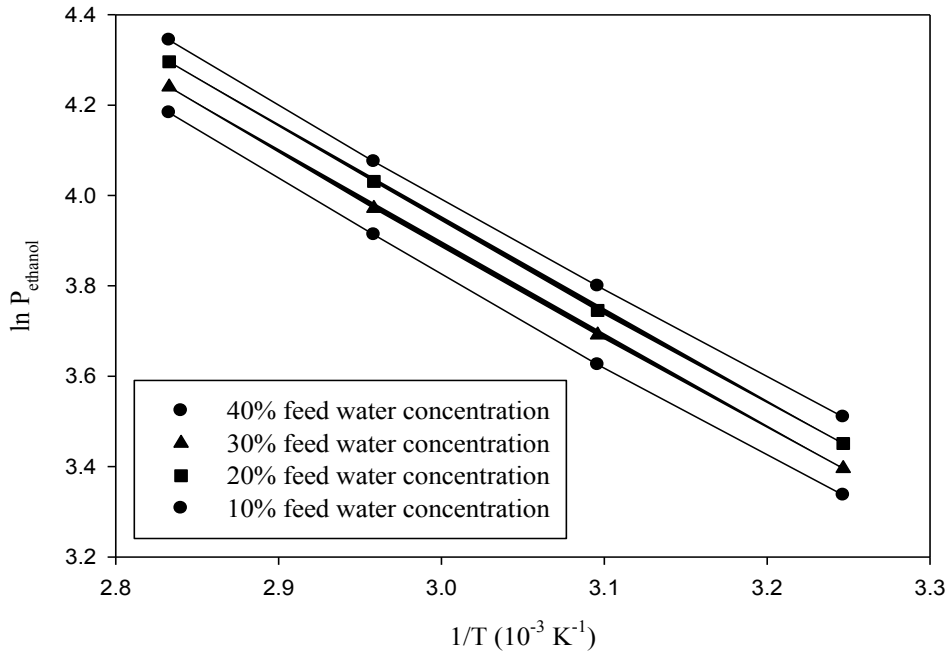


Figure 20 Temperature dependence of ethanol permeance at various feed water concentrations using Arrhenius-type relationship.

4.3.4.5 The effect of operating temperature and feed water concentration on permeance of water (P_{water})

Water permeance has been plotted in figure 21 versus the feed water content. Following the same trend of the other permeating species, it can be observed that permeances increased with temperature and feed water concentration. The values of water permeances were lower than ethanol permenaces, because of the extremely high hydrophilicity property of the water. An apparent activation energy of water permeance of 10.98 kJ/mol was found from the average value of the slopes at different water concentrations in the feed (range of variation: 10.88-11.13 kJ/mol) (figure 22).

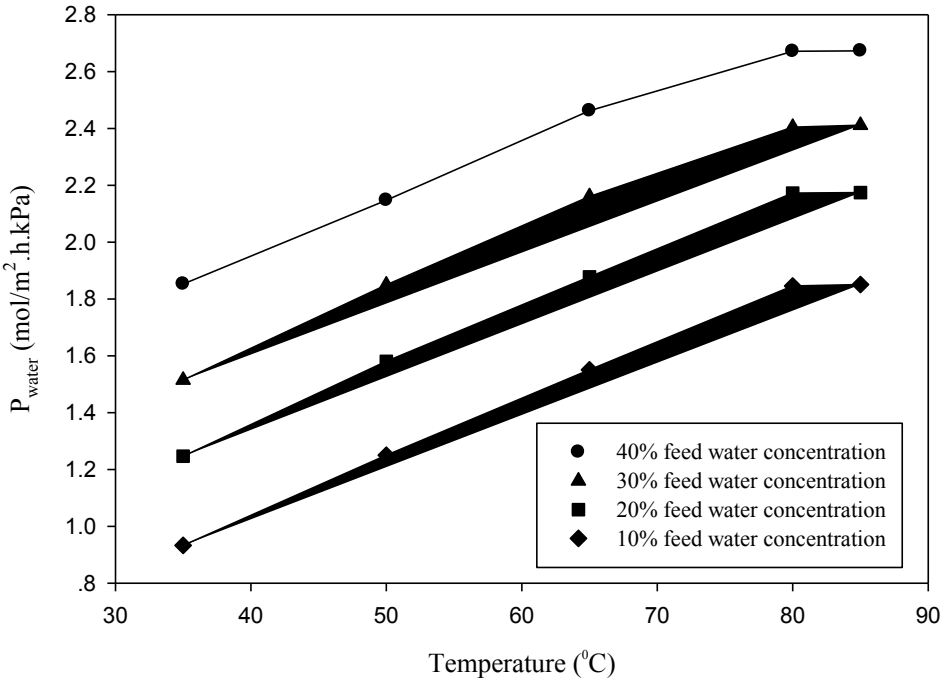


Figure 21 Influence of operating temperatures on water permeances at different feed water concentrations.

