การแยกและการทำกรดแล็กติกให้บริสุทธิ์จากน้ำหมักโดยออสโมซิสผันกลับ

นางสาวณัฏฐ์นิรินธน์ พันธุมจินดา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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SEPARATION AND PURIFICATION OF LACTIC ACID FROM FERMENTATION BROTH BY REVERSE OSMOSIS

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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ณัฏฐ์นิรินธน์ พันธุมจินดา : การแยกและการทำกรดแล็กติกให้บริสุทธิ์จากน้ำหมักโดยออสโมซิสผันกลับ (SEPARATION AND PURIFICATION OF LACTIC ACID FROM FERMENTATION BROTH BY REVERSE OSMOSIS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. ณัฏฐา ทองจุล, 112 หน้า.

งานวิจัยนี้ได้มีการประยุกต์ใช้ระบบการกรองแบบออสโมซิสผันกลับโดยนำแผ่นกรอง 2 ประเภท คือแผ่นกรองออสโมซิสผันกลับสำหรับน้ำกร่อยและแผ่นกรองออสโมซิสผันกลับสำหรับน้ำทะเล มาใช้ร่วมกันเป็นลำดับขั้นในการแยกและการทำกรดแล็กติกให้บริสุทธิ์จากน้ำหมัก การทดสอบเริ่มต้นจากการทดลองใช้สารละลายจำลอง 3 ชนิด คือ แคลเซียมแลคเตท โซเดียมแลคเตทและแอมโมเนียมแลคเตท เพื่อศึกษาหาภาวะที่เหมาะสมของแผ่นกรองออสโมซิสผันกลับสำหรับแยกกรดแล็กติก จากผลการทดลองพบว่าความดันที่ใช้ส่งผลต่อการแยกกรดแล็กติกและเกลือของแล็กติกผ่านแผ่นกรองออสโมซิสผันกลับสำหรับน้ำกร่อยตามทฤษฎีของดอนแนนและพันธะไฮโดรเจนที่เกิดขึ้นระหว่างกระบวนการแยก ซึ่งส่งผลให้มีการห้ามผ่านของประจุสองบวกผ่านแผ่นกรอง ดังนั้นแคลเซียมไอออนส่วนใหญ่ในสารละลายจึงถูกกักไม่ให้ผ่านแผ่นกรองออสโมซิสผันกลับสำหรับน้ำกร่อย เนื่องจากคุณสมบัติการแพร่ผ่านที่ต่ำและปฏิสัมพันธ์ระหว่างประจุของแคลเซียม (ประจุสองบวก) และประจุของไอออน (กลุ่มคาร์บอนิล) บริเวณผิวแผ่นกรอง ในขณะที่ประจุหนึ่งบวกสามารถสร้างพันธะไฮโดรเจนกับกลุ่มคาร์บอนิลบริเวณผิวแผ่นกรอง ดังนั้นประจุของโซเดียมและแอมโมเนียมจึงสามารถผ่านแผ่นกรองออสโมซิสผันกลับสำหรับน้ำกร่อยได้ ต่อมาแผ่นกรองออสโมซิสผันกลับสำหรับน้ำทะเลจะถูกนำมาใช้ต่อเนื่องจากแผ่นกรองประเภทแรกเพื่อกำจัดน้ำออกจากสารละลาย เพื่อทำให้กรดแล็กติกที่ได้มีความเข้มข้นที่สูงขึ้น จากผลการทดสอบการใช้แผ่นกรองทั้ง 2 ประเภทร่วมกันพบว่ากรดแล็กติกที่ได้ให้ค่าความบริสุทธิ์ถึง 99.2% และมีประสิทธิภาพในการเก็บกักกรดแล็กติกสูงถึง 50.5% สำหรับสารละลายจำลองแคลเซียมแลคเตท จากผลลัพธ์ดังกล่าวงานวิจัยนี้จึงนำเสนอกระบวนการแยกและการทำกรดแล็กติกให้บริสุทธิ์จากน้ำหมัก โดยออกแบบให้กระบวนการทั้งหมดเป็นการใช้ระบบการกรองโดยแผ่นกรอง การกรองด้วยไมโครฟิวเตรชันใช้ในการกำจัดจุลินทรีย์ การกรองด้วยอัลตราฟิลเตรชันใช้ในการแยกโปรตีน และการกรองด้วยออสโมซิสผันกลับใช้ในการแยกและการทำกรดแล็กติกให้บริสุทธิ์จากน้ำหมัก กระบวนการดังกล่าวได้มีการพัฒนาเพื่อเข้าสู่ระดับอุตสาหกรรมด้วยกำลังการผลิตกรดแล็กติก 100,000 กิโลกรัมต่อปีโดยนำโปรแกรมจำลองเพื่อการออกแบบระบบด้านการผลิตและการวิเคราะห์ด้านเศรษฐศาสตร์ (SuperPro® Designer software) ร่วมกับภาวะที่เหมาะสมที่ได้จากผลการทดลองในระดับห้องปฏิบัติการมาเป็นเครื่องมือวิจัยเพื่อประเมินหาเทคโนโลยีและกระบวนการทางเลือกใหม่ๆ หาขนาดของเครื่องจักรที่เหมาะสม หาปริมาณสารเคมีและสาธารณูปโภคที่ใช้ รวมถึงการประมาณต้นทุนการลงทุนและการดำเนินงาน ผลจากงานวิจัยสามารถสรุปได้ว่าการแยกและการทำกรดแล็กติกให้ บริสุทธิ์จากน้ำหมักด้วยกระบวนการกรองโดยแผ่นกรองแบบคู่ขนาน (In-parallel membrane based process) มีประสิทธิภาพในการเก็บกักกรดแล็กติกสูงสุดและมีค่าความบริสุทธิ์ของกรดแล็กติกที่สูงเมื่อเปรียบเทียบกับผลิตภัณฑ์กรดแล็กติกที่จำหน่ายเชิงพาณิชย์ ผลการวิจัยยังสรุปได้อีกว่าจำนวนเครื่องจักรที่ใช้มีผลต่อต้นทุนการผลิตทั้งในด้านการลงทุนและการดำเนินงาน กระบวนการกรองโดยแผ่นกรองแบบคู่ขนานลดต้นทุนการดำเนินงานในกระบวนการแยกและการทำกรดแล็กติกให้บริสุทธิ์จากน้ำหมักได้ถึง 23.33-31.29 % สำหรับทั้ง 3 ชนิดของน้ำหมักแคลเซียมแลคเตท โซเดียมแลคเตท และแอมโมเนียมแลคเตท

สาขาวิชา เทคโนโลยีชีวภาพ ลายมือชื่อนิสิต .....

ปีการศึกษา 2560 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

5572873223 : MAJOR BIOTECHNOLOGY
KEYWORDS: LACTIC ACID / FERMENTATION BROTH / DOWNSTREAM RECOVERY / MEMBRANE FILTRATION / ECONOMIC ANALYSIS / REVERSE OSMOSIS / PROCESS SIMULATION

NATNIRIN PHANTHUMCHINDA: SEPARATION AND PURIFICATION OF LACTIC ACID FROM FERMENTATION BROTH BY REVERSE OSMOSIS. ADVISOR: ASSOC. PROF. NUTTHA THONGCHUL, Ph.D., 112 pp.

Brackish water reverse osmosis (BWRO) and seawater reverse osmosis (SWRO) membranes were used in the 2-stage reverse osmosis (RO) unit to recover, pre-purify, and pre-concentrate lactic acid. Calcium lactate (CaLAC), sodium lactate (NaLAC), and ammonium lactate (NH_4LAC) were used as model feed solutions. The operating pressure showed a pronounced effect on lactate passage through the first BWRO unit, and the Donnan exclusion effect and hydrogen bonding were responsible for cation rejection. Calcium ions were rejected at the BWRO unit because of low diffusion rate and charge interaction at the surface. However, monovalent ions formed hydrogen bonds with the carbonyl group of the membrane that allowed passage across the membrane. The second SWRO unit was for pre-concentrating lactic acid. A high lactate purity of 99.2% with a total recovery of 50.5% was acquired from calcium lactate feed solution. Lower purity with higher lactate recovery was obtained when the feed solution was sodium lactate and ammonium lactate. Process and cost models for lactic acid recovery from fermentation broths at an annual capacity of 100,000 kg were developed as a research tool in evaluating an alternated process technology. The models were developed using SuperPro® Designer software by gathering the optimized data from the laboratory scale experiments. Sizing of unit operations, chemicals and utility consumptions, and estimation of capital and operating costs with the cost breakdown analysis were acquired from the simulation. Membrane based process design was proposed in this study. The processes mainly consisted of microfiltration for cell removal, a series of ultrafiltration for eliminating proteins, and the integrated reverse osmosis systems to recover, preconcentrate, and prepurify lactic acid. Among the 3 proposed process designs, in-parallel membrane based process exhibited the highest lactic acid recovery yield while the purity remained sufficiently high in comparable to the commercial grade products. The number of unit operations was found to be responsible to high production cost both investment and operating costs. Omitting centrifugation and ultrafiltration at 30 kDa molecular weight cut-off with integrated brackish water reverse osmosis membrane in parallel units in the design could lower the operating cost by 23.33-31.29% for different fermentation broths entering the downstream processing units.

Field of Study: Biotechnology Student's Signature .....

Academic Year: 2017 Advisor's Signature .....

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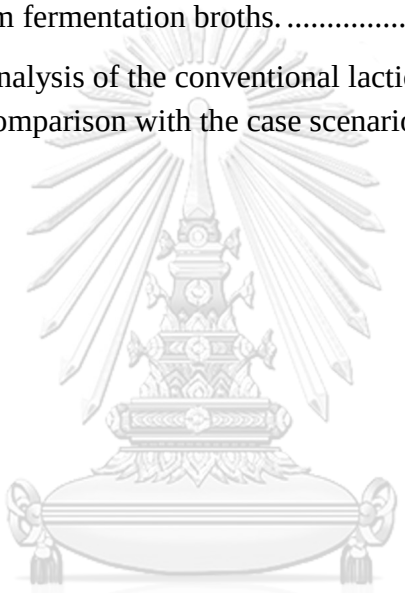


LIST OF FIGURES

LIST OF ABBREVIATIONS

A	=	The effective membrane surface area (m^2)
C_B	=	Concentration at fluid bulk (kg/m^3)
C_w	=	Concentration at the membrane interface (kg/m^3)
C_{feed}	=	Concentration of the solute in the feed (kg/m^3)
C_{perm}	=	The concentration of the solute in the permeate (kg/m^3)
C_{retn}	=	The concentration of the solute in the retentate (kg/m^3)
C_{BWP}	=	Lactic acid or ion concentrations present in the permeate left the BWRO unit (g/L)
C_{BWR}	=	Lactic acid or ion concentrations present in the retentate left the BWRO unit (g/L)
$C_{LA,SWF}$	=	Lactic acid concentrations present in the feed solution entering the SWRO unit (g/L)
$C_{LA,SWP}$	=	Lactic acid concentrations present in the permeate left the SWRO unit (g/L)
D	=	Diffusivity of the protein in the solvent (m^2/ s)
F_{LA}	=	The initial mass of lactic acid present in the feed solution (g)
F_i	=	The initial mass of the ion present in the feed solution (g)
G	=	Acceleration due to gravity (m/s^2)
I	=	Dimensionless van't Hoff factor
J	=	Permeate flux / Solvent flux (m/s)
J_{LA}	=	Lactic acid flux (g/ m^2h)
J_w	=	The water flux (m/s)
Lp	=	Hydraulic permeability (m/s Pa)
Lpo	=	Water permeability ($m^3.s^{-1}.m^{-2}.bar^{-1}$)
M	=	Molarity (molar concentration of the solution)
m_{LA}	=	The lactic acid mass passing through the membrane (g)
m_i	=	The ion mass passing through the membrane (g)
n	=	Rotation speed, RPM
$m_{LA,SWR}$	=	The mass of lactic acid product remained in the retentate left the SWRO unit (g)
$m_{T,\ SWR}$	=	The total mass of ions remained in the retentate left the SWRO unit (g)
P_{LA}	=	The purity of lactic acid product in percentage (%)
P_1	=	The pressure at the module inlet (bar)
P_2	=	The pressure at retentate side (bar)
P_3	=	The pressure at permeate side pressure, assumed uniform (bar)

ΔP	=	The pressure difference (bar)
ΔP_{TM}	=	Trans-membrane pressure difference (Pa)
Q_{feed}	=	Volumetric flow-rate of the feed
Q_W	=	Volumetric flow-rate of the permeate (water)
r	=	Distance from the axis of rotation (m)
R	=	Radius (m)
R%	=	The rejection of solute
R_{LA}	=	Lactic acid rejection (%)
$R_{LA,SWRO}$	=	lactic acid recovery in percentage (%)
R_i	=	Ion rejection (%)
$R_{overall\ (RO)}$	=	Overall recovery of RO unit in percentage (%)
$R_{overall\ (solid)}$	=	Overall recovery of solid removal unit in percentage (%)
RC	=	Rejection coefficient
Rt	=	Gas constant 0.08205746 (L atmK^{-1} mol^{-1})
S_{LA}	=	Lactic acid separation in percentage (%)
S_i	=	The ion leakage in percentage (%)
S_{cen}	=	The lactic acid separation on centrifuge in percentage (%)
S_{MF}	=	The lactic acid separation on microfiltration in percentage (%)
S_{UF}	=	The lactic acid separation on ultrafiltration in percentage (%)
t	=	The time (h)
T	=	Temperature (ºK)
X	=	Distance from the membrane
Z	=	Membrane thickness (m)
ω	=	Angular velocity (radians/s)
ε	=	Membrane porosity, dimensionless
μ	=	Viscosity of the permeate (Pa s)
$\boldsymbol{\pi}$	=	Osmotic Pressure (atm)
δ	=	Thickness of the boundary layer for diffusion

CHAPTER I
INTRODUCTION

1.1 Introduction

According to lactic acid (LA) becoming an important monomer which is highly required for the production of biodegradable product in several applications. Lactic acid (LA) has been extensively used in food, cosmetic, and pharmaceutical industries due to its chiral structure containing both carboxyl and hydroxyl groups that can be converted into many useful chemicals. It was suggested that the recent increasing demand of lactic acid was due to the production of the biodegradable polylactic acid (PLA). From "Lactic Acid Market by Application (Biodegradable Polymer, Food, & Beverage, Personal Care & Pharmaceutical) & Polylactic Acid Market by Application (Packaging, Agriculture, Automobile, Electronics, Textile), & by Geography – Global Trends & Forecast to 2020", the global PLA market is projected to reach USD 5.16 billion by 2020 with the growth at a CAGR of 20.9% during the forecast period. Europe is estimated as the largest market for PLA while Asia-Pacific is projected to be the rapid-growing market. It was suggested that government regulations and growing environmental concerns are the keys driven of PLA market. The expansion of PLA market thus drives the continual growth of lactic acid market to serve as the building block in PLA synthesis. From the report, the global lactic acid market is projected to reach USD 3.82 billion by 2020, growing at a CAGR of 18.6% during the forecast period. Therefore, the developments of lactic acid production and purification process are economically investment. Lactic acid (LA) can be produced via chemical synthesis or microbial fermentation. In chemical synthesis the petroleum feedstocks are converted to a racemic mixture of lactic acid under drastic conditions. However, it has limitation on petroleum supplied and environmental pollution. While fermentation process utilizes the renewable biomass and feedstocks under the mild conditions and high yield and productivity of lactic acid can be accomplished; thus, the process is considered green (Castillo Martinez et al., 2013). In the lactic acid production acid via bacterial fermentation process, pH normally decreases upon lactic acid formation producing by lactic acid bacteria while the operating pH must be controlled at the range of 5.5-6.5 as the lactic acid bacterial growth preference (Vaidya et al., 2005). To maintain the pH for optimal growth of lactic acid bacteria in fermentation process, the addition of alkali, as the neutralizing agent, is importantly required. However, neutralization gives lactate salt at the end of fermentation. This generates 2 lactate species (free lactic acid and lactate salt) in the fermentation broth. At the end of the fermentation, lactate salts and free lactic acid must be recovered in the form of free acid in the downstream operation units.

To convert lactate salts resulting from neutralization process to be in the form of free lactic acid and salt ions, acidification process is required. This can be done by the addition of mineral acids such as sulfuric acid (H_2SO_4) and hydrochloric acid (HCl). Using different acid to acidify gives the different broth compositions (1) when using H_2SO_4, insoluble or partial soluble sulfate salts are obtained while (2) using HCl, the resulting chloride salts are highly soluble in water. In the present invention, H_2SO_4 is the preferred mineral acid. Nonetheless, H_2SO_4 commonly causes insoluble sulfate salts that become a major impurity in the feed stream entering the downstream process after acidification process. It should be noted that lactic acid fermentation broth contains several components besides lactic acid product; therefore, the downstream recovery and purification of lactic acid after fermentation is of concern (Sikder et al., 2012). Therefore, In order to ease the downstream recovery of lactic acid, prior removal of sulfate salt is necessary (Datta and Henry, 2006) Referring to previous studies, there are different compounds to control pH such as calcium carbonate ($CaCO_3$), calcium hydroxide $Ca(OH)_2$, sodium hydroxide (NaOH) and ammonium hydroxide (NH_4OH) (Hetényi et al., 2011; Nakano et al., 2012). Among three neutralizing agents, $CaCO_3$ has the lowest solubility property and NH_4OH has the highest solubility. Therefore NH_4OH and NaOH are more suitable for bipolar membrane separation processes such as microfiltration (MF), electrodialysis (ED) and ion exchange since the precipitates are not formed rapidly after acidification by H_2SO_4 resulting in less precipitates at the membrane surface that eventually lower the competition in binding to the counter ions on the resin/membrane surface. On the other hand, $CaCO_3$ and $Ca(OH)_2$, which is commonly used to maintain the optimal pH in the fermentation broth, gives a large amount of calcium sulfate (gypsum) as a byproduct after acidification with H_2SO_4 in the solid stream. Thus, gypsum process (using $CaCO_3$ and $Ca(OH)_2$ for pH control) is well-suited for centrifugation technique (Qin et al., 2010). Not only the production of lactic acid by fermentation process has been developed continuously but the implementation of lactic acid recovery and purification process has been also studied widely. Lactic acid product required for biodegradable polymer grade must provide the sufficiently high purity. Many downstream processes have been developed to achieve the targeted product purity and recovery yield, either by a single unit operation or the combined units. In the recovery and purification of lactic acid from the fermentation broth, many major process steps are involved: e.g., primary recovery, product purification, and finishing processes. The first step in primary recovery units involves cell separation by centrifugation or microfiltration. There are general techniques to reduce impurities to get the desired purity of the final product with the possibly low loss.

Those techniques include centrifugation (Hu et al., 2017), distillation (Joglekar et al., 2006), extraction (Khunnonkwao et al., 2012), crystallization (Tait et al., 2009), evaporation (Petrides et al., 2002), adsorption (ion exchanger) including membrane filtration (microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO) and electrodialysis (ED) (Rodrigues et al., 2017).

Each technique provides different specific functions such as, operating conditions, specification, running cost, and definitely pros and cons in lactic acid production. Therefore, most of the downstream process designs involve many steps in order to achieve the targeted product specifications. Several downstream techniques have been studied and applied as a single unit or combine unit to acquire both technical and cost effectiveness. However, most techniques still have some limitation. Both distillation and ED have high energy consumption in a large-scale operation because of the low volatility of lactic acid and high electricity loading, respectively. When charged compounds such as amino acids and other organic acids are present, the separation efficiency of ED decreases as a result of membrane fouling. Moreover, ED fails to reject divalent ions such as calcium and magnesium; therefore, it is not suitable for recovering lactic acid from the conventional calcium base fermentation process where lactate salts are present in the form of calcium lactate and lactic acid (Khunnonkwao et al., 2012; Lee et al., 2006). Thus, it is good to separate the culture broth from the gypsum process (Kim et al., 2012). In extraction, the toxicity of solvent and product contamination is concerned (Wasewar et al., 2004). Crystallization maybe requires mixed solvent and other equipment to separate solid crystal from system (Tait et al., 2009). Using ion exchangers to recover lactic acid requires large amounts of chemicals, enzymes, and process water during the resin regeneration and washing steps. This eventually generates a large effluent loading in wastewater treatment. In addition, precautions should be taken in the pretreatment steps before feed enters the ion exchangers so as to avoid fouling and resin deterioration (Joglekar et al., 2006). Purified free lactic acid then enters the evaporator where water is removed, resulting in concentrated lactic acid as the finished product (Wojtyniak et al., 2016). As previously mentioned, process integration is necessary in order to achieve good recovery performance as well as cost effectiveness. Membrane separation provides the advantages of low energy consumption and low toxicity. Nevertheless, a single membrane unit cannot fulfill lactate recovery, purification, and concentration of lactic acid. The typical membrane filtration techniques suffer from fouling problem at membrane surface. Therefore, single unit is not sufficient to separate all kinds of contaminants in the product stream due to the individual pore size (Pal et al., 2009). Typically, NF is used prior to the product finishing step (evaporation in case of lactic acid recovery and purification). NF is applied for removal of trace ions and small, neutral molecules from free lactic acid solution (Ghaffar T., 2014). On the other hand, RO is generally applied for removal of water in previous literatures (Pal et al., 2009).

From the mentioned problems and limitations of the recovery techniques, both conventional and alternated techniques are reviewed in order to develop the new approach that provides the simple and cost effective process. Among the several techniques, RO, one of membrane separation processes, is suitable for removing organic and inorganic compound from aqueous solution by the principles of molecular size and driven force (Senthilmurugan and Gupta, 2006). RO successfully adopted to remove the ions from water in wastewater treatment such as ammonia nitrogen (NH_4^+), sulfate (SO_4^{2-}), sodium (Na^+), chloride (Cl^-), iron (Fe^{3+}), including manganese (Mg^{2+}) in several industrial applications (Huang et al., 2011) and widely applied in desalination also (Ding et al., 2015). This is because RO is developed for removing the low molecular weight organic and inorganic compounds in water feed solution since it can provide the specific pore size less than 0.001 μm and the molecular weight cut-offs (MWCOs) less than 100 Daltons. As a result, all impurities, such as aqueous salts, metal ions, and other small particles, are limited to pass through RO membrane. The impurities are still remained in the concentrate while the salt and water permeate through an RO membrane by solution-diffusion transport mechanism when the applied pressure is higher than the osmotic pressure (Shenvi et al., 2015). The applications of RO can be classified as in table 1.1. In separation of inorganic / organic compounds, it was reported that $CaSO_4$ and Fe^{2+} were separated from the natural water using the composite RO membrane. In this study, it was found that both $CaSO_4$ and Fe^{3+} are the insoluble salt. Not only causing the mineral impurities, they also reacted and $Fe(OH)_3$ was formed. This strongly promoted membrane fouling. To minimize fouling of these 3 species, composite RO membrane was introduced with the fouling inhibitor sodium carboxymethylcellulose. This eventually improved RO performance resulted in effective separation process (Kavitskaya et al., 2000). More investigation on inorganic (ionic) reduction by RO was done using aqueous sulfate solution as the model solution. It was reported that higher than 99.55% sulfate is rejected (Bódalo et al., 2004). RO was also used to separate the aqueous solution containing both inorganic (NaCl, NaBr, and KBr) and organic compounds (phenol, 2,4-dinitrophenol and pentachlorophenol). Both were successfully separated from the solution under the specific tested conditions (Senthilmurugan and Gupta, 2006). RO has been continuously studied for organic salt separation. Ethanol, butanol, acetic acid, lactic acid, oxalic acid, and butyric acid were prepared in the aqueous mixture and tested. It was reported that all above mentioned compounds were rejected by RO. The % rejection of lactic acid was 99.2 (Diltz et al., 2007). RO was also applied to concentrate lactic acid. A tubular thin-film composite membrane was successfully developed for concentrating lactic acid from fermentation broth. It was found that the permeate flux was increased as a result of the increasing the driving force. While the permeate flux was decreased when the pH was higher than 5-6, resulting in the rejection percentage of lactic acid and residual sugars higher than 97% (Schlicher and Cheryan, 2007).

Table 1.1 Applications of reverse osmosis (RO) on lactic acid production.

Membrane process	Application	Results
(1) Reverse osmosis (RO) (Diltz et al., 2007)	Concentrating lactic acid from aqueous mixture	99.2% rejection of lactic acid from mixture
(2) Combined ultrafiltration (UF) with reverse osmosis (RO) (Yorgun et al., 2008)	Concentrating lactic acid from the permeate stream collected from UF	Highest protein recovery in UF stage Highest lactic acid recovery in RO stage
(3) Combined nanofiltration (NF) with reverse osmosis (RO) (Li et al., 2008)	Concentrating lactic acid from the permeate stream collected from NF	Highest lactose retention in NF stage (97%) Highest lactic acid recovery in RO stage (nearly 100%)

(2), (3) cheese whey production

Among 3 membranes (UF, NF, and RO), previous studies revealed that RO could effectively be applied for lactic acid concentration from the fermentation broth (Orozco et al., 2014). With smaller pore sizes than the UF and NF membrane, thus the RO membrane provides high efficiency in the rejection of lactic acid. From the literatures, RO is one of the effective techniques well employed in water purification and waste water treatment. However, it was partially involved in lactic acid recovery process as the concentration unit where water was removed. To date, there is no report studying RO for lactic acid recovery and purification though many applications in salt removal have been studied by RO. The basic concept in lactic acid recovery and purification shares the common background with water purification where ions are removed from water. Therefore, in this work RO technique will be employed for lactic acid separation and purification. In this study, a 2-stage RO membrane-based process was developed for recovering, purifying, and concentrating free lactic acid from fermentation broth by applying the appropriate pressure higher than the osmotic pressure of the species of interest. From the principle of the RO process, solute transport occurs by diffusion through the membrane depending on molecular size and charge (Bellona et al., 2004). With smaller pore sizes than the NF membrane, the RO membrane provides high efficiency in the rejection of monovalent ions (Datta and Sablani, 2007). It was also reported that some trace organic compounds, such as neutral molecules smaller than the molecular weight cut-off of the membrane, leaked and passed through the membrane (Košutić and Kunst, 2002). By applying an operating pressure sufficiently higher than the osmotic pressure of the molecules, such molecules can pass through the RO membrane. At the proper pH, free lactic acid was supposed to recover and purify from its salts and other trace ions at the first RO unit (free lactic acid passed across the first RO membrane to the permeate side whilst the cation salts remained in the retentate). The second RO unit was for pre-concentrating lactic acid solution.