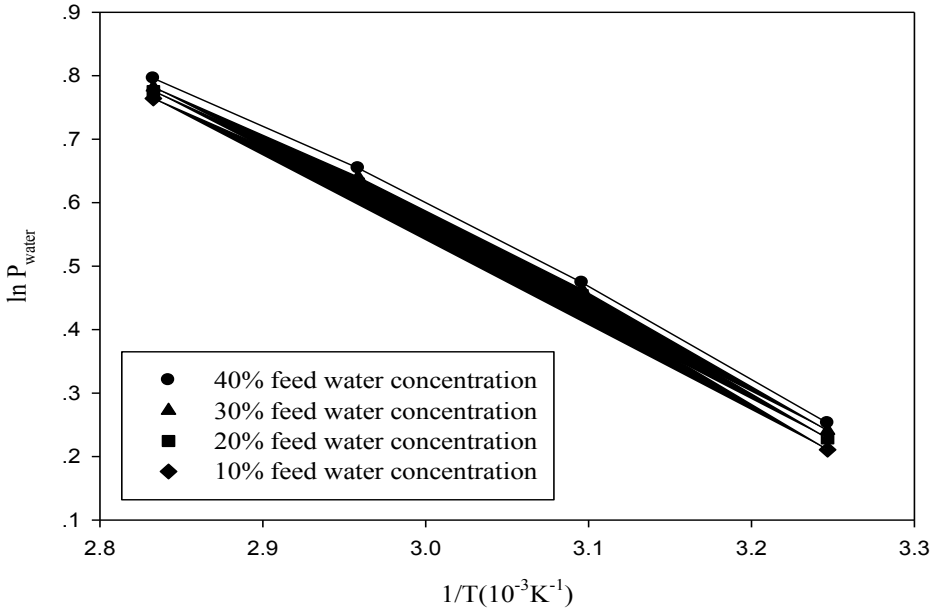


Figure 22 Temperature dependence of water permeance at various feed water concentrations using Arrhenius-type relationship.

4.3.4.6 The effect of operating temperature and feed water concentrations on separation factors

In figure 23, separation factors of the four different feed compositions have been plotted as a function of operating temperatures. In general, it was showed that separation factor exhibited an inverse trend compared with permeation fluxes. As a result, separation factors decreased at higher temperature of the feed solution. Therefore, process optimization to obtain the most suitable condition for this esterification-assisted pervaporation technique. Separation factor is strongly dependent on not only the temperature, but also on the feed water concentrations. In addition, the higher swelling degree of the membrane pore in contact with organic compounds facilitates the permeability of the different components leading to decrease in the separation factor. In addition, the separation factor increased with decreasing feed water composition of the same operating temperature. The reason for this different behavior from separation factor could be that while selectivity is mainly dominated by the membrane transport properties, separation factor takes into account the membrane transport properties and thermophysical properties of the feed mixture. Ethyl lactate concentration in feed increases with decreases feed water concentration, consequently the ethyl lactate permeance increases with decreasing water concentration in the feed. Separation factors of different feed water concentrations at 35 ^{o}C were obtained in the range between 2.7-3.4, and this characteristic was obtained for the other operating temperatures.

Regarding to the effect of temperature, it can be seen that temperature has a greater influential effect on separation factor compared to the effect of feed water concentration. In this system, separation factors dramatically decreased at elevated

temperature. The separation factors were obtained in the range between 1.3-3.3 depending on the temperatures and feed water concentrations. At 40% feed water concentration and temperature from 35 to 85 ^{o}C; for example, separation factor decreased from 3.3 to 1.7 which correspond to approximately 50% of the separation efficiency. However, separation factor is not the issue of interest because the permeation flux of ethyl lactate is the most important. This is because the required flux is related to the required membrane area and directly involves the investment cost.

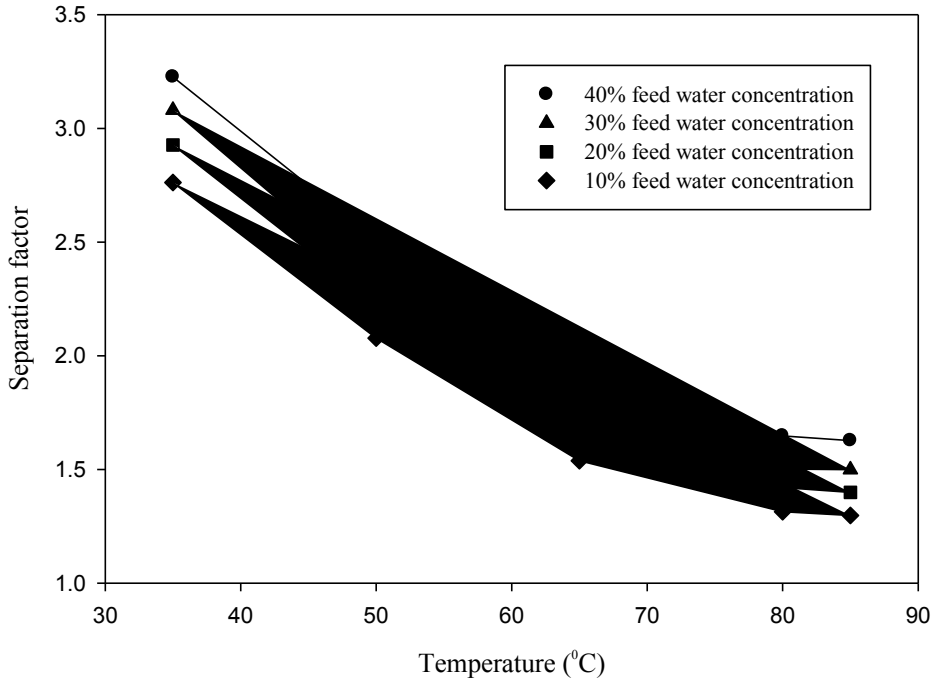


Figure 23 Influence of operating temperatures on separation factor at different feed water concentrations.

4.4 The hydrolysis of ethyl lactate

The lactic acid yield of hydrolysis process is shown in figure 23, under the operating temperature of 80°C and the molar ratio of water to ethyl lactate was 15:1. The hydrolysis reactor was coupled with a distillation column. In hydrolysis reaction, the ethyl lactate will react with water to produce lactic acid and ethanol. Therefore, ethanol and water in the reaction can be removed by the distillation column. Hydrolysis is a simple process, and variation of operating parameter is not necessary. The results showed that the maximal value of lactic acid was obtained at 91%w/v L-(+)-lactic acid. The productivity of L-(+)-lactic acid was approximately 179.83 g/L.h.

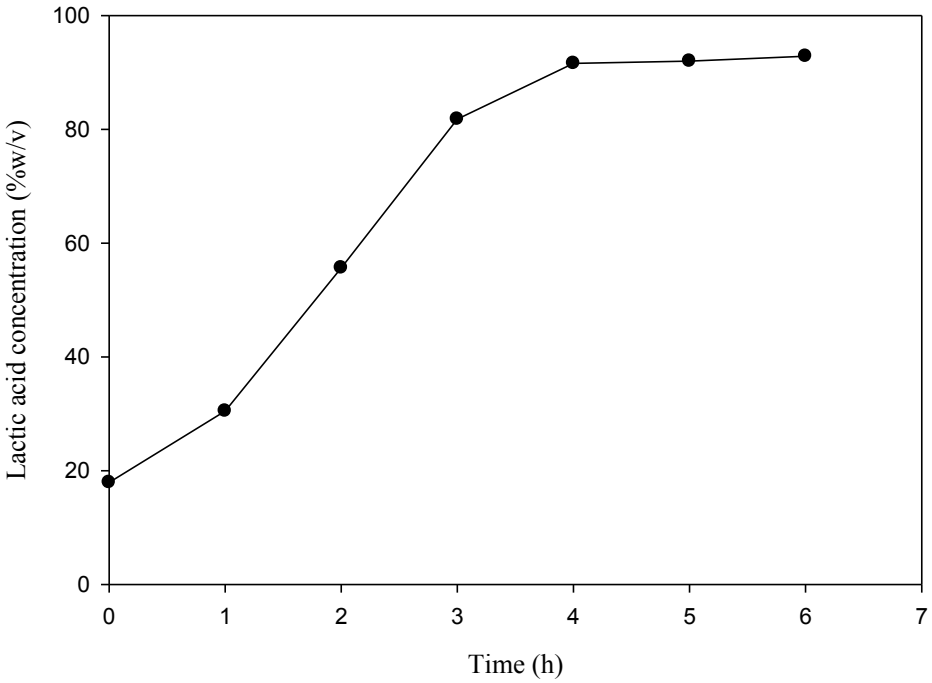


Figure 24 Time profiles of the lactic acid concentration on hydrolysis process (temperature was 80°C, the molar ratio of water to ethyl lactate was 15:1).