Compared with the other techniques mentioned previously, RO filtration did not require chemicals, enzymes, and process water during the operation. The low volumetric rate of the exit stream from this 2-stage RO unit resulted in size reduction of the evaporator and lower capital and operating expenditures. To proficiently optimize the performance of RO technique for lactic acid separation and purification process, the fermentation broth must be prepared properly before entering the separation and purification process since it is definitely helpful to reduce the tasks in downstream process. Undesired particles remaining in lactate fermentation broth should be firstly removed. The compositions of the fermentation broth depend mainly on the upstream operation process including the microbes used in fermentation, the substrates and medium compositions including the pH control agent. Lactic acid in the fermentation broth is typically in the form of lactate salt due to pH control at the optimal condition for growth and lactic acid production during fermentation. Acidification is commonly required to recover lactic acid and precipitate some ions contained in the fermentation broth. Depending on the pH control agent and the acidifier, free lactic acid and salts of the pH control agent occur after acidification. The microbial cells and solid are removed by centrifugation leaving the aqueous stream containing free lactic acid and other soluble components. After insoluble solid removal unit, MF and UF units are required since the broth compositions still contain soluble proteins and other soluble organic matters in excess. This strongly affected the efficiency of RO separation unit. From above mentioned, the recovery process design mainly depends on the feed stream compositions left from the broth. Nonetheless, the production cost increases as many unit operations and steps involved in the recovery process. Ideally, the downstream lactic acid recovery process should provide simple operation, high process performance, high cost effectiveness, and low environmental impact. In addition, the integrated design should be able to accept a variety of lactic acid fermentation broths that might be obtained from different fermentation process. Therefore, simple process that can be adapted for all the different feed streams in commercial lactic acid plant is preferred. Among the unit operations mentioned above, membrane filtration process provides the beneficial outcomes in low energy requirement, low chemical consumptions, and low effluent generation; thus, being considered as the green process. The objective of this study was to provide the technical insights on the membrane based process technology as an alternative to recover lactic acid from the fermentation broths. It is attempted to develop the simple solid removal (both soluble and insoluble) and to adapt RO for lactic acid separation and purification via process optimization. This typical process design, at least 6 process steps are required. Thus, the short chain downstream processing train is preferred. Since employing less stage would be economically beneficial for industrial scale. The possibility of downstream processing platforms will be investigated and compared in term of the efficiency and production cost.

Many process simulation tools have been continuously applied in chemical industries for developing and optimizing the design and operation of integrated process since 1960s. When a new process model is proposed, process simulators can generate the model that predicts the equipment sizing, process scheduling, and economic evaluation (Petrides et al., 2002). Thus, the simulation results are often used as one of the tools to guarantee the process feasibility at the initial stage of development. Intelligen's SuperPro® designer software (Version 9.5) was used as the simulation tool. Thus the goal of this study is to develop the novel membrane based process for lactic acid separation and purification that can achieve high purity of lactic acid in comparison to the commercial product. The proposed designs reflect the ability to compare the modified process with the base case design to help researchers in developing the process integration and intensification in lactic acid recovery technology. Thus, three major separation and purification processes, consisting of (1) lactic acid fermentation broth preparation process (2) solid removal process and (3) simultaneous lactic acid separation and purification process, will be optimized. The parameters affecting the efficiency will be also determined. The following process flow diagram describes the base case design for separating and purifying lactic acid from fermentation broth as figure 1.1.

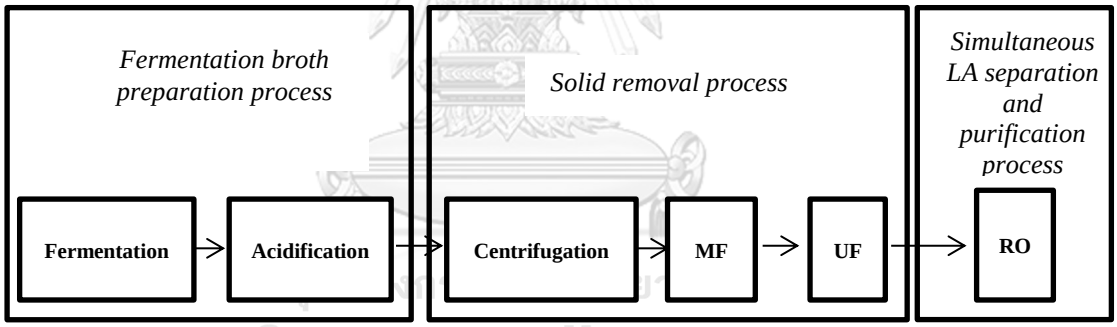


Figure 1.1 Base process flow diagram.

1.2 Objectives

1.2.1 To apply reverse osmosis technique to simultaneously separate and purify lactic acid from the fermentation broth

1.2.2 To optimize the solid removal section for separating insoluble matters from the fermentation broth for lactic acid recovery

1.2.3 To propose the novel membrane based process for lactic acid recovery

1.2.4 To evaluate the economic assessment for industrial scale.

1.3 Scope of Research

The scope of this thesis is summarized in Figure 1.2.

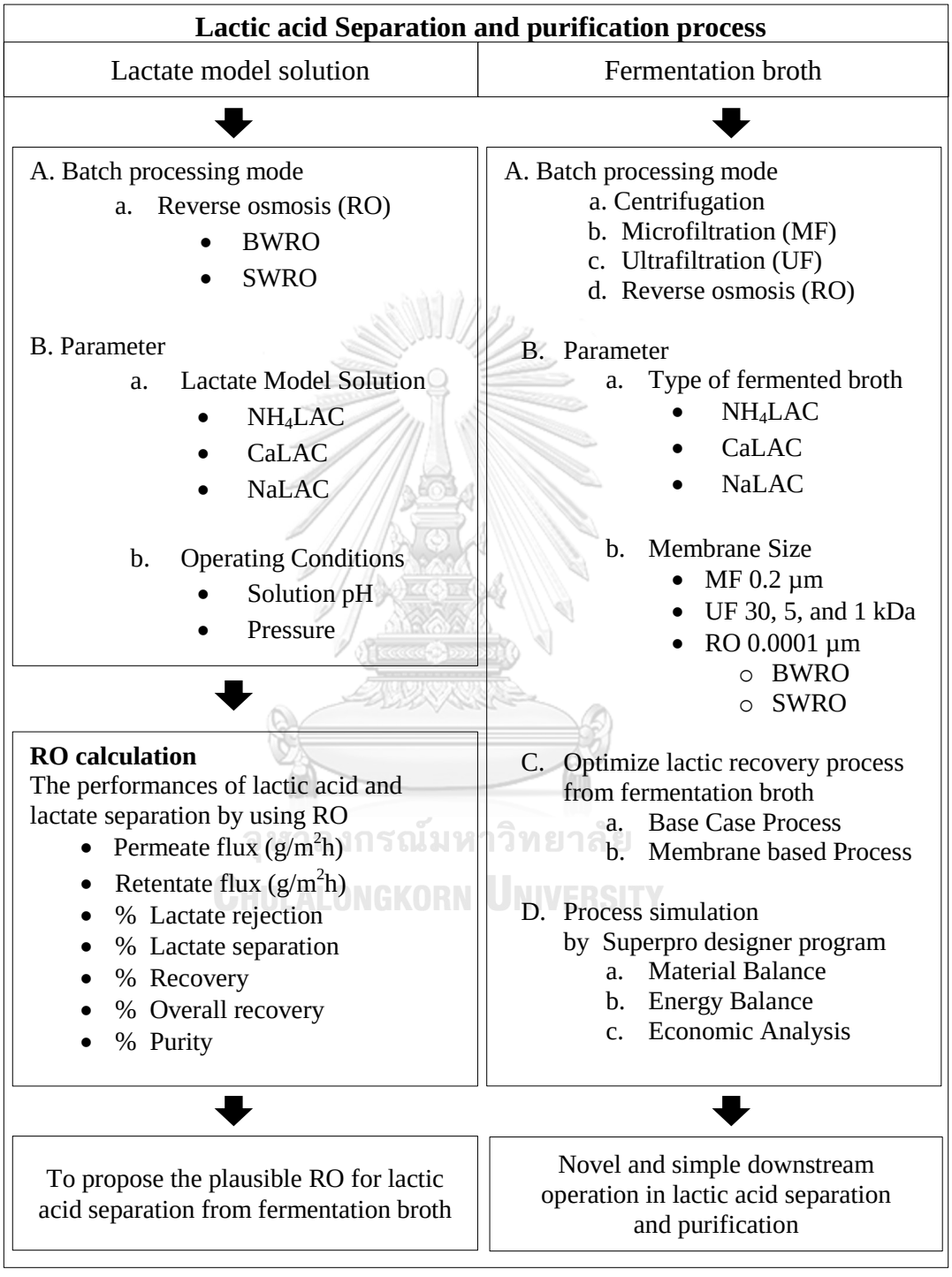
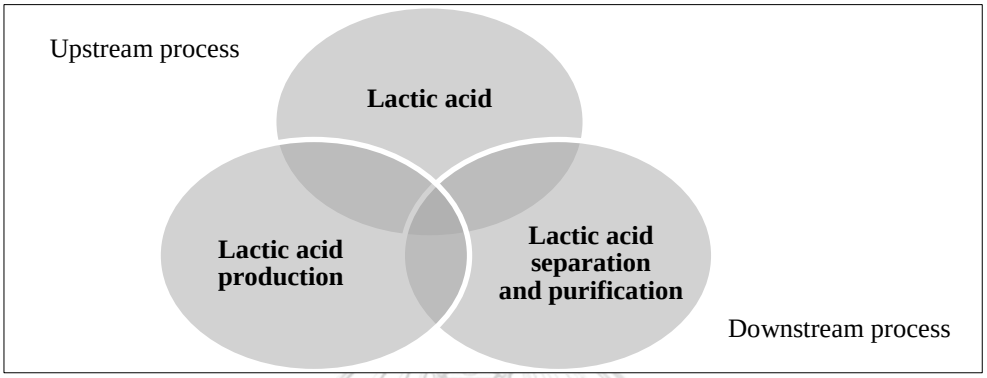


Figure 1.2 Scope of thesis.

CHAPTER II
THEORETICAL AND LITERATURE REVIEW

This chapter contains the literature reviews of lactic acid production process (upstream) as well as lactic acid separation and purification process (downstream) used in the thesis.



Firstly, the characteristics of lactic acid will be reviewed in the part of the background, the physical and chemical properties including lactic acid production (especially, fermentation process which uses to produce lactic acid as feed stream for separation and purification process). The ionization of lactic acid and the structures and chemical properties of lactate salts resulting from fermentation process are also described. In the part of separation and purification process, the advantages and disadvantage of lactic acid conventional separation and purification technique are mentioned the same as the results of conventional processes are referenced and compared. The potential techniques approached lactic acid separation and purification from fermentation broth via fermentation, consisting of centrifugation, microfiltration (MF), ultrafiltration (UF) and reverse osmosis (RO), will be introduced. The theory, characteristic and operation of four techniques mentioned above will be reviewed. The previous studies will be mentioned for experimental references. The mass transfer mechanisms in membrane separation process will be discussed. Parameters, used to determine the performance of lactic acid separation and purification process such as permeate flux and retentate flux, % rejection, % separation, % recovery, % overall recovery and % purity, will be performed. The problem occurred during membrane process, such as inorganic salt precipitation and concentration polarization, will be declared. Furthermore, the process simulation method by superpro designer program will be explained.

2.1 Lactic acid

2.1.1 Introduction

In 1780, lactic acid (LA) was found by C.W. Scheele in sour milk. It was initially considered as a milk component. Then, Lavoisier named this milk component “acide lactique”. Later, Pasteur discovered the fact that it was not a milk component and it was in fact related to fermentation metabolite, which is generated by microorganisms. In 1839, Fremy studied the usefulness of carbohydrates such as sucrose, lactose, starch and dextrin to produce lactic acid by fermentation (Vijayakumar et al., 2008). In 1881, Fermi obtained lactic acid by a microbial process from fermentation resulting in its industrial production in United States (Castillo Martinez et al., 2013). Two major biotechnological processes for the production of lactic acid that are usually related are lactic acid fermentation and product recovery and purification.

2.1.2 Physical and chemical properties

Lactic acid (2-hydroxypropionic acid, CH3CHOHCOOH) is an organic acid (John et al., 2007). There are two optical isomers of lactic acid, which are L(+)-lactic acid and D(–)-lactic acid, providing three different structures, which are an optically pure L(+)-, optically pure D(–)-lactic acid or a racemic DL-lactic acid (Gupta et al., 2007) as the stereoisomers (Figure 2.1). In particular, an optically pure L(–)-lactic acid is preferable by the food and pharmaceutical industries due to the fact it can be metabolized by the human body (Castillo Martinez et al., 2013). In contrast to D (–)-lactic acid, it is a harmful to human metabolism, affecting acidosis and decalcification. Moreover, the chemical and cosmetic industry requires one of the pure isomers or a mixture of both, according to the application. The racemic DL-lactic acid is always produced by chemical synthesis from petrochemical resources while an optically pure L (+)- or D(–)-lactic acid can be obtained by the microbial fermentation of renewable resources.

In addition, lactic acid is the most important hydroxycarboxylic acid due to it containing of both carboxylic (–COOH) and hydroxyl groups (–OH) (Varadarajan and Miller, 1999) that can be converted into different potentially useful chemicals. Generally, the conversions of lactic acid provide many beneficial chemical substances such as acrylic acid by dehydration, pyruvic acid by dehydrogenation, 1,2-propanediol by hydrogenation and lactate ester by esterification, including PLA Poly-lactic acid by polymerization (Fan et al., 2009).

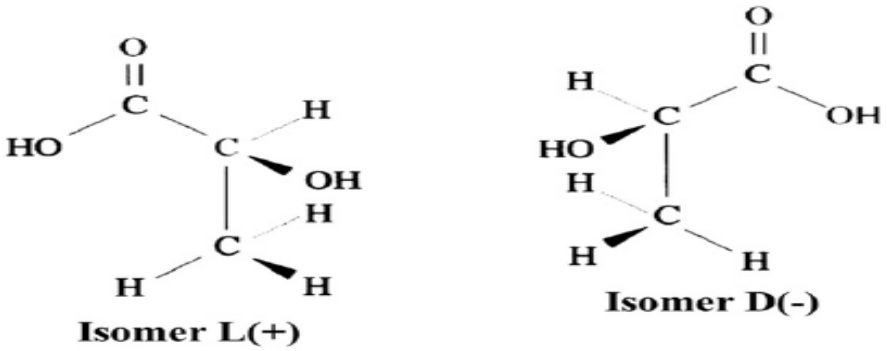


Figure 2.1 The stereoisomers of lactic acid.

2.1.3 The production of lactic acid

LA can be alternatively produced by chemical synthesis or fermentation

2.1.3.1 Chemical synthesis

Chemical synthesis of lactic acid is mainly based on the hydrolysis of lactonitrile by strong acid. The presence of lactonitrile is produced by the reaction between hydrogen cyanide and acetaldehyde and then hydrolyzed to lactic acid, either by concentrated hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) as the following reactions (Narayanan et al., 2004). This process yield provides a racemic mixture of the 2 isomers (John et al., 2007)

(1) Addition of Hydrogen Cyanide

$$\underset{\text{(acetaldehyde)}}{CH_3CHO} + \underset{\text{(hydrogen cyanide)}}{HCN} \longrightarrow \underset{\text{(lactonitrile)}}{CH_3CHOHCN}$$

(2) Hydrolysis by H_2SO_4

$$\underset{\text{(lactonitrile)}}{CH_3CHOHCN} + H_2O + \underset{\text{(sulfuric acid)}}{\tfrac{1}{2}(H_2SO_4)} \longrightarrow \underset{\text{(lactic acid)}}{CH_3CHOHCOOH} + \underset{\text{(ammonium salt)}}{\tfrac{1}{2}(NH_4)_2SO_4}$$

2.1.3.2 Fermentation

The fermentative production can directly lead to the structures of optically pure or the racemic lactic acid depending on the strain being used (Narayanan et al., 2004). Lactic acid fermentation is required in two major steps as mentioned below:

(1) Fermentation and neutralization process

$$\underset{\text{(carbohydrate)}}{C_6H_{12}O_6} + \underset{\text{(calcium hydroxide)}}{Ca(OH)_2} \rightarrow \underset{\text{(calcium lactate)}}{Ca^{2+}(2CH_3CHOHCOO^-)} + 2H_2O$$

During the fermentation process, pH normally decreases upon lactic acid formation produced by lactic acid bacteria, while the operating pH must be controlled at a range of 5.5-6.5 as the lactic acid bacterial growth preference (Vaidya et al., 2005). To maintain the pH for the optimal growth of lactic acid bacteria in the fermentation process, the addition of alkali, as the neutralizing agent, is importantly required. However, neutralization by alkali gives lactate salt at the end of fermentation. Different neutralizing agents to control the pH, such as calcium carbonate ($CaCO_3$), sodium hydroxide (NaOH) and ammonium hydroxide (NH_4OH), are effectively used in lactic production (Hetényi et al., 2011). The following equations describe the different forms of lactate salts depending on the agents used at the end of fermentation (Nakano et al., 2012; Narayanan et al., 2004).

$CaCO_3$: $\underset{\text{lactic acid}}{2CH_3CHOHCOOH} + \underset{\text{calcium carbonate}}{CaCO_3} \rightarrow \underset{\text{calcium lactate}}{(CH_3CHOHCOO)_2Ca} + H_2O + CO_2$

NaOH: $\underset{\text{lactic acid}}{CH_3CHOHCOOH} + \underset{\text{sodium hydroxide}}{NaOH} \rightarrow \underset{\text{sodium lactate}}{(CH_3CHOHCOO)Na} + H_2O$

NH_4OH: $\underset{\text{lactic acid}}{CH_3CHOHCOOH} + \underset{\text{ammonium hydroxide}}{NH_4OH} \rightarrow \underset{\text{ammonium lactate}}{(CH_3CHOHCOO)NH_4} + H_2O$

To convert lactate salts resulting from neutralization process to be in the form of free lactic acid and salt ions, acidification process is required. This can be done by the addition of mineral acids such as sulfuric acid (H_2SO_4) and hydrochloric acid (HCl). Using different acid to acidify gives different broth compositions: (1) when using sulfuric acid, insoluble or partial soluble sulfate salts are obtained; (2) using hydrochloric acid, the resulting chloride salts are highly soluble in water. In the present invention, H_2SO_4 is the preferred mineral acid. Nonetheless, H_2SO_4 commonly causes insoluble sulfate salts that become a major impurity in the feed stream entering the downstream process after the acidification process. In order to ease the downstream recovery of lactic acid, prior removal of sulfate salt is necessary

(2) Acidification process (Hydrolysis by H_2SO_4)

$$2Ca^{2+}(CH_3CHOHCOO^-) + H_2SO_4 \rightarrow 2CH_3CHOHCOOH + CaSO_4$$

(calcium lactate) (sulfuric acid) (lactic acid) (calcium sulfate)

During the acidification process, strong acid is required to convert lactate slats to be free lactic acid. H_2SO_4 is preferred due to the lower volatility and the lower solubility of sulfate in aqueous solution, which eases the solid removal. The acidification reactions with the resulting free lactic acid and sulfate salts are shown below:

$CaCO_3$: $(CH_3CHOHCOO)_2Ca + H_2SO_4 \rightarrow 2CH_3CHOHCOOH + CaSO_4$

NaOH: $2(CH_3CHOHCOO)Na + H_2SO_4 \rightarrow 2CH_3CHOHCOOH + Na_2SO_4$

NH_4OH: $2(CH_3CHOHCOO)\ NH_4 + H_2SO_4 \rightarrow 2CH_3CHOHCOOH + (NH_4)_2SO_4$

NH_4OH and NaOH are more suitable for the bipolar membrane separation processes, including microfiltration (MF), electrodialysis (ED), and ion exchange, since the precipitates are not formed rapidly after acidification by H_2SO_4. This result in less precipitates at the membrane surface that eventually lower the competition in binding to the counter ions on the resin/membrane surface. On the other hand, $CaCO_3$, which is commonly used to maintain the optimal pH in fermentation broth, gives a large amount of calcium sulfate (gypsum) as a byproduct after acidification with H_2SO_4 in the solid stream. Thus, the gypsum process (using $CaCO_3$ for pH control) is well-suited for the centrifugation technique (Qin et al., 2010).

2.1.4 Microorganisms for biotechnological production of lactic acid

Several microorganisms, classified into bacteria, fungi, yeast, cyanobacteria, and algae, have achieved one or more improvements over others in the production of lactic acid. Especially, bacteria and fungi are frequently reported in lactic fermentation (Litchfield, 1996). However, lactic acid fermentation by lactic acid bacteria (LAB) is preferred due to the limitation of fungal morphology.

2.1.5 Raw materials for biotechnological production of lactic acid

LAB requires some elements for growth, such as carbon and nitrogen sources, in the form of carbohydrates, amino acids, vitamins, and minerals (Hofvendahl and Hahn-Hagerdal, 2000). This is based on high lactic acid yields, optimum biomass production, negligible by product formation, fast fermentation rate, less pre-treatment, easy downstream processing and low cost.

2.1.5.1 Carbon source

A number of different renewable resources, such as sweet sorghum, corn, wheat, molasses, cassava and cellulose, have been used for the lactic acid fermentation to provide a pure sugar for fermenting lactic acid.

2.1.5.2 Nitrogen source

Nitrogen is available in the form of amino acids, peptides and inorganic compounds that can be added to the culture media as peptone, yeast extract, urea or ammonium sulfate (Nancib et al., 2005).

2.1.5.3 Mineral and vitamin source

Mineral elements, such as Mg, Mn and Fe, are provided in the medium in the form of salts ($MgSO_4$, $MnSO_4$ and $FeSO_4$) and vitamins present in yeast extract (Buyukkileci and Harsa, 2004).

2.1.6 The ionization of lactic acid

Lactic acid dissociates in water resulted in ion lactate and H^+. Hydronium ion (H_3O^+) was presented in the solution, which is from lactic acid ionization and water autoionizaion. The equilibrium equation describes the ionization of lactic acid as below:

$$CH_3CHOHCOOH\ (aq) \longleftrightarrow H^+\ (aq) + CH_3CHOHCOO^-(aq)$$

$$CH_3CHOHCOOH\ (aq) + H_2O\ (l) \longleftrightarrow H_3O^+\ (aq) + CH_3CHOHCOO^-(aq)$$

The ionization of lactate salts occurred during the fermentation process show that both sodium lactate and ammonium lactate provide the behaviors of monovalent cations (Na^+ and NH_4^+) whereas calcium lactate provides the behavior of divalent cation (Ca^{2+}) as the following equation.

$$Ca(CH_3CHOHCOO)_2\ (s) \leftrightarrow Ca^{2+} + 2CH_3CHOHCOO^-\ (aq)$$

$$Na(CH_3CHOHCOO)\ (s) \leftrightarrow Na^+ + CH_3CHOHCOO^-\ (aq)$$

$$NH_4(CH_3CHOHCOO)\ (l) \leftrightarrow NH_4^+ + CH_3CHOHCOO^-\ (aq)$$

As the theory of ionization, it was referred that divalent cation has a lower solubility than monovalent cation due to the combination of steric hindrance and ionic interactions. Among three different lactate compounds can be implied that calcium lactate (CaLAC) has less solubility than sodium lactate (NaLAC) and ammonium lactate (NH_4LAC) in the solution at the certain condition after the neutralization (Tu et al., 2011). Moreover, the reports on the characteristics of ion solubility indicated that organic compounds tend to increase in solubility at high pH due to it causes higher degree of ionization as the theory of acid-base equation as below:

$$CH_3CHOHCOOH\ (aq) \leftrightarrow H^+\ (aq) + CH_3CHOHCOO^-(aq)$$

$$pH = pKa + \log \frac{[CH3CHOHCOO-]}{[CH3CHOHCOOH]}$$

$$pH = -\log Ka + \log \frac{[CH3CHOHCOO-]}{[CH3CHOHCOOH]}$$

Where; Ka (lactic acid) = 1.4 x 10–4 , pKa (lactic acid) = 3.86

2.1.7 The properties of compound relating the lactic acid fermentation

Table 2.1 The properties of lactic acid and lactate.

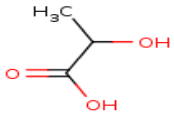
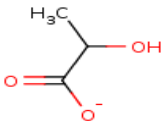
Chemical structure		
	Lactic acid	**Lactate**
Formula	$C_3H_6O_3$	$C_3H_5O_3$
Composition	C (40%), H (6.71%), O (53.29%)	C (40.45%), H (5.66%), O (53.89%)
Molecular weight	90.08 g/mol	89.08 g/mol
Solubility in water	Miscible	-
Color in solution	Colorless	Colorless
Density (25°C)	1.15 g/ml	-
Particle Size	0.00075 µm	-
pH (0.1 N)	2.4	-
Polar surface area	57.53	60.36

Table 2.2 The properties of lactate compounds.