Figure 25 shows the images of samples taken during purification steps. The left was the evaporated fermentation broth which contained several organic acid impurities including a small amount of acetic acid. The middle was the permeate from the esterification-assisted pervaporation technique showing a clear and colorless characteristic. The right was the purified L-(+)-lactic acid with its light yellow color. This color was the result of high acid concentration which also made the solution very viscous. After this purification process, the L-(+)-lactic acid is suitable to be further investigation for the polymerization step with the main goal of bioplastic production. For figure 26 (A) shows the HPLC chromatogram of fermentration broth. This result showed the fermentation broth mainly contains impurities such as sugar(glucose) and other organic acid especially acetic acid. Therefore, this impurities have to be remove during purification process. In addition, HPLC chromatogram of enantiomer separation confirmed the homofermentative characteristic of the *P. pentosaceus* (figure 26 (B)). Acetic acid was not detected because it was removed from the system during distillation in the form of ethyl acetate. Moreover, only 2 peaks of D-(-)- and L-(+)-lactic acid were detected with the concentration ratio of 5 to 95 %, respectively. This result showed a very high optical purity of L-(+)-lactic acid was produced.

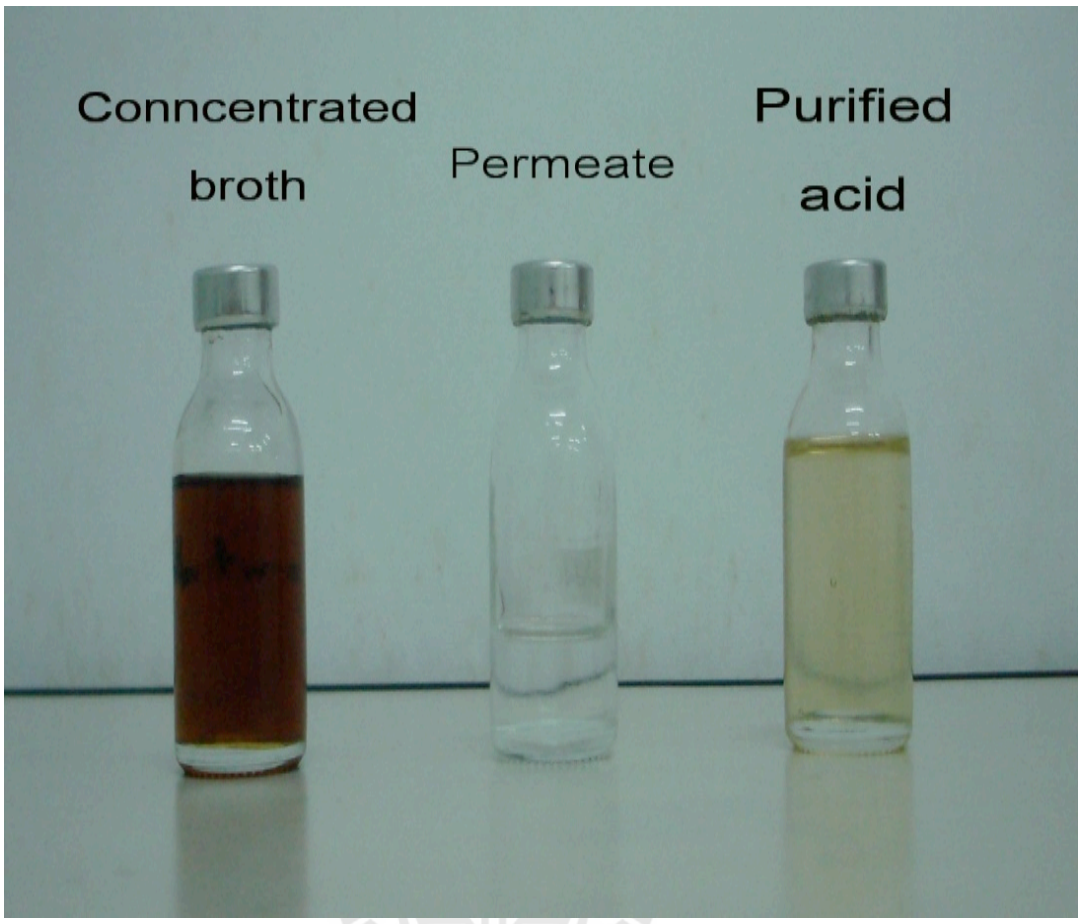
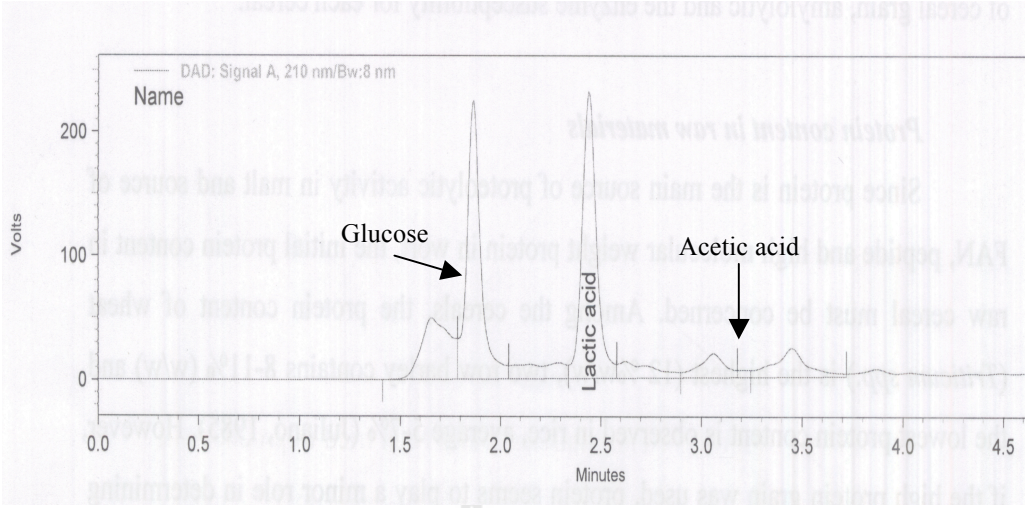
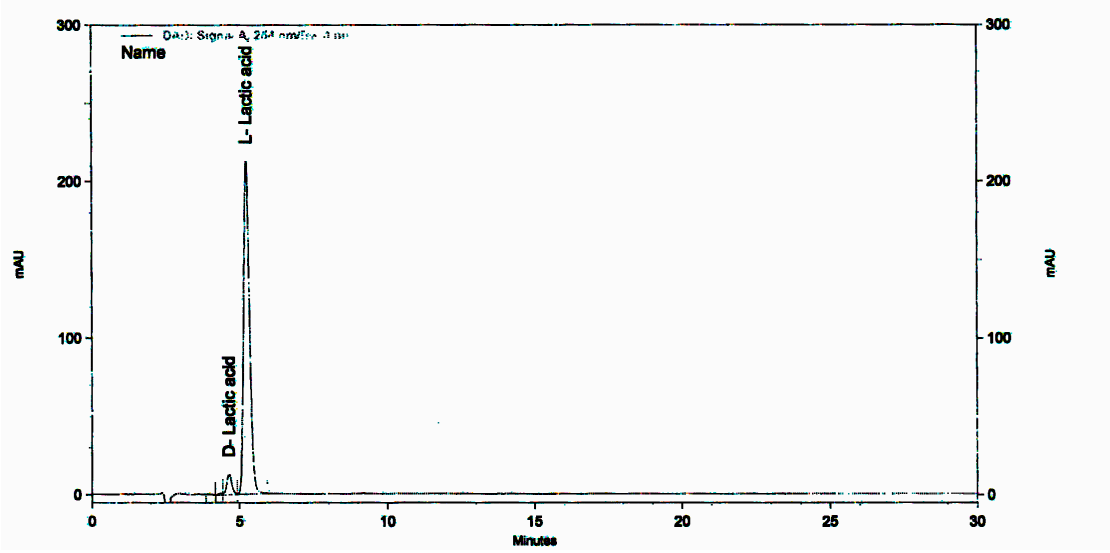


Figure 25 Image of samples taken during purification processes of lactic acid showing the concentrated fermentation broth (Left), permeate (Middle), and purified L-(+)-lactic acid after hydrolysis (Right).



(A)



(B)

Figure 26 HPLC chromatogram of fermentation broth (A) and chiral separation shows more than 95% optical purity of purified L-(+)-lactic acid (B).