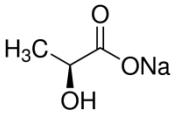
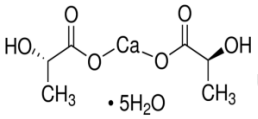
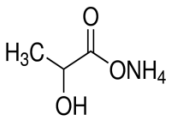
Chemical structure			
	Sodium lactate	**Calcium lactate**	**Ammonium lactate**
Formula	$C_3H_5NaO_3$	$C_6H_{10}O_6Ca.5H_2O$	$CH_3CH(OH)CO_2NH_4$
Composition	C (32.15%) H (4.5%) Na (20.52%) O (42.83%)	C (16.44%) H (6.9%) Ca (18.28%) O (58.39%)	C (33.64%) H (8.47%) N (13.08%) O (44.81%)
Molecular weight	112.06 g/mol	308.29 g/mol	107.11 g/mol
Solubility in water	15 g /100 ml	9 g / 100 ml	225 g/ 100ml
Color in solution	Colorless	Colorless	Colorless
Density (25°C)	1.33 g/ml	-	1.054 g/ml
Particle Size	-	≈ 50 µm	-
pH	6 – 8	6 – 8	6 – 8
Polar surface area	60.36	60.36	60.36

Table 2.3 The properties of neutralizing agents.

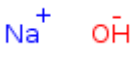
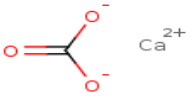
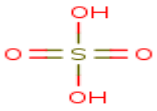
Chemical structure	 **Sodium hydroxide**	 **Calcium carbonate**	 **Ammonium hydroxide**
Formula	NaOH	$CaCO_3$	NH_4OH
Composition	H (2.52%) Na (57.48%) O (40%)	C (12%) Ca (40.04%) O (47.96%)	H (14.38%) N (39.97%) O (45.65%)
Molecular weight	40	100.08	35.05
Solubility in water	109 g/100 ml (20 °C)	0.0014 g/ 100 ml (25 °C)	Miscible
Color in solution	Colorless	white	Colorless
Density (25°C)	2.13 g/ml	2.71 g/ml	0.91 g/ml
pH (0.1 N)	12.88	9.91	11.12
Polar surface area	0.00	63.19	0.00

Table 2.4 The properties of acidifying agent.

Chemical structure	 **Sulfuric acid**
Formula	H_2SO_4
Composition	H (2.06%) O (65.25%) S (32.69%)
Molecular weight	98.08
Solubility in water	Miscible
Color in solution	Colorless
Density (25°C)	1.84 g/ml
pH (0.1 N)	1.01
Polar surface area	74.60

http://www.chemicalize.org
http://www.aqion.de/

2.2 Lactic acid separation and purification process

Lactic acid requires higher grades in commerce. Thus, well-purified lactic acid should minimize the impurities in the fermentation medium in order to reduce the levels of impurities present during recovery/separation and the purification process (Vijayakumar et al., 2008). Due to lactic acid fermentation broth containing several impurities such as microbial cells, residual sugar, nutrients, ions, other organic acids and color (Joglekar et al., 2006). The pretreatment step is importantly required to remove undesired products in order to obtain more purity in the lactic acid before the following steps of lactic acid/lactate separation and purification from the fermentation broth.

2.2.1 Centrifugation

2.2.1.1The theory of centrifugation

The centrifugal separation is the separation process for the heterogeneous mixtures of phases that differ from each other in density difference, particle size and shape under the effect of the Earth's gravity. Centrifuges are classified by function or by structure. The types of centrifuges according to the function are solid-liquid separation and liquid-liquid separation. The types of centrifuges separated by the structure are tubular, disc-bowl and basket centrifuges (Berk, 2013).

Applications for centrifugation include the sedimentation of microbial cells and viruses, as well as the separation of subcellular organelles, such as the isolation of macromolecules like DNA, RNA, proteins, or lipids. Biological substances, such as microorganism cells and precipitated forms of proteins, are easy to separate by centrifugation following the solid-liquid separation process. After applying the centrifugal force (g-force), the centrifuge tubes are spun. The centrifugal action creates an induced gravitational field in an outward direction relative to the axis of rotation and this drives the particles or precipitated matter towards the bottom of the tube. Thus, microbial cells and protein, which are heavy phase in liquid suspension, may fall to the bottom. The matter that falls to the bottom is called precipitation and the liquid above the solid is called supernatant. The principle of separation by centrifugation is shown in figure 2.2 and 2.3.

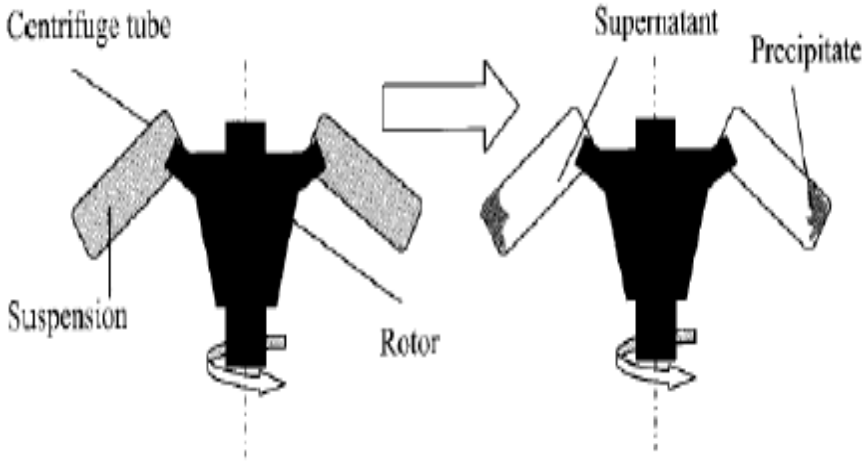


Figure 2.2 A basic principle of a centrifuge.

The simplest form of separation by centrifugation is differential centrifugation. Particles of different densities or sizes in a suspension will sediment at different rates (v), with the largest, most dense particles sedimenting the fastest followed by the less dense, smaller particles. The sedimentation rates can be increased by using centrifugal force (F). A suspension of cells subjected to a series of increasing centrifugal force cycles will yield a series of pellets containing cells of a decreasing sedimentation rate.

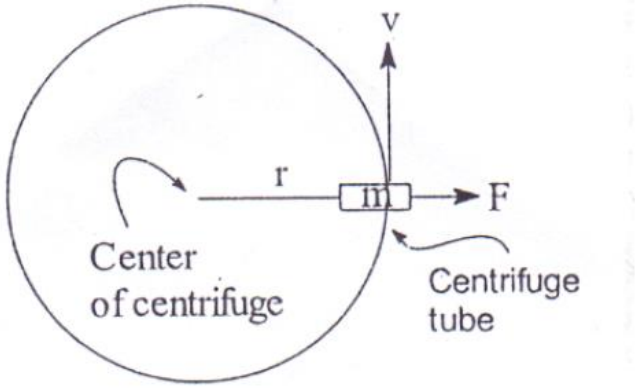


Figure 2.3 Illustration of the principle of Centrifugation.

When a liquid suspension is rotated at a certain speed or revolutions per minute (RPM), the centrifugal force causes the particles to move radially away from the axis of rotation. The force on the particles is called Relative Centrifugal Force (RCF). For example, an RCF of 1000 x g refers that the centrifugal force applied is a thousand times stronger than gravity. The Relative Centrifugal Force (RCF) value depends on the rotation speed as well as the manner in which the centrifuge tubes are held by the rotor. The calculation on RCF as formula below

$$\boldsymbol{RCF} = \frac{r\omega^2}{g} = \frac{r(2¶n)^2}{g} = \frac{r(2 * 3.14 * (n/60))^2}{9.81} = 1.12 * 10^{-3} rn^2 = 1.12 * 10^{-3} r(RPM)^2$$

Where:
r is distance from the axis of rotation (m)
ω is angular velocity (radians/s)
g is acceleration due to gravity (m/s^2)
n is rotation speed, RPM

The certain rotor speed achieved the separation of biological substances, is recommended as the common centrifuge classes and applications in table 2.5.

Table 2.5 Centrifuge classes and applications.

	Centrifuge classes		
	Low speed	**High speed**	**Ultra/micro-ultra**
Maximum Speed (rpm x 10^3)	10	28	100/150
Maximum RCF (x10^3)	7	100	800/900
Pelleting applications			
Bacteria	Yes	Yes	Yes
Animal and plant cells	Yes	Yes	Yes
Nuclei	Yes	Yes	Yes
Precipitates	Some	Most	Yes
Membrane fractions	Some	some	Yes
Ribosomes / Polymers	-	-	Yes
Macromolecules	-	-	Yes
Viruses	-	Most	Some

2.2.1.2 The researches on centrifugation in lactic acid production

The summary of the different methods for cell harvesting (bacteria and fungi cell) in lactic acid production such as centrifugation, microfiltration and pellet precipitation, proved that the uses of cell recycling by centrifuge potentially provided the total amount of lactic acid concentration over than 100 g/l (Abdel-Rahman et al., 2013). Therefore, centrifugation was continuously used in the process of cell harvesting/ removal in lactic acid production as the data provided in table 2.6.

Table 2.6 Cell harvesting by centrifuge based on the strain and condition**.**

Year	Research By	Strain	Operating condition			
		Type	Optical	Rpm	Temp (ºC)	Time (min)
2004	Tong	*Lactobacillus rhamnosus ATCC 10863*	L	8,000	4	20
2006	Altaf	*Lactobacillus amylophilus GV6*	L	8,000	-	20
	Ding	*Lactobacillus casei LA-04-1*	-	4,500	-	10
	Gao	*Lactobacillus rhamnosus NBRC 3863*	-	5,500	-	40
	Wang	*Lactobacillus*	L	3,000	-	5
2007	Altaf	*Lactobacillus amylophilus GV6*	L	12,000	-	15
	Gao	*Lactobacillus rhamnosus NBRC 3863*	L	5,500	-	40
	Resa	*Lactobacillus casei*	-	4,000	37	10
	Shen	*Lactobacillus bulgaricus BCRC 10696*	-	6,000	-	20
2008	Zhang	*Lactobacillus coagulans*	L	4,000	-	10
2009	Gao	*SaccharomycescerevisiaeOC-2T T165R*	L	8,000	-	5
	Gao	*Saccharomyces cerevisiae OC-2T T165R*	L	5,000	-	5
	Maeda	*Lactobacillus acidophilus strain TS1*	-	8,000	-	1
	Wee	*Lactobacillus sp. RKY2*	-	13,000	-	20
2010	Chai Ng	*Lactobacillus acidophilus BCRC 17002* *Lactobacillus casei subsp BCRC 12248*	- -	3,000	-	10
2011	Edward	*Lactobacillus plantarum*	-	10,000	-	-
	Cagno	*Pediococcus pentosaceus SWE5* *Lactobacillus plantarum FP3*	- -	10,000	4	10
	Gao	*Saccharomyces cerevisiae OC-2T T165R*	L	10,000	-	3

Table 2.6 Cell harvesting by centrifuge based on the strain and condition (con).

Year	Research By	Strain	Operating condition			
		Type	Optical	Rpm	Temp (ºC)	Time (min)
2012	Dey	*Lactobacillus delbruckii NCIM-2025*	L	12,000	-	15
	Oguntoyinbo	*Lactobacillus plantarum ULAG11*	-	4,000	-	5
	Sikder	*Lactobacillus plantarum NCIM 2912*	L	12,000	4	15
	Vukovic	*Lactobacillus rhamnosus ATCC 7469*	L	4,500	-	20
2013	Nguyen	*Lactobacillus paracasei LA104*	L	8,000	37	20
		Lactobacillus coryniformis ATCC 25600	D			
	Watanabe	*Lactobacillus rhamnosus M-23*	L	5,000	4	10
	Wang	*Escherichia coli HBUT-D*	D	10,000	4	5
	Wouters	*Lactobacillus plantarum IMDO 788*	-	16,000	4	20
		Lactobacillus sakei IMDO 1358	-			
2014	Abdel-Rahman	*Enterococcus mundtii QU 25*	L	7,190	4	15
	Ashraf	*Lactobacillus casei 290*	-	6,000	15	4
	Chookietwattana	*Lactobacillus Plantarum MSUL 903*	-	10,000	-	10
	Komesu	*Lactobacillus plantarum*	-	5,000	25	15
	Kumar	*Lactobacillus delbrueckii subsp. bulgaricus DSMZ 20081*	D	5,000	-	8
	Nionelli	*Lactobacillus plantarum*	-	10,000	4	10
	Tosungnoen	*Lactobacillus Plantarum MSUL 702*	-	10,000	15	20
	Sharma	*Lactobacillus plantarum NCDC 414*	-	10,000	4	10
	Ye	*Bacillus coagulans JI12*	L	4,000	-	10
	Zhang	*Bacillus coagulans IPE22*	-	10,000	-	10
2015	Esteban-Torres	*Lactobacillus plantarum*	-	8,000	4	15

2.2.2 Membrane based separation process

2.2.2.1 The theory of membrane based separation process

The principle of membrane-based separation processes is mainly based on selective permeability either by size exclusion or solute diffusion (Mohr et al., 1988). The solution is forced through a porous of filtration media (membrane). The particles that are larger than the porous surface of the membrane are retained. The objective of membrane filtration may be the removal of undesirable solid particles from a liquid product or, alternatively recovery of a solid product from a solid/ liquid mixture. Surface membrane filtration processes are classified into two categories: dead-end filtration and cross-flow filtration. With dead-end filtration, the direction of suspension flow is normal to the filter surface. The particles are stopped (come to a dead-end) on the filter surface and accumulate as a cake. The flux decreases rapidly due to the accumulation of particles on the filter layers (Figure 2.4). On the other hand, with cross-flow filtration, the direction of suspension flow is parallel to the filter surface. The retained particles are carried forward by the flowing suspension maintaining a high velocity of flow. It does not eliminate the particle boundary layer completely but it does lead to higher flow rates (Figure 2.5.) (Berk, 2013).

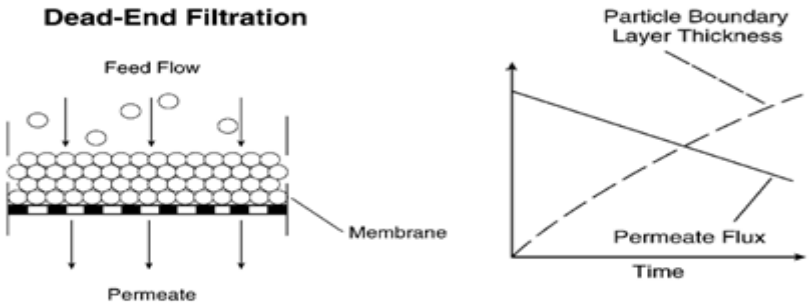


Figure 2.4 The characteristics of dead-end filtration.

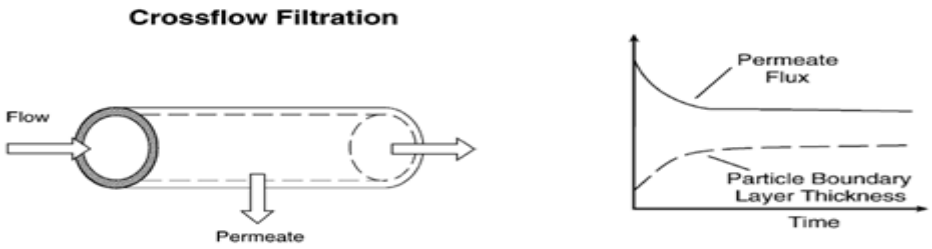


Figure 2.5 The characteristics of cross-flow filtration.
(Ref U. S. Department of Energy)

Microfiltration (MF) and ultrafiltration (UF) are applied in filtration processes in which particle size is practically the sole criterion for permeation or rejection. In contrast, reverse osmosis (RO) membranes separate particles at a molecular level, and their selectivity is considered on the chemical nature of the particles. The driving force for material transport through the membrane in MF, UF and RO processes is a pressure difference. These processes are called pressure-driven membrane processes. The approximate ranges of separation and typical operation pressures for the pressure-driven membrane processes are given in figure 2.6 and 2.7. (Berk, 2013)

Typical Range of Application of Pressure-Driven Membrane Separation Processes

Process	Typical operating pressure range: MPa	Limit particle size range: nm or (molecular weight)
MF	0.1– 0.3	100–10 000
UF	0.2–1.0	1–100 (10^{2}–10^{6} Da)
NF	1–4	0.5–5.0 (10^{2}–10^{3} Da)
RO	3–10	(10^{1}–10^{2} Da)

Figure 2.6 The typical range of application of pressure-driven membrane separation process

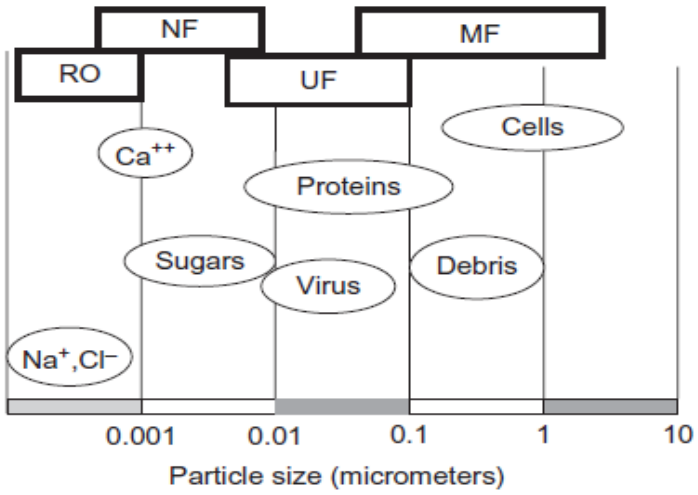


Figure 2.7 Separation range of pressure-driven membrane processes

The driving force transportation through the membrane is the pressure drop across the membrane (Transmembrane pressure difference, TMPD). As figure 2.8, the pressure at the permeate side is practically uniform while the pressure at the retentate side relates the direction of the flow.

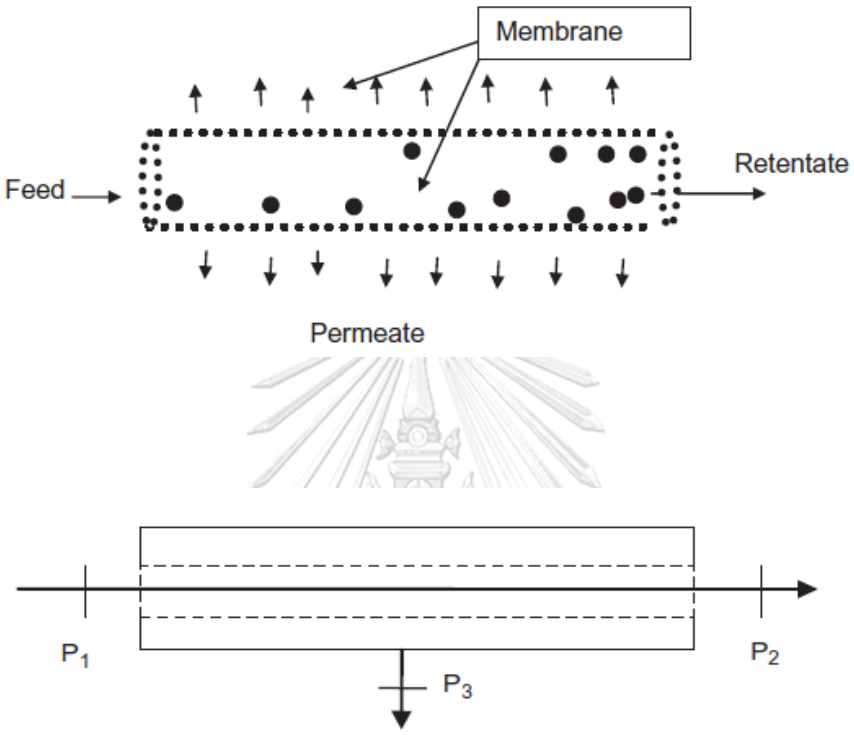


Figure 2.8 Definition of the trans-membrane pressure drop (TMP).

Trans-membrane pressure difference (TMPD) can be calculated by the following formula

$$\boldsymbol{TMPD} = \frac{P1 + P2}{2} - P3$$

Where

P1 is the pressure at the module inlet (bar)

P2 is the pressure at retentate side (bar)

P3 is the pressure at permeate side pressure, assumed uniform (bar)

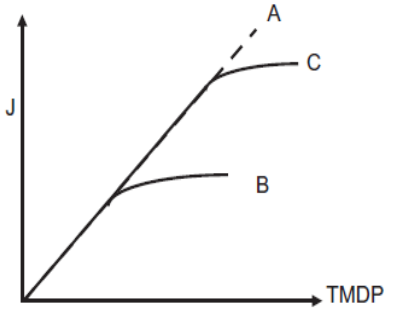
2.2.2.2 Microfiltration (MF) and Ultrafiltration (UF)

2.2.2.2.1 The characteristic of MF and UF membrane process

The membranes used in these filtration processes are porous. Thus, the transport of permeate through the membrane follows the basic principles of flow through porous media (size exclusion). Thus, if the particle size is larger than the membrane pores size, it definitely cannot pass through the membrane. MF is practically used to separate the micron-sized particles, whereas UF is basically used to remove macromolecules such as proteins (Datta and Sablani, 2007). Mass transfer through MF and UF membranes are described as being solvent transport, following Darcy's Law and solute transport below (Berk, 2013):

(1) Solvent transport

$$\boldsymbol{J} = L_p \Delta P_{TM} \quad ; \quad L_p = \frac{\varepsilon R^2}{8\mu z}$$



Where

J is permeate flux ($^{m}/_{s}$)

Lp is hydraulic permeability, ($^{m}/_{sPa}$)

ΔP_{TM} is transmembrane pressure difference *(Pa)*

ε is membrane porosity, dimensionless

Z is membrane thickness (m), R is radius (m)

μ is viscosity of the permeate (Pa s)

The straight line A represents the theoretical behavior according to the equation. The curve B depicts the typical behavior observed in reality. The decline in flux may be explained by concentration polarization, fouling membrane compaction. The curve C shows that increasing the flow-rate results in increasing the flux.

(2) Solute transport

The solute rejection (%R) and the sieving coefficient (S) of a membrane are defined as follows

$$\boldsymbol{R}\% = (1 - S) * 100 \quad ; \quad \boldsymbol{S} = \frac{C_{perm}}{C_{retn}}$$

Where

R% is the rejection of solute

C_{perm} is the concentration of the solute in the permeate, kg/m^3

C_{retn} is the concentration of the solute in the retentate, kg/m^3

2.2.2.3 Reverse osmosis (RO)

2.2.2.3.1 The characteristic of RO membrane process

Osmosis is the explanation of the spontaneous transfer of water from a dilute into a concentrated solution through a membrane, as shown in figure 2.9. In order to stop the osmotic transfer of water into a solution, a certain pressure, called osmotic pressure, must be exerted against the direction of the transfer. Application of a pressure stronger than the osmotic pressure causes water transfer in the opposite direction, transferring from the concentrated solution to the less concentrated medium, which is called reverse osmosis, as shown in figure 2.10 (Berk, 2013).

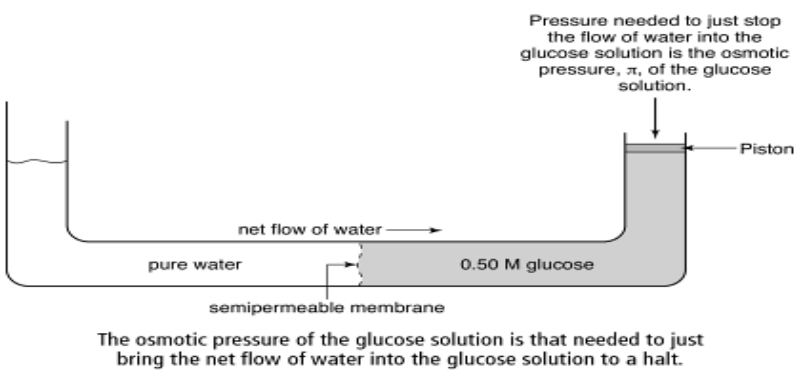


Figure 2.9 Osmosis.

An important fact about osmosis and osmotic pressure is that the osmotic pressure of pure water (pure solvent) is zero. Solutions have osmotic pressure but pure solvents do not have osmotic pressure.

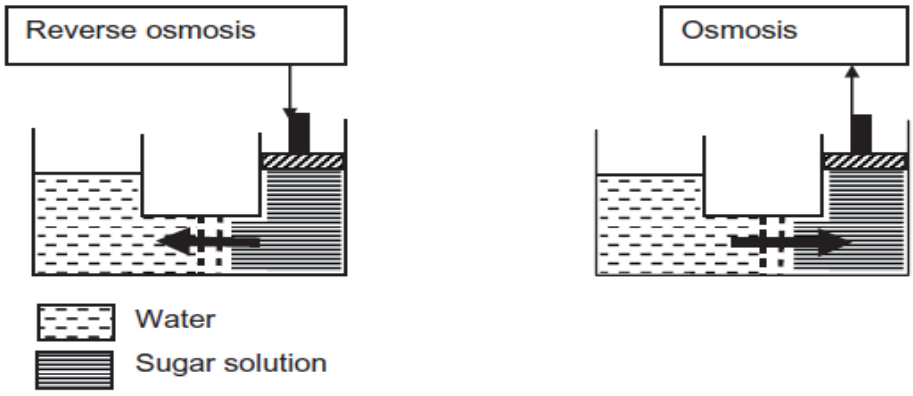


Figure 2.10 Osmosis and reverse osmosis.

The mechanism of separation by reverse osmosis membrane is not relative to just size exclusion but also solution diffusion affecting the solute transportation. In the solution-diffusion mechanism, solutes are diffused and then absorbed into the membrane structure; thus, the relative rates of the adsorption desorption and diffusion of the solution, including the electrostatic repulsion interaction between the solute charges and membrane surface charges, are the factors to control the separation by RO (Datta and Sablani, 2007). The same as MF and UF, RO is operated under the pressure-driven process. However, the pressure applied must overcome the osmotic pressure (π) in order to the solution transfer. Osmotic pressure (π) is defined below as;

$$\boldsymbol{\pi} = iMR_tT$$

Where
$\boldsymbol{\pi}$ is osmotic Pressure (atm)
i is dimensionless van't Hoff factor
M is molarity (molar concentration of the solution)
Rt is gas constant 0.08205746 (L atmK^{-1} mol^{-1})
T is temperature (^oK)

(1) Solvent transport

Mass transfer in RO membrane is described by the molar flow, mass flow or volumetric flow per unit time for 1 unit of area as follow;

$$\boldsymbol{J = Flux} = \frac{Transfer\ rate}{Transfer\ area}$$

(2) Solute transport

Total rejection of solute, the concentration ratio achieved by RO membrane is shown as follow;

$$\frac{C_{retn}}{C_{feed}} = \frac{Q_{feed}}{Q_{feed} - Q_w}$$

Where
C_{feed} is concentration of the solute in the feed
C_{retn} is concentration in the retentate
Q_{feed} is volumetric flow-rate of the feed
Q_W is volumetric flow-rate of the permeate (water)

Furthermore, the water permeability ($m^3.s^{-1}.m^{-2}.bar^{-1}$) will be determined. The water permeability quotation is described as follow;

$$L_{po} = \frac{J_w}{\Delta P}$$

Where
Lpo is water permeability (m3.s-1.m-2.bar-1)
J_w is the water flux (m/s)
ΔP is the pressure difference (bar).

Referring to the principle of the solute transport in RO membranes is caused by the diffusion through a membrane pore upon sieve effect and charge effect (Tsuru et al., 1991). Some evidence reported that certain trace organic compounds cannot be removed during the RO process completely due to the fact low molecular weight (MW) organic compounds, such as neutrals and acids, show a MW smaller than the molecular weight cut-offs (MWCOs) of the membranes tested. Thus, some of organic compounds can still be moved to RO permeates (Bellona et al., 2004). It is implied that the smallest membrane pore size will not always guarantee the highest solute rejection, especially for low MW non-charged organics. Two important mechanisms, indicating the salt or solute rejection in RO membrane filtration, are restricting solute diffusion across the membrane (charge effect) and chemically hindering the transport of solutes through pores (sieve effect) (Košutić and Kunst, 2002). Many researchers also reported on the rejection of organic solutes by RO membranes, stating that it was influenced by feed pH, solute charge (associated with acid or base dissociation coefficient, pKa or pKb) and membrane surface charge (Bellona et al., 2004). The combinations of positively charged ions (cations) and negatively charge ions (anions) in the solution could be contacted with the strong negatively charged membrane resulting in the cation concentration in the membrane being greater than in the bulk solution. In contrast, the anions concentration in the membrane becomes less than in the bulk solution. A strong negatively charged membrane will produce a greater repulsive force than a weak negatively charged membrane. This electrical reaction is known as the Donnan potential, occuring at the boundary between the membrane and the feed solution (Bartels et al., 2005) as figure 2.11 demonstrates below:

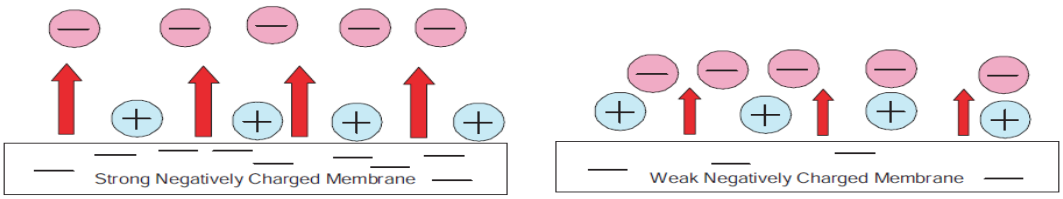


Figure 2.11 Donnan potential reaction on negatively charged membrane (Bartels et al., 2005).

Feed of pH has been indicated as one of the most significant factors influencing the rejection of compounds in the membrane filtration process. This is because changes of feed pH directly relate to the amount of ion concentration in solution and the characteristic of ionization. Moreover, the pH of the solution and its isoelectric point (IEP) directly affects the membrane charge (González et al., 2008). Thus, the changes in pH can cause an effect to the separation of acids, including their behavior towards the membrane changing also. The pH effect on the amount of ion concentration was explained by the addition of acid or alkali by adjusting pH from the neutral pH of solution. Sodium hydroxide (NaOH), as an alkali, is basically used to adjust pH, which causes an increase in Na^+ concentration with an increasing pH (Timmer et al., 1993). Therefore, the adding of acid (HCl) or alkali (NaOH) causes a dramatically large amount of ion in the solution by presenting higher molecules ions (Na^+, H^+ and OH). Then, the researcher concluded that at a pH with a low ion concentration, the permeate concentration or solute leakage was enhanced. Meanwhile at pH with a high ion concentration, the permeate concentration or solute leakage was reduced. This was due to the larger hydrated ions sizes, compared with the molecules, would reduce the diffusion rate, resulting in lower permeate concentrations (Liew et al., 1995). In addition, the pH factor also affects the hydration and absorption capacity of the solution on membrane. H^+ ion is formed by the hydrogen-bonds with the carbonyl groups of the polyamide membrane, which can then help to promote the ionic passage. A pH lower than the pKa (pKa of lactic acid = 3.86) can improve the diffusion of compounds through the membrane due to the hydrogen bonds between protonated acid and membrane. In contrast, a pH higher than the pKa can increase the rejection rate of compounds (Morin Couallier et al., 2006). At pH values lower than pKa, lactic acid is presented in an undissociated form. On the other hand, lactic acid is completely dissociated at higher pH values affecting the rejection by charged membranes (Bartels et al., 2005). The amount of undissociated lactic acid and lactate anions can be calculated following the Henderson–Hasselbalch equation below:

$$CH_3CHOHCOOH\,(aq) \quad \longleftrightarrow \quad H^+(aq) + CH_3CHOHCOO^-(aq)$$

$$pH \;=\; pKa \;+\; \log \frac{[CH3CHOHCOO-]}{[CH3CHOHCOOH\,]}$$

Therefore, the adjustment of pH value, by adding alkali or acid, will be significantly related to the chemical compositions of total ions in solution. This is due to the total ion value being relatively based on a function of pH variances. Besides the pH factor, ionic strength of the solution is also reported to be one of key factors affecting salt removal by RO membrane (Oo and Song, 2009). **S**alt rejection is significantly influenced by membrane charge and feed solution composition (feed ionic strength) contributing in the variation of ion passage through RO membrane following Donnan's effect theory. Donnan potential is dependent on the rejection of anions while attracting the cations. The cation ion at the membrane surface shields the repulsive force of the membrane's negative charge on the anions in the bulk solution. Thus, a solution with a higher concentration of divalent cation shows the weakest Donnan potential leading to an increase of salt rejection at the membrane (Bartels et al., 2005), as figure 2.12 demonstrates below:

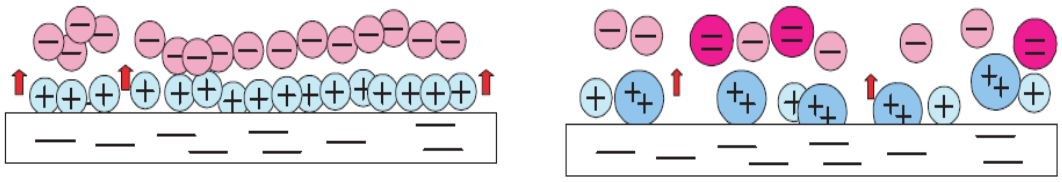


Figure 2.12 Donnan potential reaction on negatively charged membrane (-) between monovalent cation (+) and divalent cation (++) (Bartels et al., 2005)

The diameters of the hydrated ions, such as H^+, Na^+ and NH_4^+, result from the diffusion rate and permeate concentration. Among H^+, Na^+ and NH_4^+, monovalent cation of Na^+ has the biggest ion size; therefore, it provides a lower rate of diffusion and a lower permeate concentration. In contrast, monovalent cation of H^+ and NH_4^+ ions could be formed the hydrogen-bonds with the carbonyl groups of the polyamide membrane promoting the ionic passage through RO membrane. In conclusion, the changes in molecules concentration, ion concentration (Liew et al., 1995) and ionic strength (ionic type) significantly cause an effect on the solutes permeability during the RO process at certain operating pressures; following the concept of ion transportation across a membrane by Donnan's effect theory (Schäfer et al., 2004). Previous studies also confirmed that the permeate flux and the rejection of lactic acid/lactate by membrane resulted from the effects of skin shrinkage in concentrated solutions. Moreover, the sorption of lactic acid/lactate by the membrane is influenced by the conventional effects of charge and solute size, as well as osmotic differences between concentrate and permeate streams (Freger et al., 2000). In addition to factors of pH and ion strength, the operating conditions such as feed pressure and flow rate are also indicated to be the significant key indicators of solute rejection by RO membrane filtration**.** Further investigation on scale formation and membrane fouling are reported using the following criteria (Kim and Hoek, 2005; Tilak G., 2010)

(1) Inorganic salt precipitation – inorganic salts resulted from neutralization and acidification strongly affects RO process due to membrane fouling. This leads to flux decline from the scale blockage which limits the RO performance and membrane life span. The major factors including cross flow velocity, TMP, permeate recovery, concentration polarization, the presence of metal ion contaminants as well as the operating conditions (fluid flow rate, pH, and temperature) are responsible to scale formation; thus, significantly affect RO performance.

(2) Concentration polarization (Figure 2.13) is also one of the important factors influencing the performance of RO because it represents the accumulation of rejected solute at the membrane surface. It is directly governed by solute properties, membrane properties, and hydrodynamics. The adverse effects of concentration polarization are decreasing water flux, increasing solute flux, rejection of RO permeates, solute precipitation, diverted membrane properties, fouling and blocking at membrane surface, and shortened membrane lifespan. Concentration polarization is explained the accumulation of rejected solutes at the membrane surface that the solute concentration at the membrane wall is higher than the bulk feed solution. When water passes through the membrane, the flow of solute to the membrane surface is larger than the diffusion of the solute. As a result, the concentration of the solutes at the membrane wall increases.

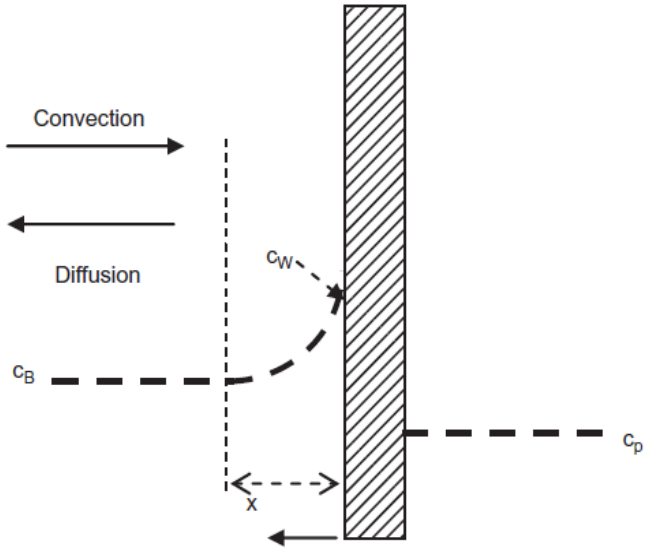


Figure 2.13 Concentration Polarization Concentration Profile.

At steady state, the local concentration does not change with time, Therefore the effects must be in equilibrium. Assuming Fick's Law for the back-diffusion, the steady-state condition as follows (Berk, 2013).

$$\boldsymbol{J} \cdot \boldsymbol{C} = -D\frac{dc}{dx} = \frac{J}{D}\int_0^{\delta} dx = \int_{CW}^{CB}\frac{dC}{C}$$

Integration

$$\boldsymbol{J} = \frac{D}{\delta} \ln \frac{C_W}{C_B} = K_L \ln \frac{C_W}{C_B}$$

Where

C_w is concentration at the membrane interface, kg/m^3

C_B is concentration at fluid bulk, kg/m^3

J is solvent flux, m/s

D is diffusivity of the protein in the solvent, m^2/ s

X is distance from the membrane

δ is thickness of the boundary layer for diffusion.

2.2.2.3.2 RO Membrane materials

Commercial RO membranes often employed in bioprocess classified by the material used and properties the table 2.7.

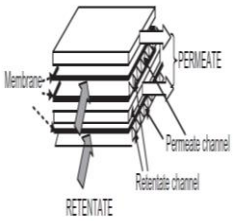
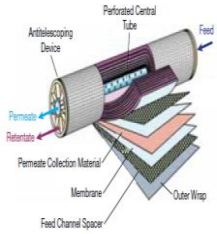
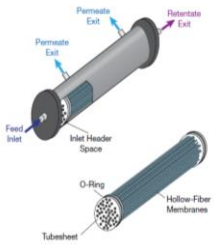
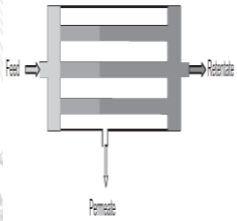
Table 2.7 RO membranes material used and properties.

Properties	Cellulose Acetate Membrane (CA)	Aromatic Polyamide Membrane	Polyamide thin film composites (TFC)
Surface Layer (μm)	0.2-0.5	0.1-1.0	0.001-0.1
Water permeability	High	Higher water flux than CA membranes	High
Organic rejection	Low	Higher rejection of water-soluble organics and salt rejection than CA membrane	Reject some low molecular weight organics than CA
pH	4-8	4-11	2-11
Operating Pressure	30 kg/cm^2	15 kg/cm^2	15 kg/cm^2
Temperature	Maximum 35 ºC	Maximum 35 ºC	Maximum 45 ºC
Membrane Fouling	Low	High	High
Cost	Low	High	High

2.2.2.3.3 RO Membrane configurations

Four main types of membrane configurations are usually adopted in the industry as provided in the table 2.8.

Table 2.8 RO membrane configuration.

1.Plate and Frame 	Simplest configuration, consist of two end plates, the flat sheet membrane, and spacers	**3. Spiral Wound** 	Flows on one side of the membrane. Permeate is collected on the other side
2. Hollow Fiber 	Membrane is on the inside of a tube, and the feed solution is pumped through the tube	**4. Tubular** 	Stainless steel tubes in shell and tube construction

2.2.2.3.4 RO Membrane categories

Reverse osmosis membranes can be separated into three categories by referring to their applications and uses as detailed below:

(1) Tap water reverse osmosis membranes (TWRO); A few impurities or dissolved inorganic solids such as salts, fluoride, chloride, nitrate and sulfate are found in water. It contained about 200 - 500 ppm salt solution at an operating pressure of 3 – 6 bar. The quality of the filters and membranes used in the RO system is around 92-98 % rejection.

(2) Brackish water reverse osmosis membranes (BWRO); It contains a higher salinity concentration levels than tap water but not as much as sea water. Brackish water commonly refers to the condition of where fresh water meets sea water. It was generally operated within a 500 – 30,000 ppm salt solution at an operating pressure of 2 – 17 bar. It rejects around 98 – 99 % of impurities or salts from salt solution.

(3) Sea water reverse osmosis membranes (SWRO); This membrane type has a very high salinity of around 32,000 – 50,000 ppm at an operation pressure of 40 – 70 bar. SWRO is successfully used for water treatment in many industries with the result of a salt rejection percentage higher than 99%.

2.2.2.3.5 RO Membrane selectivity

The selectivity of membrane can be predicted by many theories and knowledge as summarized below (Baker, 2004).

(1) Valences of ions; in general, Monovalent ions are retained less than divalent ions and multivalent ions

(2) Dissolved gasses; the dissolved gasses can easier pass through permeation side normally.

(3) Ionization; when the acid or base is in the ionized form, the rejection will be high but the rejection will be low in the nonionized form.

(4) Molecular weight; the greater molecular weight of neutral organic solutes will be more retained by RO membrane

(5) Negative rejection coefficient; it can happen when a solute concentration in the permeation side is higher than in the feed side.

2.2.2.4 The researches on membrane based separation process

The applications of MF, UF and RO for several industries provided as the table 2.9 and the applications of MF, UF and RO for lactic acid production provided as the table 2.10.

2.2.2.4.1 Applications of MF, UF and RO for several industries.

Table 2.9 Applications of MF, UF and RO for several industries.

Membrane	Application	Results
MF	Separation of phosphorus from wastewater (PO_4^{3-}, Zn^{2+}, Ca^{2+}, Mg^{2+})	Phosphate removal was 99.7% The % rejection was 99.7,1.24, 23.65 and 14.26 respectively (Zhang et al., 2006)
RO	Separation of nitrogen from domestic wastewater	The separation efficiency was 95% for total nitrogen. (Bilstad, 1995)
RO	Separation of $CaSO_4$ and Fe^{3+} from natural water	$CaSO_4$ and Fe^{3+} were separated from the natural (Kavitskaya et al., 2000)
RO	Purification of phosphoric acid solutions	46.3% permeation and 99.3% of rejection of cationic impurities (González et al., 2002)
RO	Separation of sulfate content in aqueous solutions	Sulfate concentrations were between 0.145-25.455 kg/m^3 Rejection was higher than 99.55% were obtained in all cases (Bódalo et al., 2004)
RO	Separation of inorganic and organic compounds	Phenol, 2,4-dinitrophenol (DNP), pentachlorophenol (PCP), NaCl, NaBr and KBr were separated from aqueous solutions (Senthilmurugan and Gupta, 2006)
MF with RO	Separation sodium from tannery water	82% reduction of sodium (Bhattacharya et al., 2013)
UF with RO	Separate of ions from influent and effluent water	The rejection of manganese, iron and ammoniacal nitrogen were 95.2%, 96.9% and 76.9% respectively (Huang et al., 2011)
UF with RO	Separation of impurities from the metal finishing industry	91.3 - 99.8% rejection of the contaminants such as metal elements, organic, and inorganic compounds (Petrinic et al., 2015)

2.2.2.4.2 Applications of MF, UF and RO for lactic acid production.

Table 2.10 Applications of MF, UF and RO for lactic acid production.

Membrane	Application	Results
MF	Separation of cell from lactic acid fermentation broth	The total amount of cell concentrations was 81.5g dry cell/l (Taniguchi et al., 1987)
MF	Separation of cell from lactic acid fermentation broth	The total amount of lactic acid concentrations was 92-94 g/l (Oh et al., 2003)
MF	Cell recycling from lactic acid fermentation broth	Yield, productivity and biomass were 4.23%, 315.64% and 8.88% higher than batch fermentation. (Lu et al., 2012)
UF	Separation of cells and proteins for recycling in lactic acid production	36 g/Lh of productivity and 90 g/L of lactic acid concentration (Xavier et al., 1995)
UF	Separation of cells and proteins for recycling in lactic acid production	57 g/Lh of productivity and 92 g/L of lactic acid concentration (Kwon et al., 2001)
UF	Separation of cells and proteins for lactic acid fermentation broth	100% protein retention by UF (MWCO 25 kDa) (Torang et al., 1999)
UF	Separation of cells and proteins from cheese whey production to produce lactic acid	94.5% of the lactose conversion 0.65 g. of lactic acid per g. of lactose used (Julien and Whitford, 2006)
RO	Concentrating lactic acid	97% rejection of lactic acid and residual sugars (Schlicher and Cheryan, 2007)
RO	Concentrating lactic acid from aqueous mixture	99.2% rejection of lactic acid from mixture (Diltz et al., 2007)
UF with RO	Concentrating lactic acid from the permeate stream collected from UF (cheese whey production)	Highest protein recovery in UF stage Highest lactic acid recovery in RO stage (Yorgun et al., 2008)
NF with RO	Concentrating lactic acid from the permeate stream collected from NF (cheese whey production)	Highest lactose retention in NF stage (97%) Highest lactic acid recovery in RO stage (nearly 100%) (Li et al., 2008)

2.3 Process simulation

Process simulation tools have been continuously applied in petrochemical industries for developing and optimizing the design and operation of integrated processes since 1960s. When a new process scale is required, process simulators can be used to model the required equipment size as well as to estimate the cost of equipment (Petrides et al., 2002). Various software tools were performed such as bioprocess simulator (BPS), Biopro Designer, Superpro designer, biotechnology design simulator (BDS) and batch process technology (BATCHES). The ability to handle the unit operation of both batch and continuous processes, including the specification of unit operation to bioprocessing are required as minimum functions for biochemical process simulation. SuperPro designer program (Figure 2.14 and 2.15), one of the most well-known process simulator software tools, was successfully used to demonstrate the role of simulation tools in the bioprocess design for balancing the material and energy with the function of process modeling, equipment sizing, scheduling, including economic evaluation (Julien and Whitford, 2006).

Figure 2.14 Superpro designer process simulation software.

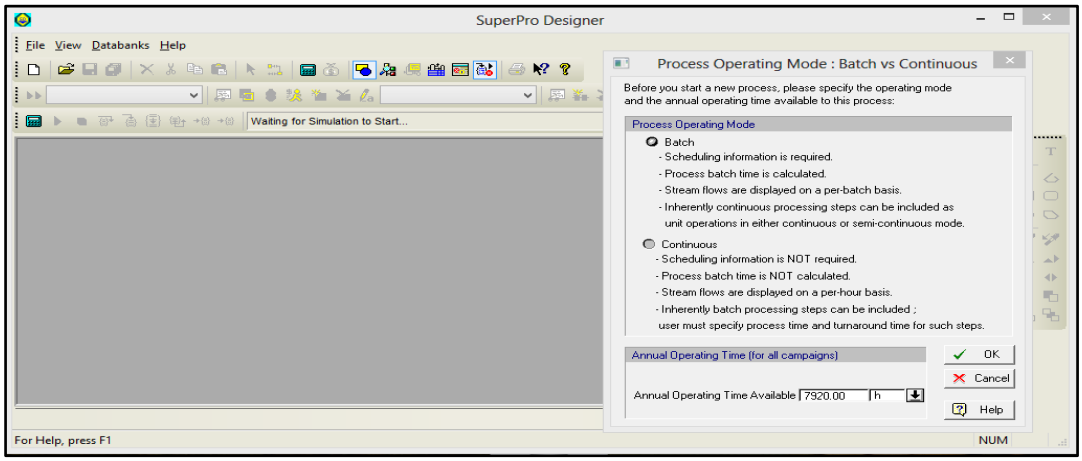


Figure 2.15 Accessing mode of process simulation.

2.3.1 Procedure

The basic steps of process simulation by superpro designer are detailed as following:

(1) Create a design simulation

When the program is completely opened, the user can create a new flowsheet for starting a simulation

(2) Specify mode of operation

When the new design case is created, the basic mode process will be selected. There are two options: batch mode and continuous operation mode.

(3) Set default physical units

Defining the measurement units will be generally used for inputting data

(4) Register components and mixtures

If the data of components and mixtures are not available in databases, the user can register pure components and mixtures for the provided process

(5) Add unit procedures

The unit procedure represents the unit operation of equipment. Thus, adding the unit procedure is required for simulation. Unit operations available in unit procedures based on the filtration procedure are offered by superpro designer, as shown in table 2.11.

(6) Add input and output streams

Stream represents the transportation of material in unit procedures. Input stream refers to the material transfers into the unit while output stream transfers go out of the unit.

(7) Specify operations

After unit procedures and streams were added completely, the operation needed to be specified and detailed within each of the unit's equipment. The specification of unit operation can be selected from the list of unit operation available in unit procedures, as shown in table 2.11.

Table 2.11 Unit operation available in unit procedures.

Filtration procedure	Operation available	
Microfiltation (batch)	Charge	Steam-in-place (SIP)
	Clean-in-place (CIP)	Transfer in
	Concentrate	Transfer out
	Hold	
Ultrafiltration (batch)	Charge	Steam-in-place (SIP)
	Clean-in-place (CIP)	Transfer in
	Concentrate	Transfer out
	Hold	
Reverse osmosis (batch)	Charge	Steam-in-place (SIP)
	Clean-in-place (CIP)	Transfer in
	Concentrate	Transfer out
	Hold	

(8) Schedule process

Scheduling is essential for the batch process consisting of the 4 steps mentioned below:

(8.1) Specification of setup time

8.1.1 User specification; if the operating time is known; the user can input the duration time by using the "set by user" function.

8.1.2 Simulation calculated; if operating time is unknown, the process time can be calculated by selecting the "calculated based on" function.

8.1.3 Master-slave relationship; if operating time is unknown, the process time can be referred to by another process or a series of processes by choosing the "set by master-slave relationship" function

(8.2) Scheduling relationship

Four scheduling relationships are classified as below

8.2.1 Beginning of the batch relationship; if the duration time of each operation is known, the user can indicate a start time relative to the beginning of the batch for a certain operation in the unit procedure.

8.2.2 Previous operations in the same procedure relationship; the start time will be scheduled according to the start or end of another operation in the same unit procedure

8.2.3 Another operation in the same procedure relationship; the other operation will be selected and then the user can specify the start time based on the starting or ending time of another operation in the same procedure.

8.2.4 Another operation in another procedure relationship; the start time of an operation is scheduled according to the start or end time of an operation in another procedure

(8.3) Process schedule information

This step is to overview all the scheduling information for the process. It presents the data for an operation, such as the set up time of batch, the operating time of batch, or the running time of process including the data for a unit procedure, such as the number of batches/cycles.

(8.4) Scheduling calculation

Based on the process schedule information (start time, duration and number of cycles), the system computes the equipment cycle times and annual operating time.