4.5 Comparative productivity of downstream processing for lactic acid

Table 3 shows the productivity of lactic acid downstream processing. This result shows that the productivity of pervaporation – assisted esterification higher more than another technique. Therefore, the pervaporation – assisted esterification Pervaporation-assisted esterification process can improve productivity of esterification reaction.

Table 3 Productivity of downstream processing for lactic acid

Downstream process technique	Literature for downstream process	Productivity (g/L.h)
Esterification using rectifying column	Sun *et al.* (2006)	118.75
Esterification using zeolite A vapour permeation membrane	Jalal *et al.* (2002)	125.00
Esterification using zeolite T vapour permeation membrane	Kazuhiro *et al.* (2002)	131.58
Esterification using pervaporation reactor	Wasewar *et al.* (2009)	140.00
Pervaporation - assisted esterification using hydrophobic membeane	This research	179.83

APPENDIX

APPENDIX

Antione equation (Hansen *et al.,* 1991)

The Antoine equation is a vapor pressure equation and describes the relation between vapor pressure and temperature for pure components. The Antoine equation is derived from the Clausius-Clapeyron relation. Chemists often use the Clausius-Clapeyron equation to estimate the vapor pressures of pure liquids or solids:

$$ln\left(\frac{P}{P^o}\right) = \frac{\Delta H}{R}\left(\frac{1}{T^o} - \frac{1}{T}\right) \quad (12)$$

where P is the vapor pressure, P° is a vapor pressure at a known temperature T°, ΔH is an enthalpy of vaporization if the substance is a liquid or an enthalpy of sublimation if it's a solid, R is the ideal gas law constant, and T is the temperature (in kelvins).

Several of the assumptions fail at high pressure and near the critical point, and under those conditions the Clausius-Clapeyron equation will give inaccurate results. Chemists still like to use the equation because it's good enough in most applications and because it's easy to derive and justify theoretically. Chemical engineers often use the more accurate Antoine equation to predict vapor pressures. The Antoine equation is a simple 3-parameter fit to experimental vapor pressures measured over a restricted temperature range:

$$log_{10}P = A - \frac{B}{C+T} \quad (13)$$

where P is the vapor pressure, T is temperature and A, B and C Antoine coefficients that vary from substance to substance. Sublimations and vaporizations of

the same substance have separate sets of Antoine coefficients, as do components in mixtures. The Antoine equation is accurate to a few percent for most volatile substances (with vapor pressures over 10 Torr). Antoine coefficients for many substances are tabulated in Table 1. The simplified form with *C* set to zero:

$$log_{10}P = A - \frac{B}{T} \qquad 12$$

Is named August equation, after the German physicist Ernst Ferdinand August (1795-1870). The August equation describes a linear relation between the logarithm of the pressure and the reciprocal temperature. This assumes a temperature-independent heat of vaporization. The Antoine equation allows an improved, but still inexact description of the change of the heat of vaporization with the temperature.

The Antoine equation can also be transformed in a temperature-explicit form with simple algebraic manipulations:

$$T = \frac{B}{A - log_{10}P} - C \qquad (14)$$

Example 1 Calculate the vapor pressure of ethanol at 78.32 °C by using the Antoine equation.

*Solution***:** From table 3 constants for Eq. (2) are *A* = 8.20417, *B* = 1642.89, and *C* = 230.300. With Eq. (2),

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

$$P = 760 \text{ mmHg}$$

The coefficients of Antoine's equation are normally given in mmHg-even today where the SI is recommended and pascals are preferred. The usage of the pre-SI units has only historic reasons and originates directly from Antoine's original publication. It is however easy to convert the parameters to different pressure and

temperature units. For switching from degree Celsius to kelvins it is sufficient to subtract 273.15 from the C parameter. For switching from millimeters of mercury to pascals it is sufficient to add the common logarithm of the factor between both units to the A parameter:

$$A_{Pa} = A_{mmHg} + log_{10}\frac{101325}{760} \quad (15)$$

$$A_{Pa} = A_{mmHg} + 2.124903 \quad (16)$$

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

Solution: The parameters for °C and mmHg for ethanol

A	B	C
8.20417	1642.89	230.300

are converted for K and Pa to

A	B	C
10.32907	1642.89	-42.85

The calculation with Eq. (2) becomes

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

$$P = 101328 \text{ Pa}$$

Extension of the Antoine equations (Hansen *et al.*, 1991)

To overcome the limits of the Antoine equation some simple extension by additional terms are used:

$$P = \exp(A + \frac{B}{C+T} + D \cdot T + E \cdot T^2 + F \cdot \ln(T)) \quad (17)$$

$$P = \exp\ (A + \frac{B}{C+T} + D \cdot \ln(T) + E \cdot T^F \quad (18)$$

The additional parameters increase the flexibility of the equation and allow the description of the entire vapor pressure curve. The extended equation forms can be reduced to the original form by setting the additional parameters *D*, *E* and *F* to 0. A further difference is that the extended equations use the e as base for the exponential function and the natural logarithm. This doesn't affect the equation form.

UNIFAC Method (Hansen *et al.*, 1991)

The fundamental idea of a solution-of-groups model is to utilize existing phase equilibrium data for predicting phase equilibrium of systems for which no experimental data are available. In concept, the UNIFAC method follows the ASOG method, wherein activity coefficients in mixtures are related to interactions between structural groups. The essential features are:

1. Suitable reduction of experimentally obtained activity coefficientdata to yield parameters characterizing interactions between pairs of structural groups in nonelectrolyte systems.
2. Use of those parameters to predict activity coefficients for other systems that have not been studied experimentally but that contain the same functional groups.

The molecular activity coefficient is separated into two parts: one part provides the contribution due to differences in molecular size and shape, and the other provides the contribution due to molecular interactions. In ASOG, the first part is arbitrarily estimated by using the athermal Flory-Huggins equation; the Wilson equation, applied to functional groups, is chosen to estimate the second part. Some of this arbitrariness is removed by combining the solution-of-groups concept with the UNIQUAC equation (see Table 4); first, the UNIQUAC model contains a combinatorial part, essentially due to differences in size and shape of the molecules in the mixture, and a residual part, essentially due to energy interactions, and second, functional group sizes and interaction surface areas are introduced from independently obtained, pure-component molecular structure data. The UNIQUAC equation often gives good representation of vapor-liquid and liquid-liquid equilibrium for binary and multicomponent mixtures containing a variety of nonelectrolytes such as hydrocarbons, ketones, esters, water, amines, alcohols, nitriles, etc. In a multicomponent mixture, the UNIQUAC equation for the activity coefficient of (molecular) component *i* is

$$ln\gamma_i = ln\gamma_i^c + ln\gamma_i^R \tag{19}$$

Where

$$ln\gamma_i^c = 1 - J_i + lnJ_i - 5q_i(1 - \frac{J_i}{L_i} + ln\frac{J_i}{L_i}) \tag{20}$$

$$ln\gamma_i^R = q_i(1 - lns_i - \sum_i \theta_i \frac{\tau_{ij}}{s_j}) \tag{21}$$

Where in addition

$$J_i = \frac{r_i}{\sum_j r_j x_j} \tag{22}$$

$$L_i = \frac{q_i}{\sum_j q_j x_j} \tag{23}$$

$$s_i = \sum_l \theta_l \tau_{li} \tag{24}$$

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

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

Activity coefficients depend not only on the subgroup properties *R* and *Q*, but also on interactions between subgroups. Here, similar subgroups are assigned to a main group, as shown in the first two columns of Table 5. All subgroups belonging to the same main group are considered identical with respect to group interactions. Therefore parameters characterizing group interactions are identified with pairs of main groups. Parameter values a_{mk} for a few such pairs are given in Table 6.

The UNIFAC method is based on the UNIQUAC equation, for which the activity coefficients are given by Eq.(19). When applied to a solution of groups, Eqs.(20) and (21) are written:

$$ln\gamma_i^c = 1 - J_i + lnJ_i - 5q_i(1 - \frac{J_i}{L_i} + ln\frac{J_i}{L_i}) \tag{25}$$

$$ln\gamma_i^R = q_i[1 - \sum_k(\theta_k \frac{\beta_{ik}}{s_k} - e_{ki} ln\frac{\beta_{ik}}{s_k})] \tag{26}$$

The quantities J_i and L_i are still given by Eq.(23) and (24). In addition, the following definitions apply:

$$r_i = \sum_k v_k^{(i)} R_k \tag{27}$$

$$q_i = \sum_k v_k^{(i)} Q_k \tag{28}$$

$$e_{ki} = \frac{v_k^{(i)} Q_k}{q_i} \tag{29}$$

$$\beta_{ik} = \sum_m e_{mi} \tau_{mk} \tag{30}$$

$$\theta_k = \frac{\sum_i x_i q_i e_{ki}}{\sum_j x_j q_i} \tag{31}$$

$$s_k = \sum_m \theta_m \tau_{mk} \tag{32}$$

$$\tau_{mk} = exp \frac{-a_{mk}}{T} \tag{33}$$

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

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

Example 3 For the ternary system ethyl lactate(1)/ethanol(2)/water(3) at 323.15 K, find γ_1 and γ_2 when $x_1 = 0.2$, $x_2 = 0.7$ and $x_3=0.1$