(8.5) Scheduling and equipment sizing

Process scheduling decisions have an impact on the size of equipment. The balance between capital investment, plant capacity and flexibility of expansion will be simulated and designed for new facilities.

(8.6) Accessing Gantt charts

This is the final step of the schedule process. The Gantt charts are supposed to help in the scheduling of simulation. The Gantt charts can be generated by superpro designer programs after all operations are scheduled and run completely.

(9) Specify labor requirement

This step required the raw input data of labor (labor-hrs/hr or labor-hrs/cycle) for estimating the economic evaluation of the process.

(10) Perform cost analysis

Component costs, stream costs, equipment costs, labor and utility costs will be specified after that the cost analysis is calculated and performed by the superpro designer program.

CHAPTER III
MATERIALS AND METHODOLOGY

3.1Simultaneous Reverse Osmosis (RO) membrane filtration unit

3.1.1 Chemicals

Calcium lactate (CaLAC), sodium lactate (NaLAC), and ammonium lactate (NH_4LAC) purchased from Sigma-Aldrich were used in this study as model solutions. These chemicals were dissolved directly in deionized water to the specified concentration (5 g/L lactic acid equivalent). This equivalent mass concentration of lactic acid resulted in different pH values of the solution, e.g., 4, 4, and 9 for CaLAC, NaLAC, and NH_4LAC solutions, respectively. To obtain the specific tested pH at 4 and 6, the pH of the model solution was adjusted by 5 M NaOH or 1 M H_2SO_4. The concentrations of the lactate species at equilibrium (both free lactic acid and its salts) were dependent on the pH. The following stoichiometry describes the presence of lactate species mimicking those that appear in the fermentation processes.

When $CaCO_3$ was used for pH control during the fermentation, both lactic acid and calcium lactate were present in the solution.

$$2CH_3CHOHCOOH + CaCO_3 \rightleftharpoons (CH_3CHOHCOO)_2Ca + H_2O + CO_2$$

When NaOH was used for pH control during the fermentation, both lactic acid and sodium lactate were present in the solution.

$$CH_3CHOHCOOH + NaOH \rightleftharpoons (CH_3CHOHCOO)Na + H_2O$$

When NH_4OH was used for pH control during the fermentation, both lactic acid and ammonium lactate were present in the solution.

$$CH_3CHOHCOOH + NH_4OH \rightleftharpoons (CH_3CHOHCOO)NH_4 + H_2O$$

3.1.2 Fermentation broth preparation

Lactate fermentation broth was prepared from the cultivation of *Bacillus coagulans* BC-013 in a 5 L stirred fermentor. An active 24-h glucose–yeast extract–peptone slant was used to prepare the bacterial suspension. The bacterial suspension (1% inoculum size) was inoculated in a preculture flask containing the preculture medium. The preculture medium contained (per liter) 10 g glucose, 15 g yeast extract, 4 g NH_4Cl, 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 5 g $CaCO_3$, and 20 mL salt solution. The compositions of the salt solution consisted of (per 10 mL) 400 mg $MgSO_4 \cdot 7H_2O$, 20 mg $MnSO_4 \cdot 5H_2O$, 20 mg $FeSO_4 \cdot 7H_2O$, and 20 mg NaCl. The preculture flask was incubated at 50 °C, 200 rpm for 3 h. After that, the preculture flask was transferred into the 5 L stirred fermenter containing 2.5 L sterile preculture medium at 10% inoculum size. The fermenter was operated at 50 °C and agitated at 300 rpm with 1 vvm air. After 3 h, 0.5 L of the fermentation medium containing (per liter) 720 g glucose was added into the fermenter. Aeration was then stopped. Three different bases, i.e., $CaCO_3$, NaOH, and NH_4OH, were used for pH control at 6. As a result, 3 different lactate salts, i.e., CaLAC, NaLAC, and NH_4LAC, were obtained in the fermentation. Fermentation was continued for 48 h until glucose depletion. Next, the fermentation broth was harvested. Cell biomass and soluble, neutral macromolecules such as proteins, sugars, etc. were removed from the fermentation broth by microfiltration and ultrafiltration. The cell-free broth obtained was later used in the 2-stage RO unit.

3.1.3 Designing and setting up the RO apparatus design

An in-house RO unit was constructed by a local Thai company (Icrotech Co., Ltd.) for use in this study. The apparatus set-up is illustrated in Figure 3.1.

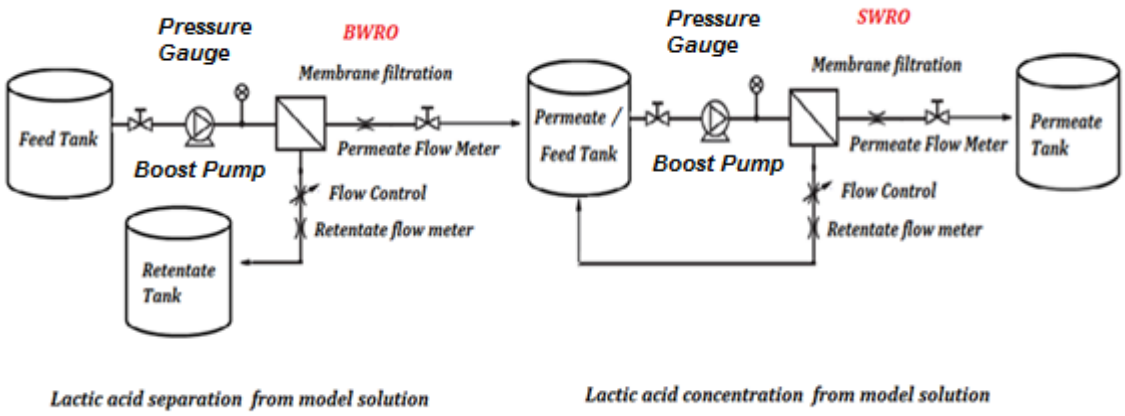


Figure 3.1 Schematic diagram of the in-house RO unit for recovery and purification of lactic acid.

Two RO membrane filtration units were subsequently connected with auxiliary instruments, including boost pumps, pressure gauges, flowmeters for feed, concentrate, and permeate, valves, and storage vessels. Negatively charged brackish water RO (BWRO) elements (DOW FILMTEC^{TM} BW60-1812-75) were installed at the first RO unit for recovering lactate from the model solution while allowing some salts to pass through the membrane at a rejection percentage of 97–99% (Table 3.1). In the second RO unit, positively charged seawater RO (SWRO) elements (DOW FILMTEC^{TM} SW30-2521) were installed for pre-concentrating recovered lactate obtained from the first RO unit where water was expelled. The rejection percentage was higher than 99.4% (Table 3.1). As a result, lactate was passed through the BWRO unit to the permeate side and later entered the SWRO unit. Lactic acid was then concentrated in the following SWRO unit and remained in the retentate.

Table 3.1 Characteristics of DOW FILMTEC^{TM} RO membrane elements.

Product name	**DOW FILMTEC^{TM} BW60**	**DOW FILMTEC^{TM} SW30**
Model	BW60-1812-75	SW30-2521
Membrane type	Polyamide thin-film composite	Polyamide thin-film composite
Membrane surface area (m^2)	0.77	1.20
Membrane surface charge	Negative	Positive
Dimension (mm × mm)	44.5 × 305	63.5 × 533
Permeate flow rate (L/h)	12	45
Average NaCl rejection (%)	97–99	99.4
Maximum applied pressure (bar)	10	55
Maximum applied temperature (°C)	45	45
pH range	2–11	2–11

Batch operation was used in the first RO unit where the feed solution entered the unit and the permeate discharged from the first unit to enter the second RO unit later on. In the second RO unit, the retentate was recycled so that most of the remaining water in the retentate was discharged into the permeate. The maximum operating pressure for the BWRO and SWRO units was set at 6 and 15 bar, respectively, owing to the pressure limit of apparatus housing, pumps, and piping systems.

3.1.4 Determining the operating conditions

To utilize the principle of RO in the recovery of lactic acid, osmotic pressure was introduced as a key factor determining the process conditions. The operating pressure was adjusted over the osmotic pressure of lactic acid so that lactic acid could pass through the BWRO membrane to the permeate, with other fermentation impurities remaining in the retentate. Eq. (1) expresses osmotic pressure as a function of molarity and temperature.

$$\pi = iMRT \quad (1)$$

where π is the osmotic pressure (bar), i is the dimensionless van't Hoff factor, M is the molar concentration of the solution/species of interest (in this case, lactic acid and its salts), R is the gas constant (0.082 L·bar/K·mol), and T is the temperature in K.
From Eq. (1), the osmotic pressures of the 3 model solutions, e.g., CaLAC, NaLAC, and NH_4LAC with 5 g/L lactate equivalent to be studied are 2.12 bar, 2.82 bar, and 2.81 bar, respectively. The osmotic pressure of lactic acid solution at 5 g/L is 1.41 bar.

The effects of pH and operating pressure on lactate separation efficiency at the BWRO unit were investigated. The lactate model solution (2 L) at 5 g/L LAC equivalent was adjusted to the tested pH values of 4 and 6 using either NaOH or H_2SO_4. The operating pressure was varied at 4 and 6 bar. Further increasing the lactate model solution to more than 5 g/L LAC equivalent resulted in higher osmotic pressure that exceeded the maximum pressure threshold in the BWRO apparatus (7 bar) and consequently led to reduced mass flux and separation efficiency. Therefore, the tested concentration of the model solution was limited at 5 g/L LAC equivalent.

The model solution (2 L) in the feed tank was fed into the first BWRO unit where free lactic acid was supposed to be separated from other impurity species, including Ca^{2+}, Na^{+}, NH_4^{+}, and SO_4^{2-} (in case the model solution was adjusted to the desired pH by H_2SO_4). The operating temperature was set at 30 °C. The apparatus was run until the collected volume of the permeate of 1.6 L was obtained. Samples (20 mL) were periodically collected from both permeate and retentate for analyses of free lactic acid concentration and ion species.

The permeate that left the BWRO unit and collected in the SWRO feed tank (1.6 L) was passed through the SWRO unit where water separation occurred, which resulted in lactate concentration in the retentate. The operating temperature was also set at 30 °C. The effects of pH and pressure on separation efficiency were determined. The tested pressure was set at 13 and 15 bar. At the first 5 min of operation, the retentate was recycled into the SWRO feed tank. After the recycling was stopped, the operation was continued until the permeate flux became zero. Samples (20 mL) were periodically collected for analyses of lactic acid and all the major remaining impurities.

3.1.5 Sample analyses

During the runs, samples from the permeate and retentate obtained in each RO unit were collected periodically for determining discharge volume, measuring pH, and analyzing substances that remained. For ion analyses, the collected samples were analyzed for concentrations of lactic acid and major impurity ions, including Ca^{2+}, Na^{+}, N (representing NH_4^{+}), SO_4^{2-}, Cl^{-}, P, and Mg^{2+}.

L-lactate ion in the sample was analyzed with a glucose–lactate analyzer (YSI2700, Yellow Spring Instruments Inc.) within the detection range of 0–2.67 g/L. The sample size of 25 μL was automatically injected into the reaction chamber where the enzymatic reaction occurred. The reading of L-lactate concentration was explained by the action of L-lactate oxidase immobilized at the membrane sensor.

An atomic absorption spectrophotometer was used to determine the metal concentration, including Ca^{2+} and Na^{+}, in the sample. The sample was prepared by dilution with 5% v/v HNO_3 solution. An air flame of 13.60 L/min along with an acetylene flame of 2 L/min was used for metal atomization of the sample before reading the atomic absorption with the atomic absorption spectrophotometer (AA280FS, Varian Inc.). Aqueous standard solutions were prepared by dilution to appropriate concentrations (2, 4, 6, and 8 mg/L for Ca^{2+}, and 5, 10, 20, and 30 mg/L for Na^{+}). The concentration of Ca^{2+} and Na^{+} in the sample was calculated by comparing the spectra with the standard calibration curves.

Nitrogen content was determined by the total Kjeldahl nitrogen technique. Nitrogen in the sample was first converted to NH_3 by metal-catalyzed acid digestion. The resulting NH_3 was separated from the sample by distillation. Released NH_3 was captured in a diluted H_2SO_4 solution. The result represented organic nitrogen after digestion and distillation in the sample. The digestion reagent (catalyst) was prepared by mixing 134 g K_2SO_4 and 7.3 g $CuSO_4$ in 134 mL concentrated H_2SO_4. After that, the volume was made up to 1 L. Digestion reagent (50 mL) was added into the sample, and digestion proceeded for 30 min (Buchi, K499). Later, 50 mL boric acid was added into the reaction mixture as the absorbent solution during NH_3 distillation (Buchi, K375). Finally, NH_3 was determined by titration with a standard solution (Buchi, K376).

Chloride was analyzed by the potentiometric method. The solubilized chloride ion in the sample was measured by a chloride ion-selective electrode during titration (Orion 720A, Labx Inc.). The sample was mixed with concentrated HNO_3 before dilution to the proper concentration. Titration was performed with a standard $AgNO_3$ solution as the reference.

Phosphorus was determined by the total phosphorus method using persulfate digestion. The sample (50 mL) was mixed with 11 N H_2SO_4 (1 mL). Next, dissolved and particulate phosphorus in the sample was digested with $(NH_4)_2S_2O_8$ (0.4 g) to convert phosphorus into orthophosphate (mixed and boiled to obtain a final volume of 40 mL). The orthophosphate concentration was measured by a spectrophotometer (Nova Spec 2, Pharmacia Biotech Inc.) using a standard calibration curve. The calibration curve was prepared from a standard phosphorus solution (0.3–1.2 mg P/L).

Sulfate ion in the sample was determined by the turbidimetric method. Sulfate ion present in the sample was converted into a $BaSO_4$ suspension under controlled conditions. The sample (80 mL) was mixed with 20 mL buffer solution containing (per liter) 30 g $MgCl_2 \cdot 6H_2O$, 5 g $CH_3COONa \cdot 3H_2O$, 1 g KNO_3, and 20 mL acetic acid (99%). Then, $BaCl_2$ was added into the reaction mixture to obtain $BaSO_4$ precipitate. The turbidity was measured by a spectrophotometer (2100P, HACH). The concentration was determined using the calibration curve of the standard sulfate solution.

3.1.6 Investigating the performance of the 2-stage RO unit

The performance of the 2-stage RO unit was evaluated using 6 criteria: mass flux of lactic acid, lactic acid separation, ion separation, lactic acid recovery, overall recovery, and purity.

The mass flux of lactic acid (J_{LA}) at the BWRO unit was calculated by the following equation.

$$J_{LA} = \frac{m_{LA,BWP}}{A \cdot t} \quad (2)$$

where $m_{LA,BWP}$ is the lactic acid mass (g) passing through the membrane, A is effective membrane surface area (m^2), and t is time (h).

The efficiency of the BWRO unit to separate lactic acid from other ions can be explained by lactic acid separation (S_{LA}) in percentage defined by Eq. (3).

$$S_{LA} = \frac{m_{LA,BWP}}{F_{LA}} \cdot 100 \quad (3)$$

where $m_{LA,BWP}$ is the lactic acid mass (g) passing through the BWRO unit and F_{LA} is the initial mass of lactic acid present in the feed solution (g).

The ion (i) leakage at the BWRO unit can be described by the separation percentage (S_i) as seen in Eq. (4).

$$S_i = \frac{m_{i,BWP}}{F_i} \cdot 100 \quad (4)$$

where $m_{i,BWP}$ is the mass of ion i (g) moving through the BWRO unit and F_i is the initial mass of the ion (g) present in the feed solution.

The efficiency of the SWRO unit to pre-concentrate lactic acid can be represented by lactic acid recovery in percentage (R_{LA}) described by Eq. (5).

$$R_{LA} = \frac{C_{LA,SWF} - C_{LA,SWP}}{C_{LA,SWF}} \cdot 100 \quad (5)$$

where $C_{LA,SWF}$ and $C_{LA,SWP}$ are lactic acid concentrations (g/L) present in the feed solution entering the SWRO unit and the permeate leaving the SWRO unit.

The overall recovery ($R_{overall}$) of lactic acid product obtained from the 2-stage RO unit can be described by Eq. (6).

$$R_{overall} = S_{LA} \cdot R_{LA} \cdot 100 \quad (6)$$

where S_{LA} and R_{LA} were defined from Eqs. (3) and (5), respectively.

The purity of lactic acid product (P_{LA}) obtained from the 2-stage RO unit can be described by the mass ratio of lactic acid and the total ions that remained in the retentate of the SWRO unit (Eq. (7)).

$$P_{LA} = \frac{m_{LA,SWR}}{m_{T,SWR}} \cdot 100 \quad (7)$$

where $m_{LA,SWR}$ and $m_{T,\ SWR}$ represent the mass of lactic acid product and the total mass of ions that remained in the retentate leaving the SWRO unit.

3.2 Simultaneous lactic acid recovery process

3.2.1 Fermentation broth preparation

Lactic acid fermentation broths obtained from the cultivation of *Bacillus* sp. at 50 °C, 300 rpm, pH 6.00 in the 5 L stirred fermentor were used as the feed solution in the downstream processing. The fermentation was conducted using different neutralizing agents including $Ca(OH)_2$, NaOH, and NH_4OH which in turn resulted in the different broth compositions at the end of the fermentation (Table 3.2).

Table 3.2 Experimental data showing the feed compositions obtained from lactate fermentation by *Bacillus* sp. to be entered the downstream processing operation.

Species/Ions present in g/L	Fermentation broth		
	CaLAC	NaLAC	NH_4LAC
Lactate	86.40	84.30	84.70
Monovalent cation			
Na^+	0.15	22.83	0.17
NH_4^+	2.19	2.23	15.59
K^+	0.67	0.86	0.82
Monovalent anion			
Cl^-	2.35	2.74	2.52
Divalent cation			
Ca^{2+}	8.35	0.35	0.38
Fe^{2+}	0.02	0.01	0.01
Mn^{2+}	0.02	0.01	0.02
Mg^{2+}	0.07	0.07	0.08
Divalent anion			
SO_4^{2-}	0.29	0.25	0.26
Multivalent cation			
P^{3+} (representing PO_4^{3-})	0.06	0.05	0.05

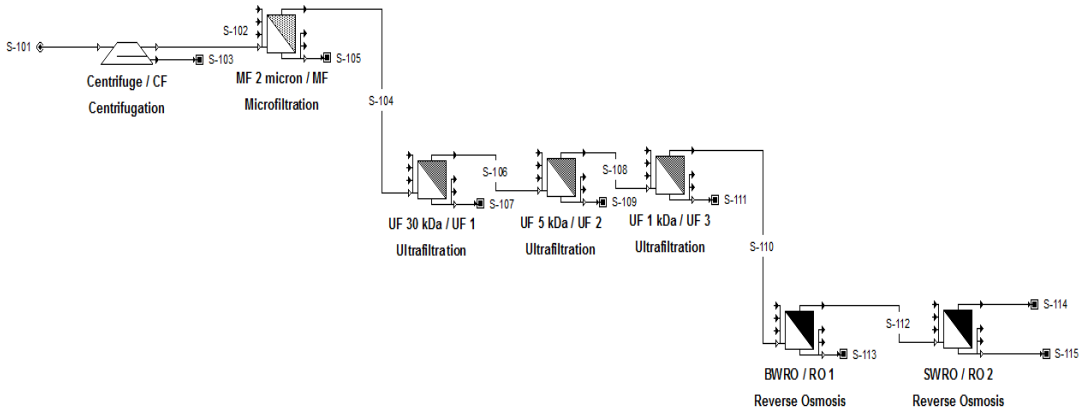
3.2.2 Process description

The simplified lactic acid recovery process schemes are shown in Figure 3.2. The general process scheme included (1) cell biomass removal from the fermentation broth left the fermentation process in the primary recovery unit. Later, (2) the proteins remained in the cell-free broth was removed during the clarification step. After that (3) the reverse osmosis (RO) units were applied to separate lactic acid from its salts and to preconcentrate before transferring to the final purification and finishing processes.

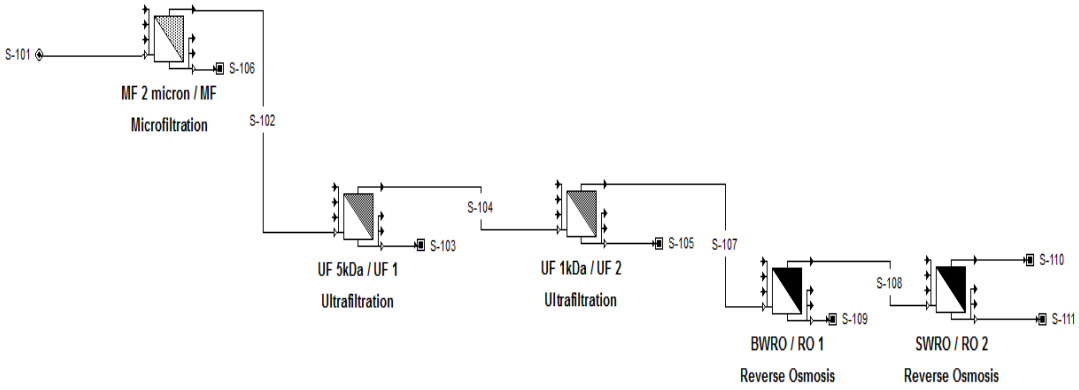
In the base process, cell biomass was removed by centrifugation (CF) and microfiltration (MF). Proteins were removed from the cell-free broth by a series of ultrafiltration (UF1, UF2, and UF3) installed with the different molecular weight cut-off (MWCO) membranes (30 kDa, 5 kDa, and 1 kDa, respectively). Lactic acid was then separated from its salts and preconcentrated in a series of reverse osmosis (RO) membrane filtration where the brackish water RO (BWRO) membrane (Dow FilmTec^{TM}, USA) and the sea water RO (SWRO) membrane (Dow FilmTec^{TM}, USA) were installed in the RO1 and RO2 units, respectively (Figure 3.2 (A)).

In the membrane based process, cell biomass was removed by MF. Later, the cell-free broth was clarified by UF1 and UF2 installed with 5 kDa MWCO and 1 kDa MWCO flat sheet membranes, respectively. Similar to those appeared in Figure 3.2 (A), lactic acid was then separated and preconcentrated by the series of RO membrane filtration (Figure 3.2 (B)). The unit operations employed in the in-parellel membrane based process for cell biomass removal and broth clarification were similar to those in the membrane based process (Figure 3.2 (B) and 3.2 (C)). To improve the recovery of lactic acid from the clarified broth, 2 BWRO membrane units were connected in parallel. The permeates from both RO1 and RO2 were combined in the mixer (MX1) before entering the SWRO membrane unit (RO3) for preconcentration. The process simulator (SuperPro Designer®, Intelligen, Inc., USA) quantified the process characteristics, energy requirements, and equipment parameters of each major equipment for the specified operating scenarios. Volumes, compositions, and other physical characteristics of input and output streams for each unit were identified. The obtained information were set as the basis of utility consumptions and purchased equipment costs for each unit item.

(A)



(B)



(C)

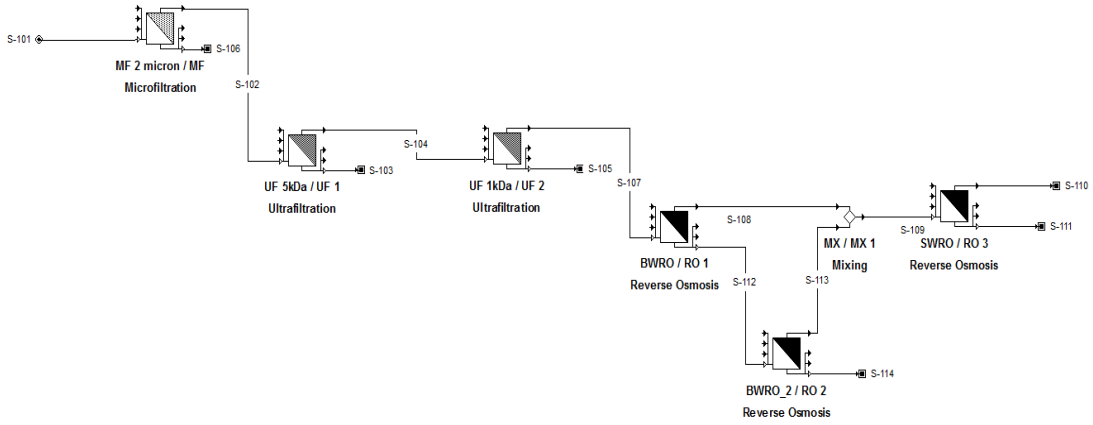


Figure 3.2 Simplified process flow diagrams displaying the major process equipment in lactic acid recovery from the fermentation broth. (A) base process; (B) membrane based process; and (C) in-parallel membrane based process.

3.2.3 Operating conditions and experimental results in laboratory scale

Among the major unit equipment, BWRO membrane unit played the key role in separating lactic acid from other ions; thus, the overall process performance relied on the operation during this process step. It should be noted that the clarified broth after passing through the UF units was diluted to the low concentration of lactic acid at 5 g/L due to the limitation of the small-scale membrane apparatus. However, the dilution step was neglected in the simulation model. Table 3.3 shows the experimental results of ion rejection coefficient (RC) obtained from the BWRO membrane unit at the laboratory scale apparatus. The information in this table was input in the simulation model for calculating mass and energy balances. The equipment models and the key operating conditions employed in the laboratory scale experiments are summarized in table 3.4.

Table 3.3 Experimental results showing ion rejection coefficient at the laboratory scale BWRO membrane unit apparatus.

Rejection coefficient	**Fermentation broth**		
	CaLAC broth	**NaLAC broth**	**NH_4LAC broth**
Lactic acid	0.748	0.546	0.417
Na^+	0.657	0.685	0.750
NH_4^+	0.666	0.641	0.469
K^+	0.673	0.768	0.891
Cl^-	0.770	0.733	0.557
Ca^{2+}	0.987	0.988	0.988
Fe^{2+}	0.855	0.852	0.750
Mn^{2+}	0.800	0.834	0.834
Mg^{2+}	0.979	0.977	0.981
SO_4^{2-}	0.914	0.931	0.917
P^{3+}	0.968	0.972	0.971

Table 3.4 The laboratory scale equipment models and their operating conditions.

Unit	Centrifuge	MF	UF			RO	
						BWRO	SWRO
Manufacturer	Kubota Corporation (Japan)	TangenX Technology Corporation (USA)				Local company in Thailand	
Configuration	Refridgerated	Flat sheet				Spiral wound	
Material	Stainless steel	Polyethersulfone				Polyamide thin-film composite	
Dimension (mm × mm)	-	210 × 475				44.5 × 305	63.5 × 533
Model	KR-20000T	XPM20A01S	XP030A01S	XP005A01S	PP001A01L	BW60-1812-75	SW30-2521
Speed (rpm)	20000	-	-	-	-	-	-
Pore size	-	0.2 μm	30 kDa	5 kDa	1 kDa	0.001 μm	0.001 μm
Surface charge	-	-	-	-	-	Negatively	Positively
Surface area (m^2)	-	0.1	0.1	0.1	0.1	0.77	1.2
Pressure (Bar) at 30 °C	-	2	2	2	2	6	15
pH range	6	6	6	6	6	4, 6	4, 6
Temperature (°C)	30	30	30	30	30	30	30
Average NaCl rejection (%)	-	-	-	-	-	97-99%	99.4%

CHAPTER IV
RESULTS AND DISCUSSION

The results and discussions of all experiments are classified into 3 sections in this chapter. Section 1 reports on the transfer mechanism of the water permeability through RO membrane after long running of lactic acid separation and purification processes. Section 2 demonstrates the potential results of RO membrane filtration for separating and concentrating free lactic acid from model solution and studies on the parameters affecting the RO separation unit through the characteristics and transfer mechanisms of three different lactate model solutions. Section 3 investigates on the simultaneous lactic acid recovery process through the characteristics and transfer mechanisms of fermentation broth and proposes the novel and simple downstream operation through mass balance and process efficiency based on process simulation. Each section, the experimental results and discussions were demonstrated separately and the conclusion is located at the end.

4.1 The water permeability rate on RO membrane filtration unit

4.1.1 Water flow rate

The water flow rate (l/m) on BWRO and SWRO unit at different testing pressure was provided in Appendix A.

4.1.2 Water permeability

The water permeability ($m^3.s^{-1}.m^{-2}.bar^{-1}$) is defined by the ratio of water flux (m/s) and the pressure difference (bar) (see Appendix B). The water permeability on BWRO and SWRO unit was calculated and then recorded as in table 4.1 and 4.2. This data represents the membrane performance changed during the previous experiment. It resulted in the evaluation of the membrane life time.

Table 4.1 Water permeability on BWRO unit.

Solution types		water permeability ($10^{-6}.m^3.s^{-1}.m^{-2}.bar^{-1}$)
1. DI water		1.19
2. Lactic acid	5 g/l	1.14
3. Lactic acid	10 g/l	1.12
4. Sodium lactate	5 g/l	1.12
5. Ammonium lactate	5 g/l	1.08
6. Calcium lactate	5 g/l	1.08
7. Sodium lactate fermentation broth	5 g/l	1.03
8. Ammonium lactate fermentation broth	5 g/l	1.03
9. Calcium lactate fermentation broth	5 g/l	1.01

Table 4.2 Water permeability on SWRO unit.

Solution types		water permeability ($10^{-7}.m^3.s^{-1}.m^{-2}.bar^{-1}$)
1. DI water		7.78
2. Lactic acid	5 g/l	7.41
3. Lactic acid	10 g/l	6.48
4. Sodium lactate	5 g/l	6.48
5. Ammonium lactate	5 g/l	6.30
6. Calcium lactate	5 g/l	6.30
7. Sodium lactate fermentation broth	5 g/l	6.30
8. Ammonium lactate fermentation broth	5 g/l	6.30
9. Calcium lactate fermentation broth	5 g/l	6.30

4.2 Simultaneous Reverse Osmosis (RO) membrane filtration unit

4.2.1 Lactate separation at the BWRO unit

A feed solution of different lactate salts, including CaLAC, NaLAC, and NH_4LAC, was prepared at an equivalent lactate concentration of 5 g/L. To obtain the specific tested pH at 4 and 6, NaOH and H_2SO_4 were added into the solution to adjust to the desired tested pH, which eventually resulted in changes in the molar concentration of chemical ions present in the model solutions as table 4.3.

Table 4.3 Molar concentration of chemical species present in different lactate model solutions containing a lactic acid equivalent of 5 g/L at pH 4 and pH 6.

Species (mol/L)	pH 4			pH 6		
	CaLAC	NaLAC	NH_4LAC	CaLAC	NaLAC	NH_4LAC
Total LAC^-	0.056	0.056	0.056	0.056	0.056	0.056
Free LA $_{(cal)}$	0.024	0.025	0.018	0.024	0.025	0.018
$LAC^-_{(cal)}$	0.032	0.031	0.038	0.032	0.031	0.038
Ca^{2+}	0.016	-	-	0.016	-	-
Na^+	-	0.031	-	0.050	0.086	-
NH_4^+	-	-	0.038	-	-	0.038
SO_4^{2-}	-	-	0.015	-	-	0.007
H_3O^+	10^{-4}	10^{-4}	10^{-4}	10^{-6}	10^{-6}	10^{-6}
OH^-	10^{-10}	10^{-10}	10^{-10}	10^{-8}	10^{-8}	10^{-8}
Total ions	0.0721	0.0821	0.1091	0.1220	0.1420	0.1010

The obtained model solutions were then transferred into the feed tank to be pumped into the BWRO unit at different operating pressures (4 and 6 bar) where lactic acid presumably passed through whereas the other cations remained in the retentate. Figure 4.1 shows the lactate mass flux passing through the BWRO membrane. The lactate mass flux increased with increasing operating pressure. It appears that the pH strongly influenced lactate transport through the BWRO membrane for CaLAC and NaLAC. On the other hand, pH showed less effect on lactate transport when compared with the operating pressure for NH_4LAC.

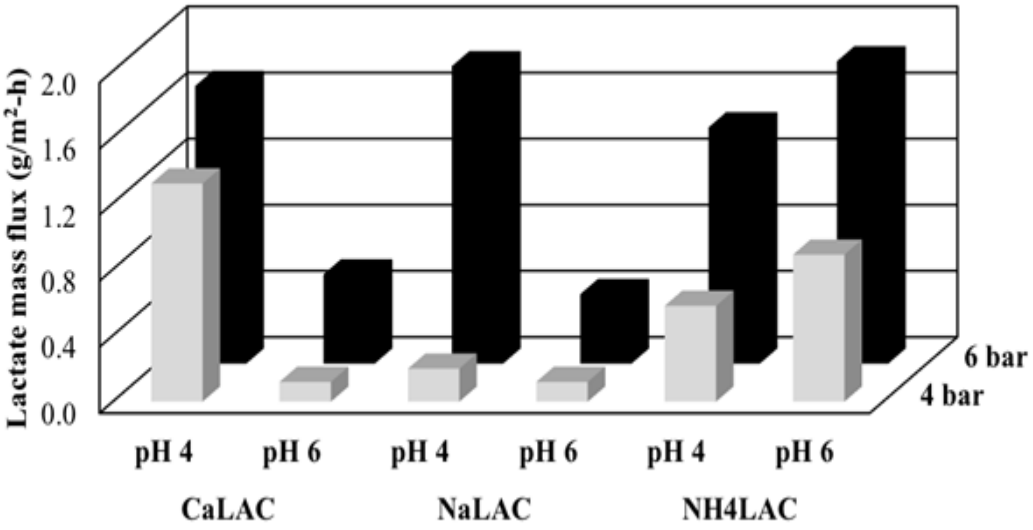
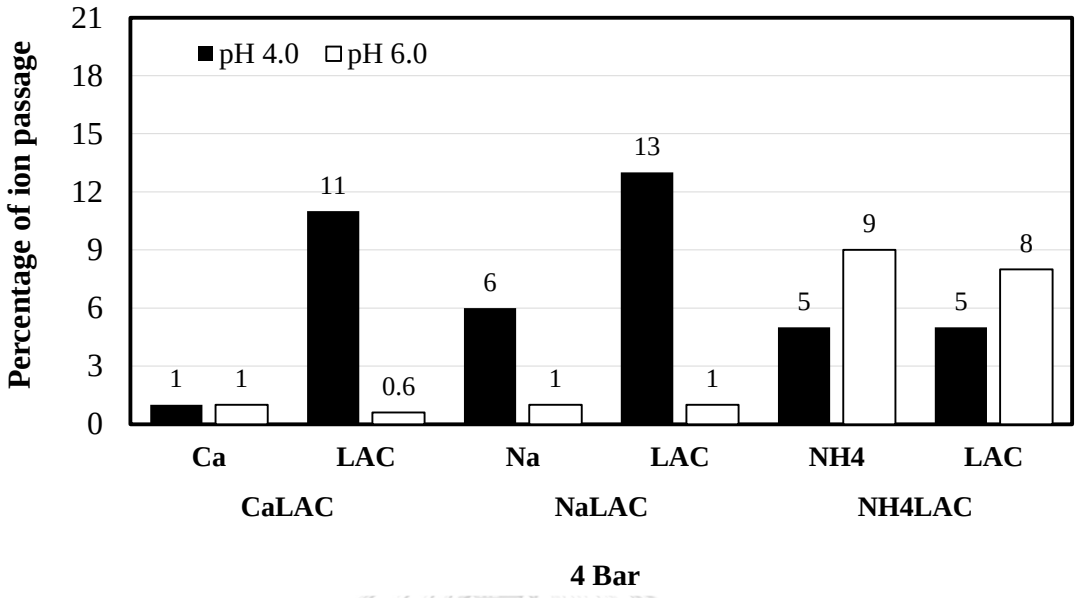


Figure 4.1 Lactate flux at the permeate of the BWRO unit operated at 30 °C. CaLAC, NaLAC, and NH_4LAC containing the initial lactate equivalent of 5 g/L at different pH values were passed through the BWRO unit at different operating pressures.

The separation efficiency of the BWRO unit displayed as in Figure 4.2. It was observed that the operating pressure exhibited a strong effect on lactate separation in the BWRO unit. High lactate separation efficiency (% lactate passage) was obtained from all 3 model solutions at 6 bar regardless of changes in pH compared with the runs using an operating pressure of 4 bar. It was suggested that increasing the operating pressure from 4 to 6 bar improved lactate separation owing to the larger difference in operating pressure and the osmotic pressure of lactic acid generating a larger driving force across the membrane (see Appendix C), which eventually resulted in a higher diffusion rate (high lactate flux as seen in Figure 4.1). Operating pressures higher than the osmotic pressure of the solution resulted in an increasing mass flux throughout the membrane and thus the separation efficiency (Freger et al., 2000). It should be noted that the osmotic pressure of the solution was increased with increasing concentration of the feed solution (González et al., 2008). Therefore, operation at a certain pressure with varied feed concentrations resulted in a different permeate flux, and eventually lactate separation efficiency, in the 3 model solutions studied (see Appendix D).

(A)



(B)

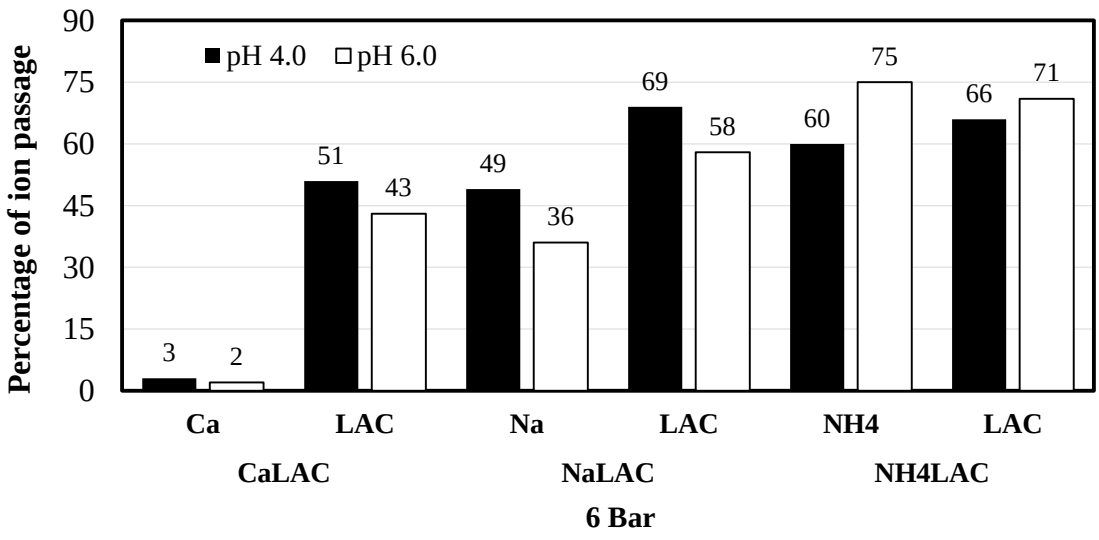


Figure 4.2 Lactic acid separation at the BWRO unit operated at 30 °C. CaLAC, NaLAC, and NH_4LAC with the initial lactate equivalent of 5 g/L were passed through the BWRO unit at different operating pressures (A: 4 bar; B: 6 bar)

From Figure 4.2, pH was found to be responsible for lactate separation in the BWRO unit. This was related to the amount of total ions present in the feed solution, which played a role in mass transport across the membrane (Table 4.3). Liew et al. claimed that as the ionic strength of the feed solution increased, the permeate flux decreased as a result of increases in osmotic pressure and viscosity (Liew et al., 1995). It was observed that the higher total ion concentration lowered the lactate flux (Figure 4.1 and Figure 4.2). At the same operating pressure, higher permeate flux resulting in significantly higher lactate separation at the BWRO unit was achieved at a lower pH with a rapid diffusion rate (pH 4 compared with pH 6) in the case of CaLAC and NaLAC feed solutions, when the total ion concentration of the feed solution was lower (Table 4.3). On the other hand, in the case of NH_4LAC, slightly increasing permeate flux and lactate separation were obtained at pH 6. This was presumably due to the slight change in total ion concentration, resulting in a similar ionic strength between the 2 pH values studied. The findings in this work confirmed that the pH and the total ion concentration of the feed solution played a role in controlling permeate flux, and thus separation efficiency, at the BWRO unit.

4.2.2 Ion rejection by the BWRO membrane

As previously mentioned, the separation of lactate from other ions was expected at the BWRO unit. Nonetheless, not only lactate species but also calcium, sodium, and ammonium ions could pass through the BWRO membrane (Figure 4.2). Several interaction mechanisms of salt passage through the membrane have been investigated, including convection, diffusion, and charge repulsion. It was claimed that both membrane charge and feed ionic strength played a significant role in salt rejection (Bartels et al., 2005). When a typical feed solution interacted at the surface of the negatively charged membrane, the ion shift was generated at the boundary between the membrane and the solution, resulting in an electrical potential known as the Donnan exclusion effect (González et al., 2008). In the case of uncharged solutes such as undissociated lactic acid, solution transport mainly occurred through diffusion and convection. The larger the difference between the operating pressure and the osmotic pressure, the larger the percentage of undissociated lactic acid that passed through the BWRO membrane. When lactic acid species were present in the dissociated form at an operating pH higher than the pK_a value (3.86), the Donnan exclusion effect governed the transport of ion species through the BWRO membrane (Dey et al., 2012). Thus, higher lactate rejection was observed in all 3 model solutions at pH 6 owing to a larger electric repulsive force by the negatively charged surface (Figure 4.2).

Considering the passage of Ca^{2+}, Na^{+}, NH_4^{+}, and H^{+} through the BWRO membrane, these cations typically bind at the membrane surface. The higher the pH, the more the dissociated lactate and the more the negatively charged membrane brings larger cations to the membrane surface (Freger et al., 2000). It was suggested that the larger ions had lower diffusion rates and thus were expected to have lower concentrations in the permeate. Size controlled ion diffusion, and the ability of ions to form hydrogen bonds with the carbonyl group of the polyamide membrane facilitated the passage of such ions (González et al., 2008; Liew et al., 1995). In addition, Tu et al. and Zaidi et al. confirmed that salt rejection by the BW30 membrane was dominated mostly by size exclusion (Tu et al., 2011; Zaidi et al., 2015). Thus, in our work, most of the Ca^{2+} ions were retained whereas Na^{+} and NH_4^{+} apparently passed through the BWRO membrane (see Appendix E).

4.2.3 Water permeability and solute rejection at the SWRO unit

Lactate concentration was determined in the SWRO unit under different operating pressures. After passing through the BWRO unit, the model solution was passed through and recirculated in the SWRO unit for 5 min. The samples were collected for analyses of lactate in both the retentate and permeate. The performance of the SWRO unit in terms of lactate recovery and water permeation is in Table 4.4.

Table 4.4 Effect of operating pressure on lactate recovery at the SWRO unit. Permeates from the BWRO unit passed through the SWRO unit at 30 °C where water was expelled yielding concentrated lactic acid solution.

Starting feed	**CaLAC**	**NaLAC**	**NH_4LAC**
At 13 bar			
Lactate at permeate (g/L)	0.05	0.20	0.09
Lactate at retentate (g/L)	6.45	9.68	7.97
Lactate rejection (%)	98	95	99
Cation rejection (%)	99	99	99
Water flux (L/m^2 h)	11.4	10.8	8.4
At 15 bar			
Lactate at permeate (g/L)	0.07	0.22	0.11
Lactate at retentate (g/L)	7.47	11.50	9.54
Lactate rejection (%)	98	95	100
Cation rejection (%)	99	99	90
Water flux (L/m^2 h)	13.2	12.0	10.8

Slightly increasing the pressure from 13 to 15 bar did not result in significant changes in SWRO performance. A slight increase in lactate concentration at the retentate with a higher water permeable flux was obtained at 15 bar. Although the operating pressure used in this study did not show a significant effect on lactate recovery owing to the limited applied pressure to the apparatus up to 15 bar, it is believed that with higher operating pressure, higher lactate rejection rate and water flux should have been obtained, resulting in increasing concentration of lactic acid product at the retentate (Oo and Song, 2009). A typical RO operation involved the removal of inorganic and organic salts from the aqueous solution (Diltz et al., 2007). The SWRO membrane used in this work was the positively charged membrane containing free amine groups; therefore, high cation rejection was expected, especially at the lower pH (pH 4) when the feed solution was more protonated and lactic acid was present more in the undissociated form. Similar to observations in the BWRO unit, evidence of some NH_4^+ leaking out from the SWRO membrane could be explained by hydrogen bonding to the carbonyl group of the polyamide membrane facilitating the passage of NH_4^+ through the permeate although Ca^{2+} and Na^+ ions were strongly rejected because of the repulsive force of the positively charged surface (González et al., 2008; Liew et al., 1995). Evidence of high rejection percentages of both cations (Ca^{2+}, Na^+, and NH_4^+) and lactate ions confirmed that the SWRO unit was successfully utilized to concentrate lactate at the retentate by expelling water through the membrane (Table 4.4).

4.2.4 Total mass balance and efficiency of lactate recovery at the 2-stage RO membrane filtration units

Figure 4.3 presents the total mass balance over the 2-stage RO units. The first BWRO unit was considered as the key operating unit where lactate ions were separated from the cations, and the second SWRO unit was for concentrating the product remaining in the retentate. From the 3 model solutions studied, it was found that more than 50% of lactic acid from the feed stream was recovered from the 2-stage RO units (Table 4.5). Compared with the other 2 feed solutions, when the feed stream was CaLAC, a lactic acid purity of 99.2% was obtained. Nonetheless, the total recovery seemed to be slightly low (50.5%). It should also be noted that the highest lactic acid purity was obtained with the lowest recovery percentage.

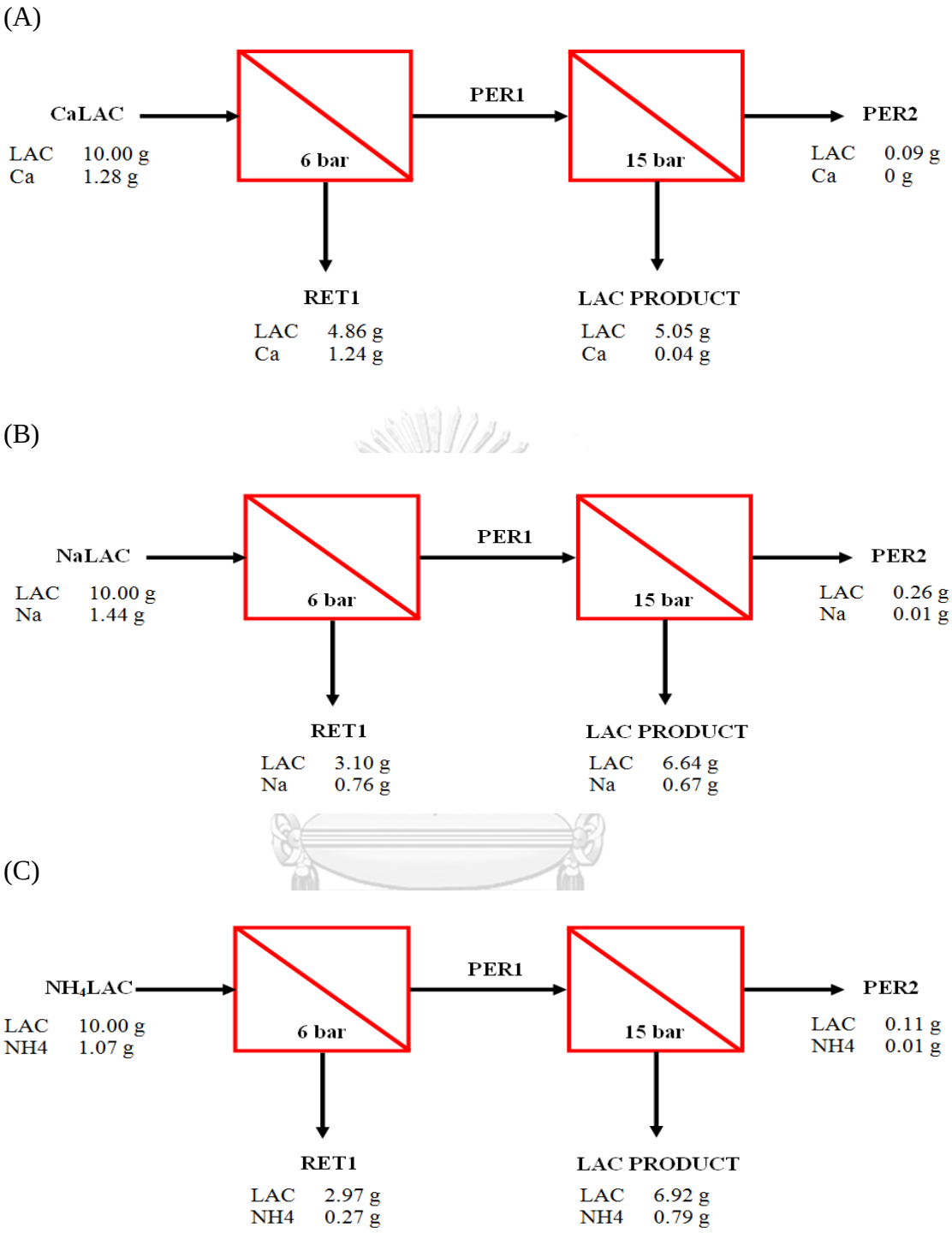


Figure 4.3 Total mass balance over the 2-stage RO units. The model solution (A: CaLAC; B: NaLAC; C: NH_4LAC) was fed into the apparatus operated under optimized pH and pressure.

Table 4.5 overall recovery and purity of lactic acid from the 3 different model solutions after passing through the 2-stage RO units operated at optimized conditions.

Feed solution	CaLAC	NaLAC	NH_4LAC
Operating conditions	pH 4, 6 bar at BWRO pH 4.6, 15 bar at SWRO	pH 4, 6 bar at BWRO pH 4.5, 15 bar at SWRO	pH 6, 6 bar at BWRO pH 5.9, 15 bar at SWRO
Total lactic acid recovery	50.5%	66.4%	70.3%
Purity (Feed)	88.6%	87.4%	90.3%
Purity (Final)	99.2%	89.9%	89.7%

In addition, the efficiency of the 2-stage RO unit was tested with the actual lactic acid fermentation broth (see Appendix F). The fermentation broths, including CaLAC broth, NaLAC broth, and NH_4LAC broth, primarily passed through the microfiltration and ultrafiltration units where cells and proteins were separated. The solutions were then diluted to obtain the equivalent concentration of lactic acid of 5 g/L before entering the RO units operated at optimized conditions determined before allowing lactic acid recovery and purification. Table 4.6 presents the efficiency of the 2-stage RO units constructed in this study on lactic acid separation and purification from the actual fermentation broths. Compared with the model solutions, the overall lactate recovery was similar whereas the purity was lower.

Table 4.6 Performance of the 2-stage RO unit to recover and purify lactic acid from the fermentation broth.