Solution:

-the subgroup involved are indicated by chemical formular:

$CH_3CH(OH)COOCH_2CH_3(1)/CH_3CH_2OH(2)/H_2O$

ethyl lactate ethanol water

-The following table shows the subgroups, their identification numbers k, Values of the subgroup parameters R_k and Q_k (from Table 6), and the number of each subgroup in each molecule:

subgroups	k	R_k	Q_k	v_{ki}		
				[1]	[2]	[3]
CH_3	1	0.9011	0.848	1	1	0
CH_2	2	0.6744	0.540	1	1	0
CH	3	0.4469	0.228	1	0	0
OH	14	1.0000	1.200	1	1	0
CH_3COO	21	1.9031	1.728	1	0	0
H_2O	16	0.9200	1.400	0	0	1

-By Eq.(25), $r_1 = 1(0.9011)+1(0.6744)+1(0.4469)+1(1)+1(1.9031)$

$= 4.9255$

-Similarly $r_2 = 2.5755$ $r_3 = 0.9200$

-In like manner, by Eq.(28),

$q_1 = 1(0.848)+1(0.540)+1(0.228)+1(1.200)+1(1.728)+0(1.400)$

$= 4.544$

-Similarly $q_2 = 2.588$, $q_3 = 1.400$

-The r_i and q_i values are molecular properties, independent of composition. Substituting known values into Eq.(29) generates the following table for e_{ki}:

subgroups	k	R_k	Q_k	v_{ki}			e_{ki}		
				[1]	[2]	[3]	[1]	[2]	[3]
CH_3	1	0.9011	0.848	1	1	0	0.1866	0.3277	0.0000
CH_2	2	0.6744	0.540	1	1	0	0.1188	0.2087	0.0000
CH	3	0.4469	0.228	1	0	0	0.0518	0.0000	0.0000
OH	14	1.0000	1.200	1	1	0	0.2641	0.4637	0.0000
CH_3COO	21	1.9031	1.728	1	0	0	0.6370	0.0000	0.000
H_2O	16	0.9200	1.400	0	0	1	0.000	0.000	1.0000

-The following interaction parameters are found from Table 6

	a_{mk}					
m	1(1)	2(1)	3(1)	14(5)	16(7)	21(11)
1(1)	0	0	0	986.50	1318.00	114.80
2(1)	0	0	0	986.50	1318.00	114.80
3(1)	0	0	0	986.50	1318.00	114.80
14(5)	986.50	986.50	986.50	0	353.50	245.40
16(7)	1318.00	1318.00	1318.00	-229.10	0	200.80
21(11)	114.80	114.80	114.80	245.40	200.80	0

-Substitution of these values into Eq.(31), with T=323.15K

	τ_{mk}					
m	1	2	3	14	16	21
1(1)	1	1	1	0.0472	0.0169	0.7009
2(1)	1	1	1	0.0472	0.0169	0.7009
3(1)	1	1	1	0.0472	0.0169	0.7009
14(5)	0.0472	0.0472	0.0472	1	0.3349	0.4679

16(7)	0.0169	0.0169	0.0169	2.0318	1	0.5372
21(11)	0.7009	0.7009	0.7009	0.4679	0.5372	1

-Application of eq.(30),leads to the values of β_{ik}

	β_{ik}					
i	1	2	3	14	16	21
[1]	1.282	1.282	1.282	0.579	0.437	1.011
[2]	0.558	0.558	0.558	0.489	0.164	0.923
[3]	0.701	0.701	0.701	0.468	0.537	1.000

-Find θ_{ik} values from eq.(31), yield:

θ_1= 0.267, θ_2 = 0.170, θ_3=*0.0165,* θ_{14} = 0.378, θ_{16} =0.0489 and θ_{21} = 0.202

-And by eq.(30), :

s_1= 1.005, s_2 = 0.640, s_3=0.768, s_{14} = 0.753, s_{16}= 0.177 and s_{21} = 0.829

-The activity coefficients may now be calculated by eq.(25) and (26)

i	[1]	[2]	[3]
$\ln\gamma_i^C$	-0.11	-0.905	0.489
$\ln\gamma_i^R$	1.327	-0.598	1.357
$\ln\gamma_i$	1.217	-0.603	1.452
γ_i	3.376	0.547	4.271

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