Feed solution	CaLAC broth	NaLAC broth	NH_4LAC broth
Operating conditions	pH 6, 6 bar at BWRO pH 5.39, 15 bar at SWRO	pH 6, 6 bar at BWRO pH 5.81, 15 bar at SWRO	pH 6, 6 bar at BWRO pH 5.96, 15 bar at SWRO
Lactate passage at BWRO	54.2%	66.9%	72.0%
Lactate rejection at SWRO	100%	97.4%	99.6%
Total lactic acid recovery	54.2%	65.2%	71.7%
Purity (Feed)	65.1%	62.8%	64.5%
Purity (Final)	86.1%	73.3%	74.7%

Various ions present in the actual fermentation broth were claimed to be responsible for the lower purity (Figure 4.4). The amount of total ions present in the feed fermentation broth was higher than that of the model solution; therefore, the Donnan exclusion effect was lowered, resulting in increasing ion passage across the BWRO membrane (Bartels et al., 2005). From the findings in this study, it can be presumably concluded that the membrane based process to recover and purify lactic acid from the fermentation broth has 2 major advantages. The first one is that no pretreatment is required for the cell-free fermentation broth before entering the 2-stage RO unit to recover, purify, and concentrate lactic acid. In general, pretreatment of the cell-free fermentation broth by acidification using H_2SO_4 is necessary for lactate recovery by the typical ion exchange resin based process. Furthermore, using the typical ion exchanger to separate lactic acid from the fermentation broth requires 3 main steps including feed stream loading (adsorption), washing (to remove unbound solution from the resins), and lactic acid elution by proper eluent (desorption) (Rodrigues et al., 2017). This resulted in the increasing consumption of chemicals, wastewater treatment, and eventually dilution of the fermentation broth after acidification. Secondly, without pretreatment of the cell-free fermentation broth and applying the 2-stage RO unit for lactic acid recovery, the volume of cell-free fermentation broth remained unchanged. Therefore, the downstream equipment sizing can be smaller compared with the typical downstream process using ion exchange resins. Although the broth had to be diluted to 5 g/L before entering the 2-stage RO unit, the performance of this unit to recover, pre-purify, and pre-concentrate lactic acid was evident. This strongly indicated the beneficial outcome of this process, especially when we could operate without the pressure limit as experienced in this work with our in-house apparatus.

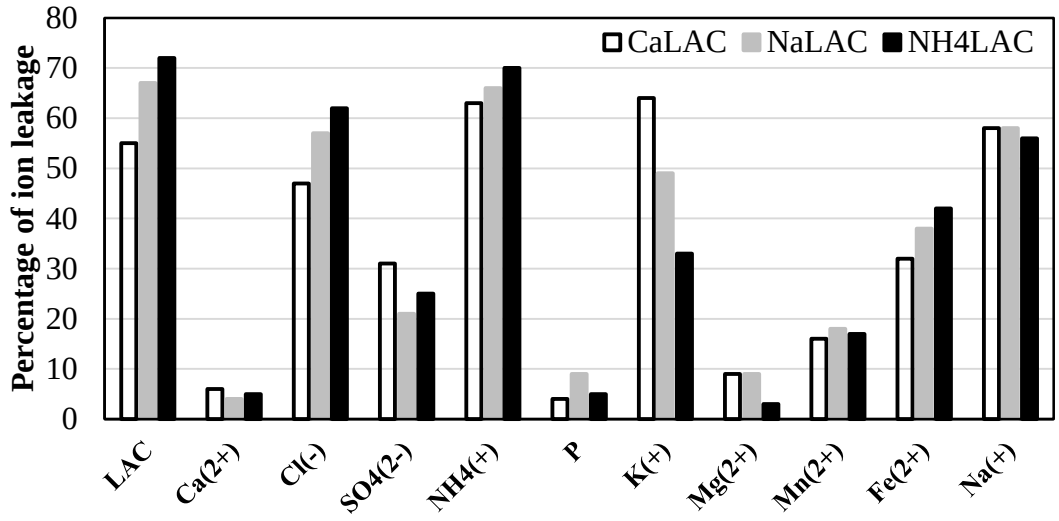


Figure 4.4 Percentage of ion leakage into the permeate. The different cell-free lactate broth solutions, including CaLAC broth, NaLAC broth, and NH_4LAC broth, entered the BWRO unit operated under the optimized conditions previously determined.

4.3 Simultaneous lactic acid recovery process

Based on the experimental data for the compositions of the fermentation broths, the operating conditions, and the performance of the proposed unit operations, lactic acid recovery yield and the product purity were estimated by the simulation model as concluded in Appendix G. Tables 4.7 and 4.8 give an overview of the key operational data in the base case process and the membrane based process (Figure 3.2 (A) and 3.2 (B)) for different feed compositions (CaLAC broth, NaLAC broth, and NH_4LAC broth). The batchwise operation was carried out at 30 °C and pH 6. By assuming the annual production of 100,000 kg lactic acid, the model predicted the overall process data, the number of each unit required and its sizing. More details on the simulated mass balance for each process flow diagram are presented in Appendix H (Tables H.1-H.3).

Table 4.7 Simulated operational data for lactic acid recovery by the base case process at the annual capacity of 100,000 kg.

Feed stream	**CaLAC broth**	**NaLAC broth**	**NH_4LAC broth**
Overall process data			
Batch capacity (kg/batch)	75.87	50.56	63.21
Number of batch runs	1,318	1,978	1,582
Annual operating time (h)	7,917	7,919	7,918
Batch time (h)	15.42	11.42	13.42
Cycle turnaround time (h)	6	4	5
Number of unit			
Centrifuge	4	4	4
MF	2	2	2
UF	6	6	6
RO	4	4	4
Equipment sizing			
Centrifuge (L/h)	94.91	90.37	96.20
MF (m^2)	36.09	36.09	36.09
UF (m^2)	36.09	36.09	36.09
RO (m^2)	3.71	3.17	3.71

Table 4.8 Simulated operational data for lactic acid recovery by the membrane based process at the annual capacity of 100,000 kg.

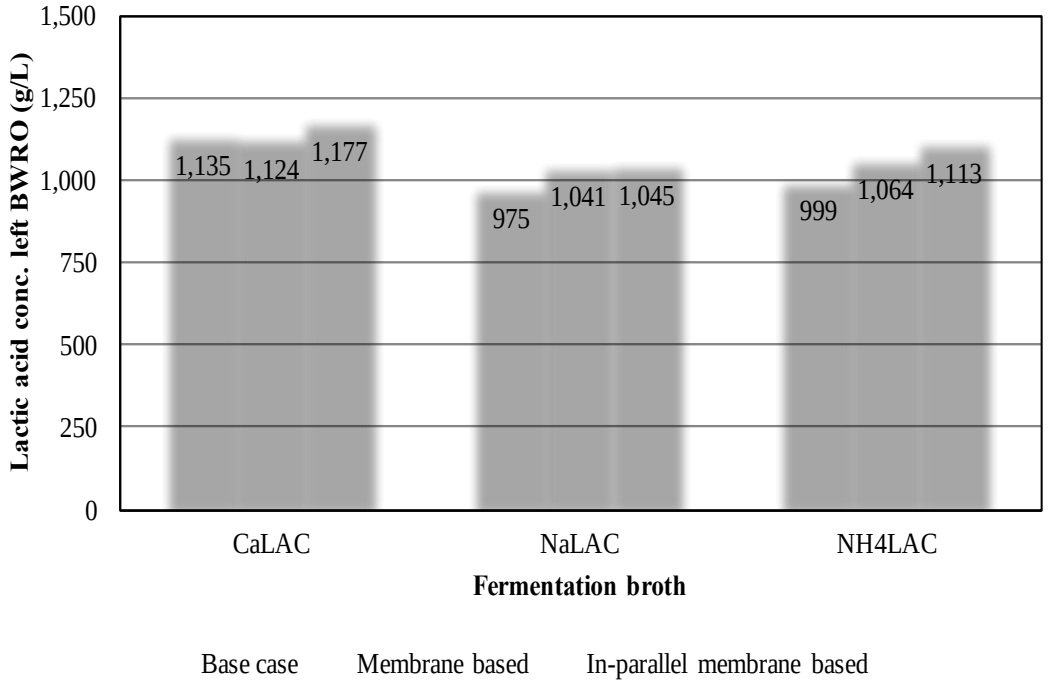
Feed stream	**CaLAC broth**	**NaLAC broth**	**NH_4LAC broth**
Overall process data			
Batch throughput (kg/batch)	75.82	50.56	63.17
Number of batches per year	1,319	1,978	1,583
Annual operating time (h)	7,918	7,916	7,919
Recipe batch time (h)	10.08	8.08	9.08
Recipe cycle time (h)	6	4	5
Number of unit			
MF	2	2	2
UF	4	4	4
RO	4	4	4
Equipment sizing			
MF (m^2)	36.0	36.0	36.0
UF (m^2)	36.0	36.0	36.0
RO (m^2)	3.70	3.70	3.70

4.3.1 Process analysis

In the base case operation, cell and insoluble materials remained in the fermentation broth were removed in the primary recovery section by centrifuge and microfiltration while only microfiltration was used in the membrane based process for removing cell biomass and insoluble materials. From the experimental data, it was found that both cell biomass and insoluble materials were completely removed from the fermentation broth resulting in the cell-free broth to be further clarified by UF. This resulted in the clarified cell-free broth without the presence of cell biomass and soluble proteins carried over to the unit operations afterward. The simulated data show that the unit operations including 1 unit of MF and 2 units of UF installed with the 5 kDa and 1 kDa MWCO membranes in the primary recovery section of the membrane based process effectively removed cell mass, insoluble materials, and proteins at the acceptable ranges similarly to those obtained from the experimental data (Phanthumchinda et al., 2017). From simulated data representing the mass balance of ions/species remained in the feed stream, less lactate loss was obtained in the membrane based process (See Appendix H, Tables H.1-H.3). Li et al. (2006) reported that most of the proteins could be separated by the UF membranes with both MWCO of 5 kDa and 20 kDa (Julien and Whitford, 2006). From the findings mentioned above, it was confirmed that centrifugation and ultrafiltration installed with the 30 kDa MWCO membrane shown in the base case process flow diagram could be omitted. This lowered the capital cost and subsequently the operating cost in the membrane based process since no centrifuge was required and the number of UF units were reduced by 2 units (Tables 4.7 and 4.8).

The stream left the primary recovery section entered the recovery section where lactic acid was separated and preconcentrated in the BWRO and SWRO membrane units. Figure 4.5 compares the simulated results on lactic acid recovery from lactate fermentation broths (CaLAC broth, NaLAC broth, and NH_4LAC broth) in the base case, membrane based, and in-parallel membrane based processes. It was found that lactic acid left the SWRO membrane unit at the high concentration (~1,000 g/L) in regardless of the recovery process and the feed stream (Figure 4.5 (A)). Changing the feed streams resulted in the different lactic acid product purity left the final SWRO membrane unit while the different recovery process did not show the strong effect on the product purity (Figure 4.5 (B)). Nevertheless, the recovery process strongly impacted the overall lactic acid product recovery (Figure 4.5 (C)). A higher lactate loss was observed in the base case process while the overall recovery yield was further improved with the membrane based and in-parallel membrane based processes, respectively. In general, the unit operations involved in the downstream product recovery are responsible for the loss of lactic acid product. Lactic acid loss was observed since cell removal and clarification step. Minimizing the unit installation in this section could help prevent such loss. As a consequence, lower lactic acid loss was obtained in the membrane based process compared with that in the base case process (Tables H.1-H.3 and Figure 4.5 (C)).

(A)



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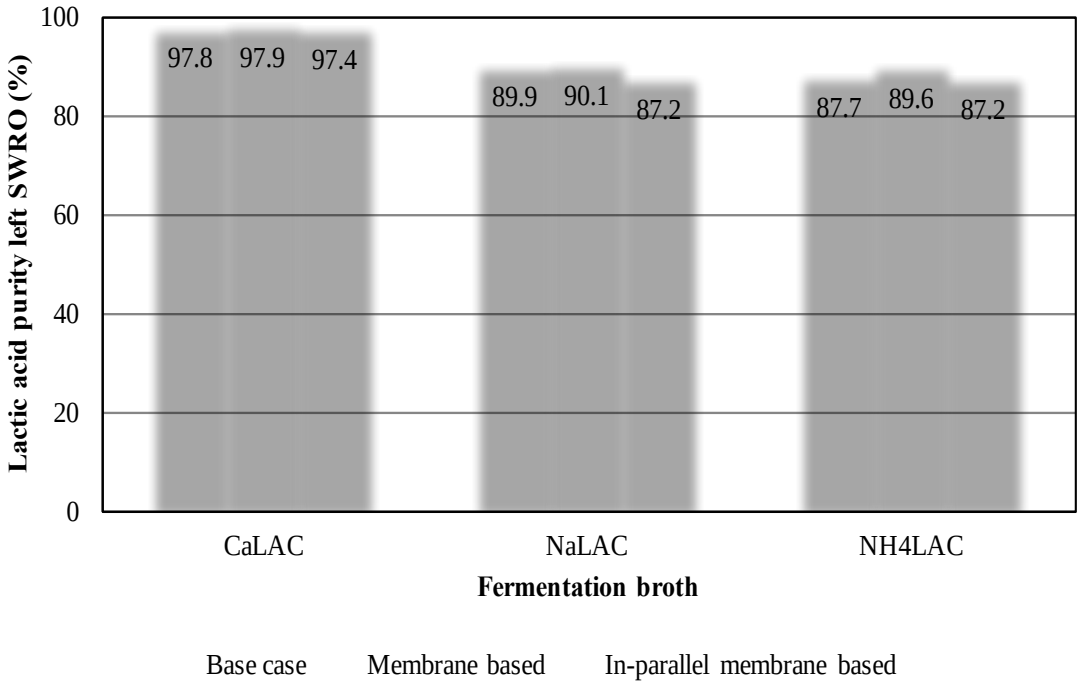

(C)

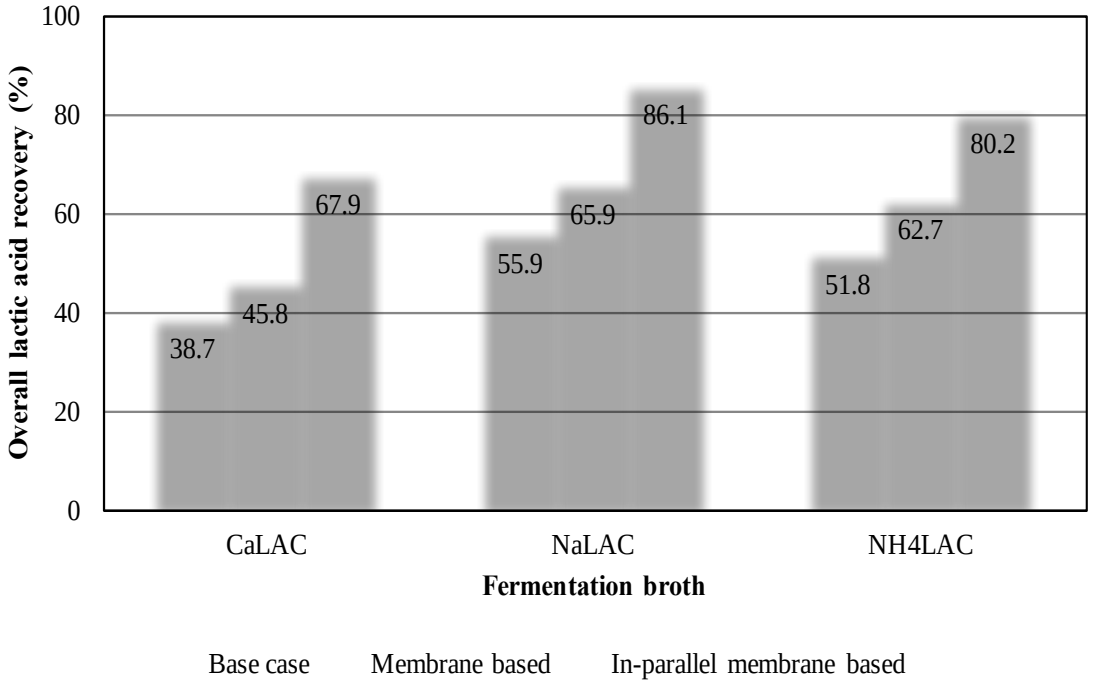

Figure 4.5 Simulated data on lactic acid recovery from the fermentation broths by 3 different downstream processing operations. (A) Lactic acid conc. left SWRO (g/L); (B) Lactic acid purity left SWRO (%); and (C) Overall lactic acid recovery (%).

In this study, major lactic acid loss occurred at the BWRO membrane unit (RO1) (Figure 4.5). To prevent the product loss, another BWRO membrane unit was installed (RO2) where lactic acid was further recovered from the retentate stream left RO1 (Figure 4.5 (C)). The permeate streams from both RO1 and RO2 in the in-parallel membrane based process were then mixed and passed across the SWRO membrane unit (RO3) where lactic acid with sufficiently high purity was preconcentrated. It should be noted that from all 3 recovery processes proposed in this study, the highest lactic acid purity with the lowest recovery was obtained for the CaLAC feed stream. When the feed stream was NaLAC broth or NH_4LAC broth, the high lactic acid recovery was obtained with the lower product purity.

Compared with the commercial grade lactic acid avaible, i.e., FCC88 with 87.5-88.5% purity, FCC80 with 79.5-80.5% purity, and FCC50 with 49.5-50.5% purity, the process flow diagrams proposed in this study provided the simulated lactic acid product at a higher purity than the commercially available products (Figure 4.5 (B)) (Gonzalez et al., 2007). Table 4.9 shows the performance of the designed process flow diagrams to recover lactic acid in this study in comparison with the previous literatures. The typical lactic acid downstream recovery processes mainly include centrifugation, filtration, extraction, and distillation. Simple operation, low capital expenditure (CAPEX) and operating expenditure (OPEX), and reduced product contamination are always considered as the keys controlling the process performance. To achieve high product purity, many downstream units are installed to remove the impurities while this eventually ends up with low product recovery due to loss in between the unit operations (Joglekar et al., 2006; Pal et al., 2009). Compared with extraction and ion exchanger which involve the consumption of solvents and adsorbents, it was observed that the membrane based processes proposed in this study not only gave a high final product concentration with the high purity comparable to the commercially available product (FCC80), membrane operation was known to be simple with low chemical consumption (Li et al., 2008).

Table 4.9 Comparison of previous lactic acid recovery processes with the simulated data obtained in this study.

DSP units	Lactic acid product				References
	Final conc. (g/L)	**Recovery at the final unit (%)**	**Purity at the final unit (%)**	**Overall recovery (%)**	
MF[1], NF[2], and ED[3]	-	-	85.6	-	(Sikder et al., 2012)
Centrifugation, Filtration, Extraction, and Evaporation	400-500	-	98.0	-	(Hu et al., 2017)
ED	-	69.5	-	69.5	(Wang et al., 2013)
Centrifugation, NF, and ED	-	73.4	-	58.2	(Kim et al., 2016)
MF, NF, ED, IEX[4], and Distillation	930	-	99.8	38.2	(Neu et al., 2016)
Evaporation	-	71.5	55.3	71.5	(Komesu et al., 2014)
Centrifugation, Filtration, Extraction, and Distillation	-	-	91.3	62.2	(Chen et al., 2012)
Extraction	-	84.3	-	84.3	(Yan et al., 2016)
Base case	1,135	100.0	97.8	38.7	This work CaLAC broth
Membrane based	1,124	100.0	97.9	45.8	This work CaLAC broth
In-parallel membrane based	1,177	100.0	97.4	67.9	This work CaLAC broth

[1]Microfiltration
[2]Nanofiltration
[3]Electrodialysis
[4]Ion Exchanger

4.3.2 Economic evaluation

Cost model integrated the data developed in lactic acid recovery process simulation model using the information from material and energy balances to describe the economic impact of the membrane based process in comparison to the base case process and those previously reported in the literatures. By assuming the batchwise operation with the annual capacity of 100,000 kg lactic acid product, a basis of 330 days per year (7920 h) operating time was used in the model. The detailed operation durations and scheduling to visualize the process were present in the Gantt chart (See Appendix I, Figure I.1-I.3). The purchased costs for the major equipment and the operating costs including utilities and labor were estimated from the default values from SuperPro Designer®. The raw material costs were obtained from the quotations from suppliers. Considering the financial investment, the project lifetime was estimated for 15 years with the construction period of 30 months and the startup period of 4 months at the inflation rate of 4%. The total fixed investment costs were estimated in Table 4.10. It was observed that the membrane based processes required lower investment cost than the base case process. It was suggested that without installation of centrifuges and UFs (at MWCO of 30 kDa) in the membrane based processes (both membrane based and in-parallel membrane based), the direct fixed capital cost was reduced by approximately 70%.

The annual operating cost was estimated for the specific downstream recovery processes with 3 different broths (Figure 4.6). It was clearly seen that the lower the process downstream units involved in the recovery process, the more the reduction of the operating cost was acquired. The base case process required the highest operating cost per unit kg of lactic acid produced in all feed streams studied (CaLAC broth, NaLAC broth, and NH_4LAC broth). The installation of centrifuge and UF with 30 kDa MWCO membrane was responsible for the high operating cost. The cost breakdown (See Appendix J) could further explain this finding from the higher cost spending on consumables and utilities in comparison with the spending in the membrane based and in-parallel membrane based processes (Figure 4.7). The results in Figure 4.6 suggested that with BWRO membrane process integration in the in-parallel membrane based process, further operating cost reduction could be obtained in all feed streams studied. The reduction was by 23.33%, 31.29%, and 27.10% in comparison to those required in the base case process for CaLAC broth, NaLAC broth, and NH_4LAC broth, respectively.

Table 4.10 Fixed investment costs for the proposed downstream processing for lactic acid recovery from fermentation broths.

Fixed capital estimate summary	**Cost (USD)**		
	Base case	**Membrane based**	**In-parallel membrane based**
1. Total Plant Direct Cost (TPDC)			
a. Equipment purchase cost	2,640,000	733,000	780,000
b. Installation	1,316,000	362,000	384,000
c. Process piping	924,000	256,000	273,000
d. Instrumentation	1,056,000	22,000	312,000
e. Insulation	79,000	73,000	23,000
f. Electrical	264,000	13,000	78,000
g. Buildings	1,188,000	330,000	351,000
h. Yard improvement	396,000	110,000	117,000
i. Auxiliary facilities	1,056,000	293,000	312,000
TPDC	**8,919,000**	**2,472,000**	**2,630,000**
2. Total Plant Indirect Cost (TPIC)			
a. Engineering	2,230,000	618,000	658,000
b. Construction	3,122,000	685,000	921,000
TPIC	**5,352,000**	**1,483,000**	**1,587,000**
3. Total Plant Cost (TPC = TPDC + TPIC)	**14,271,000**	**3,955,000**	**4,209,000**
4. Contractor's Fee & Contigency (CFC)			
a. Contractor's fee	714,000	198,000	210,000
b. Contigency	1,427,000	395,000	421,000
CFC	**2,141,000**	**593,000**	**631,000**
5. Direct Fixed Capital Cost (DFC = TPC + CFC)	**16,411,000**	**4,548,000**	**4,840,000**

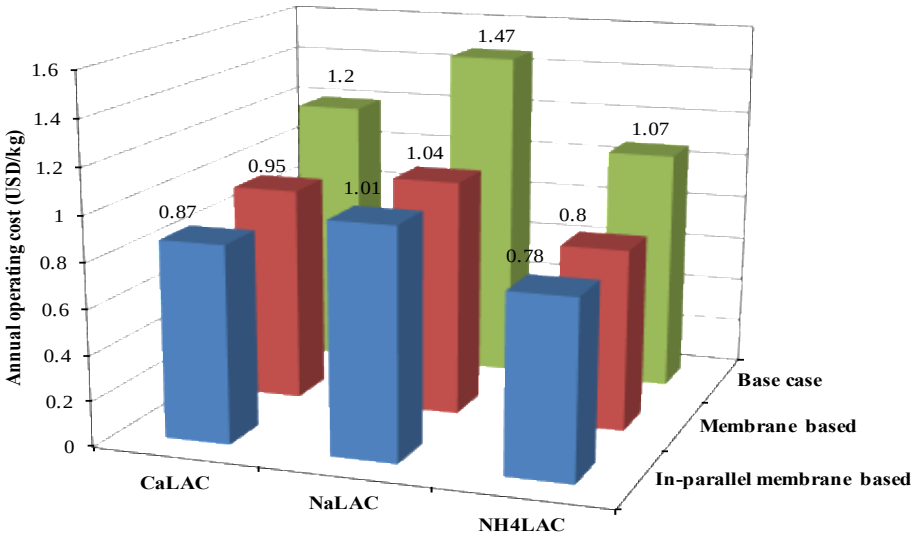

Figure 4.6 Annual operating cost for lactic acid recovery by 3 different downstream processing operations.

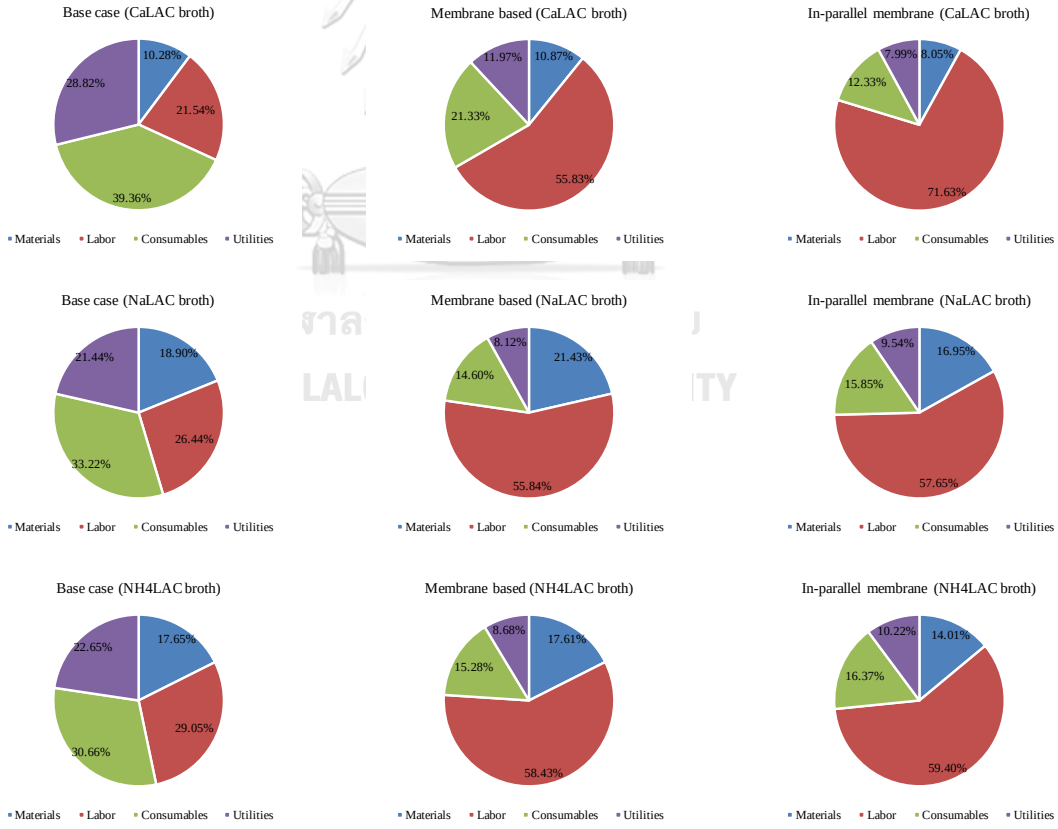

Figure 4.7 Operating cost breakdown for lactic acid recovery from fermentation broths by different downstream processings

Table 4.11 shows the unit cost of lactic acid production per kilogram by the conventional lactic acid recovery processes. The conventional recovery process usually involved acidification, solid removal, neutralization, precipitation, filtration, extraction, adsorption, distillation, and evaporation. The analysis suggested that the number of unit operations and process steps in the downstream process to provide the high purity of lactic acid production usually led to the high recovery cost. High consumption of chemicals was also responsible for the high recovery cost. For instance, a large amount of H_2SO_4 for acidification of CaLAC broth not only increased the materials/chemicals cost, it also led to an increasing effluent loading due to the formation of gypsum and wastewater during the operation (Pal et al., 2009) (Qin et al., 2010). In some separation unit such as distillation, the feed solution with high inorganic contents is prohibited; thus, the pretreatment unit prior distillation is required. This results in the additional consumption of chemicals and utilities (Chen et al., 2012). Energy conservation is mandatory in any operational process. The operation with the phase change such as distillation, evaporation, and crystallization is considered as the energy intensive process (high steam consumption, high cooling rate, and etc.). On the other hand, the membrane integrated process was suggested to be an economical route. The membrane separation process relies on the mechanical pressure driven force that does not involve in phase change. The membrane separation generally occurs at room temperature; thus, being considered as the energy saving process (Pal et al., 2009; White et al., 2002). To date, the dead-end filtration and centrifugation are partially replaced by MF because of the economical impact (Berk, 2013). It was reported that the energy consumption in RO membrane process was as low as 9-20 kWh/m^3 with the high energy saving spiral wound module (Wang et al., 2013). As a result, the proposed processes in this study based on membrane separation could provide the low unit cost of lactic acid compared with those in the previous literatures and the commercial sale price (1.0-1.8 USD/kg) (Zacharof and Lovitt, 2013). Therefore, it can be summarized from the simulated data that the proposed membrane based processes provide the new insight in lactic acid downstream recovery process. The process scenarios not only give the sufficiently high product yield and purity, but they are also considered as the economical and environmental friendly routes due to its cost effectiveness, low chemical consumption, low product contamination, low waste generation, and low energy consumption.

Table 4.11 Economic analysis of the conventional lactic acid downstream recovery processes in comparison with the case scenarios proposed in this study.

Downstream processes	Cost (USD/kg lactic acid)	References
1. Reactive extraction, re-extraction, esterification, and reactive distillation	1.59	(Posada et al., 2012)
2. Addition of lime, precipitation, dissolution in methanol, acidification to separate calcium sulfate, esterification, and hydrolysis by reactive distillation	1.40	
3. Addition of ammonium hydroxide, microfiltration, monopolar electrodialysis, bipolar electrodialysis, esterification, and hydrolysis by reactive distillation	1.74	
4. Reactive extraction	0.92	
5. Base case process	1.07	This work
6. Membrane based process	0.80	
7. In-parallel membrane based process	0.78	

CHAPTER V
CONCLUSIONS AND RECCOMMENDATIONS

5.1 Conclusions

The in-house 2-stage RO unit constructed in this study was used to recover and purify lactic acid. The acceptable amount of free lactic acid recovered with sufficiently high purity was obtained under optimized operating conditions. This unit was applicable to different fermentation broth solutions, including calcium lactate, sodium lactate, and ammonium lactate. Although the operating pressure was set at a higher value than the lactate osmotic pressure, lactate rejection was still observed at the BWRO unit where most of the lactate was expected to pass through the membrane while other ions remained in the retentate. It was found that the total ion concentration of the feed solution and the operating pH both played a crucial role in controlling ion leakage across the membrane, thereby controlling both lactate recovery and purity of this RO membrane system. From the results obtained in this study, it is suggested that by coupling this 2-stage RO unit with the upstream fermentation and primary cell and protein separation (microfiltration and ultrafiltration) units, the simple design of continuous fermentation and lactic acid recovery to achieve high productivity in long-term operations is feasible. In addition, the membrane based integrated processes were proposed for the downstream recovery of lactic acid from fermentation broths. The process economic was evaluated using the technical data previously optimized in the laboratory scale. It was found that the membrane based processes were suitable for recovering lactic acid from different fermentation broths; i.e. CaLAC, NaLAC, and NH_4LAC. Therefore, we can consider the proposed process schemes as the universal design. The membrane based processes mainly consisted of MF for cell removal, a series of UF for eliminating proteins, and the integrated RO systems to recover and preconcentrate lactic acid. The membrane based processes showed the advantageous outcomes in terms of sufficiently high product purity and recovery yield as well as the low production cost in comparison to the commercially available products. The simulation data suggested that by eliminating centrifugation and integration of the RO membrane unit, the improved recovery yield was acquired. This subsequently lowered the production cost both investment and operating costs.

5.2 Recommendations

Although low lactic acid recovery was observed in the base case and the membrane bases processes, this could be improved by the additional BWRO membrane unit to recover product loss (see Figure 5.1 and Figure 5.3). It is believed that the recovery can be further improved with stream recycle or unit integration at the BWRO membrane units. In addition, further purification to remove the monovalent ions from the lactic acid product left SWRO membrane unit can be simply conducted by installation of nanofiltration unit after the BWRO membrane unit (see Figure 5.2 and Figure 5.3).

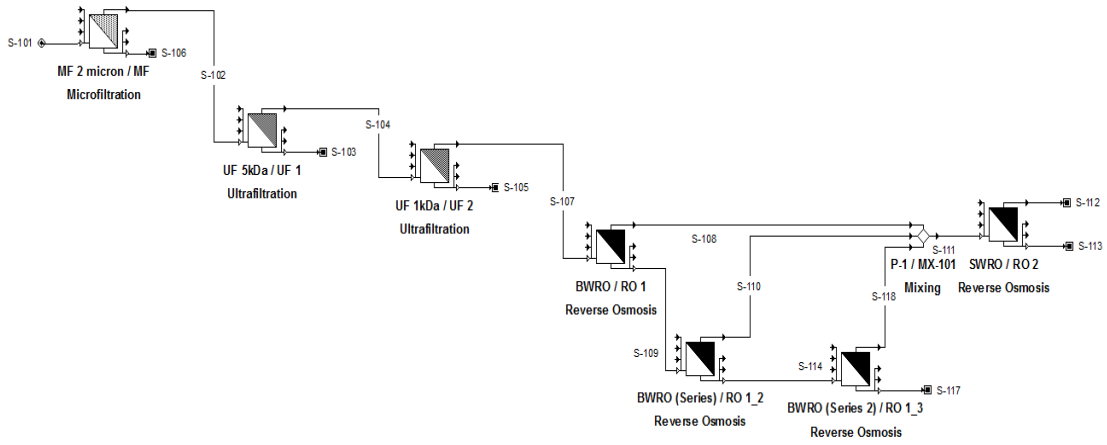

Figure 5.1 Additional BWRO membrane unit to recover product loss

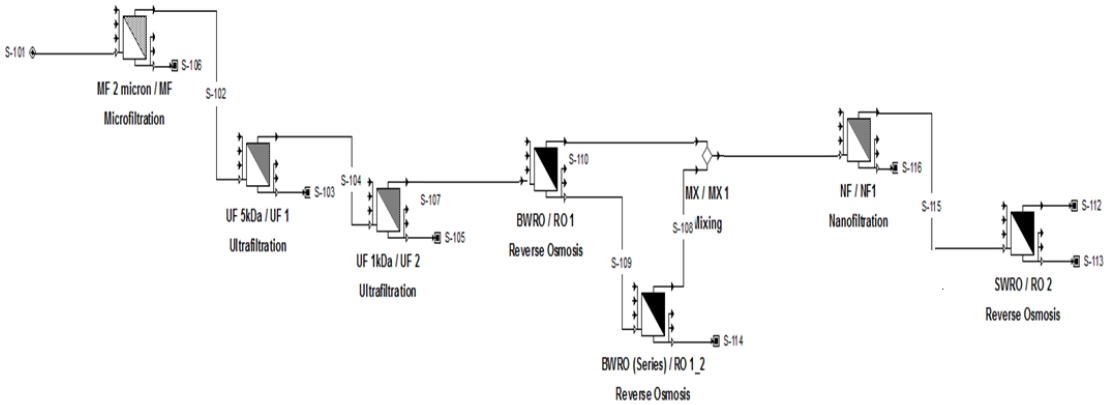

Figure 5.2 Installation of nanofiltration unit after the BWRO membrane unit

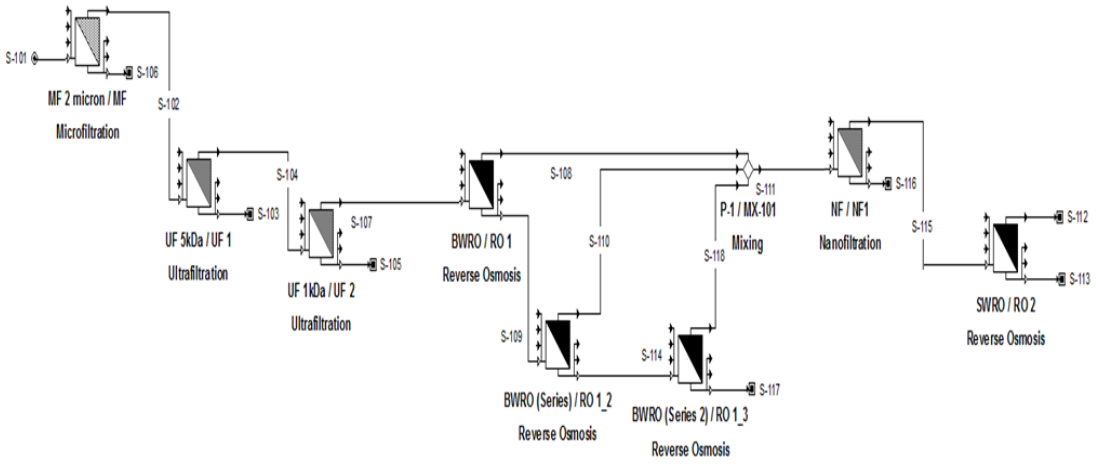

Figure 5.3 Additional BWRO membrane unit and installation of nanofiltration unit

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APPENDIX

APPENDIX A: WATER FLOW RATE

BWRO membrane module

The data of water flow rate (l/m) in this section was manually measured and reported in liter per minute unit.

Table A.1 Water flow rate (l/m) at BWRO membrane

Testing Pressure (bar)	Flow rate (l/m)	
	Permeate	**Retentate**
1	1.32	0.06
2	0.78	0.09
3	0.22	0.14
4	0	0.21
5	0	0.26
6	0	0.33

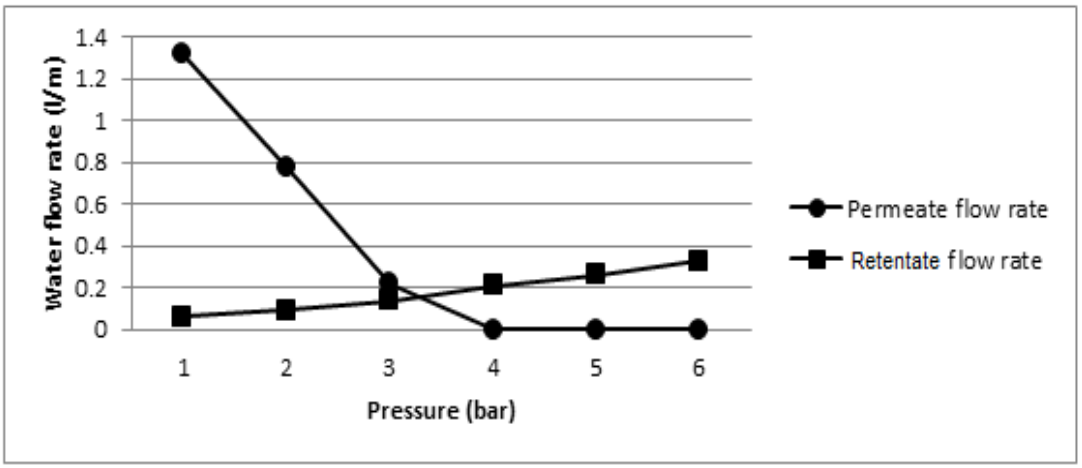

Figure A.1 Water flow rate (l/m) for BWRO membrane at different pressure (bar).

SWRO membrane module

The data of water flow rate (l/m) in this section was recorded from the flow meter which is installed in RO unit. The monitored flow rate was reported in liter per minute unit.

Table A.2 Water flow rate (l/m) at SWRO membrane

Testing Pressure (bar)	Flow rate (l/m)	
	Permeate	**Retentate**
5	1.75	5.45
6	2.22	5.30
7	2.51	5.24
8	2.70	5.25
9	2.95	5.04
10	3.26	4.95
11	3.64	4.76
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13	4.33	4.40
14	4.68	3.65
15	5.04	1.20
16	5.12	0

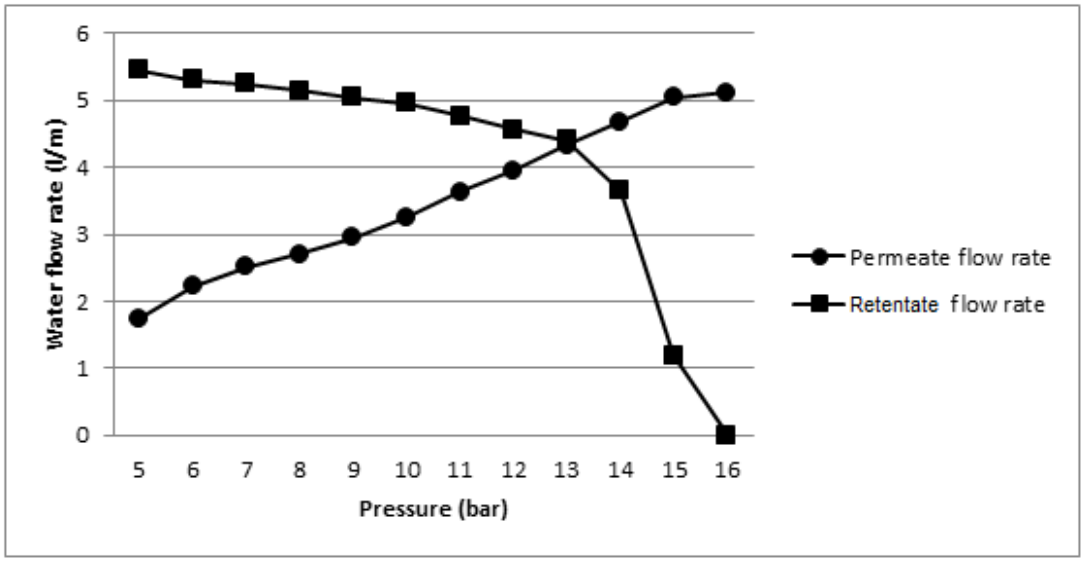


Figure A.2 Water flow rate (l/m) for SWRO membrane at different pressure (bar).

APPENDIX B: WATER PERMEABILITIES

The water permeability must be measured in order to determine BWRO and SWRO membrane performance changed during the previous experiment. The water permeability ($m^3.s^{-1}.m^{-2}.bar^{-1}$) is defined by the ratio of water flux (m/s) and the pressure difference (bar).

BWRO membrane module

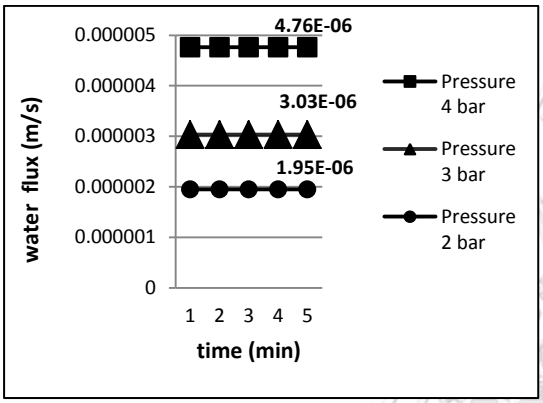


Figure B.1 Water flux rate for BWRO

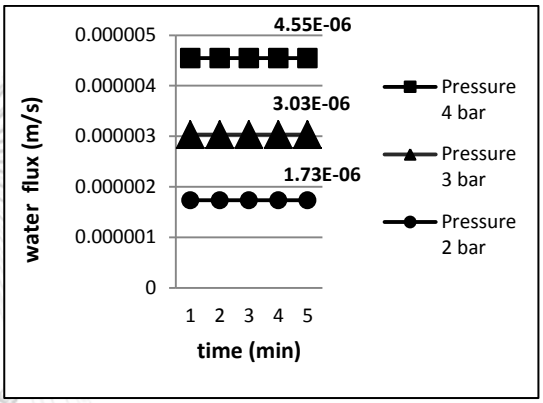


Figure B.2 Water flux rate for BWRO (Lactic acid 5 g/l)

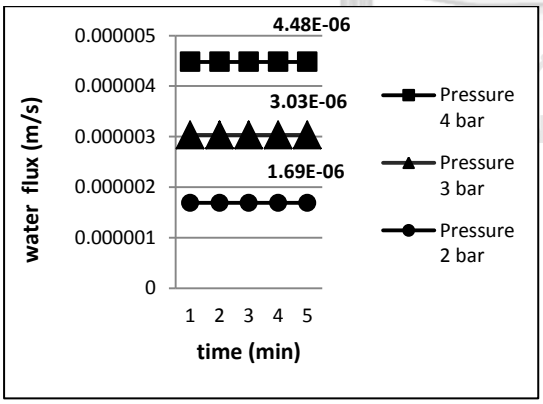


Figure B.3 Water flux rate for BWRO (Lactic acid 10 g/l)

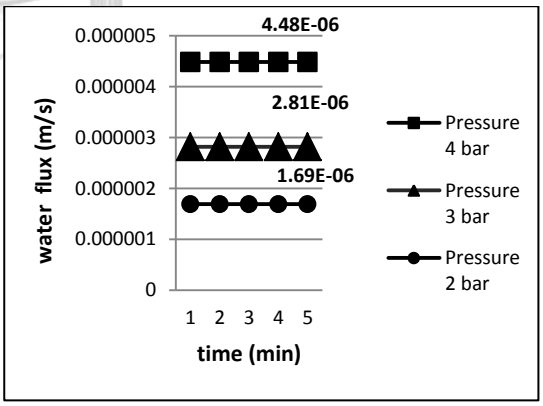


Figure B.4 Water flux rate for BWRO (Sodium lactate 5 g/l)

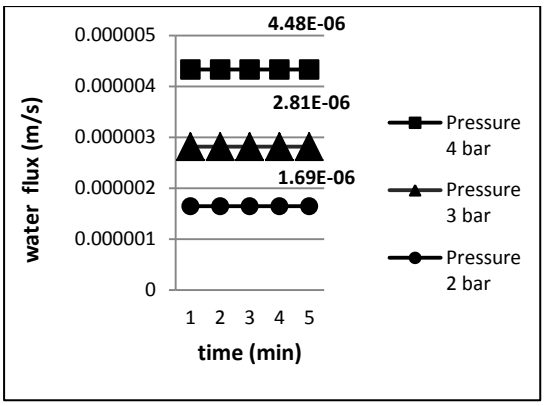


Figure B.5 Water flux rate for BWRO (Ammonium lactate 5 g/l)

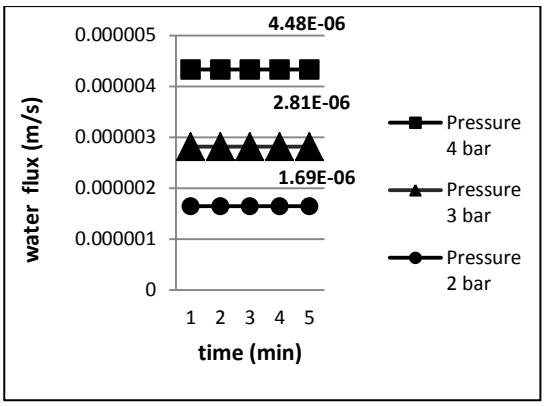


Figure B.6 Water flux rate for BWRO (Calcium lactate 5 g/l)

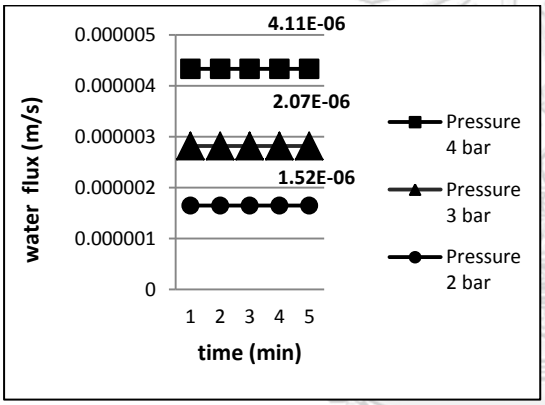


Figure B.7 Water flux rate for BWRO (NaLAC Broth 5 g/l)

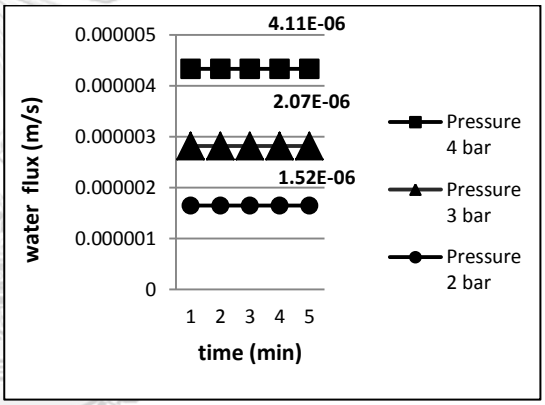


Figure B.8 Water flux rate for BWRO (NH_4LAC Broth 5 g/l)

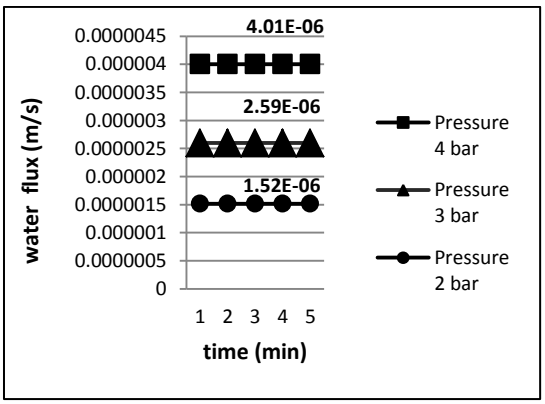


Figure B.9 Water flux rate for BWRO (CaLAC Broth 5 g/l)

SWRO membrane module

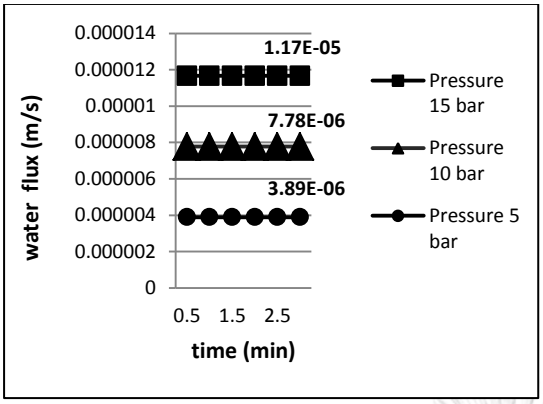


Figure B.10 Water flux rate for SWRO

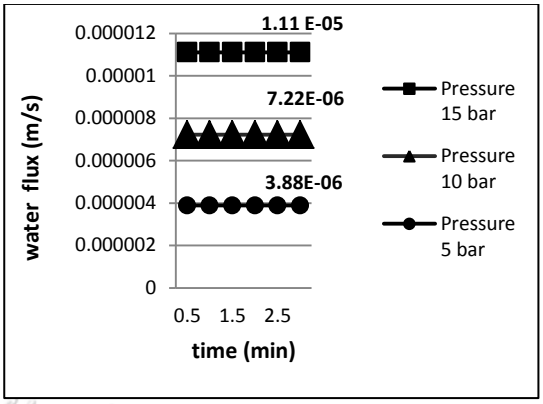


Figure B.11 Water flux rate for SWRO (Lactic acid 5 g/l)

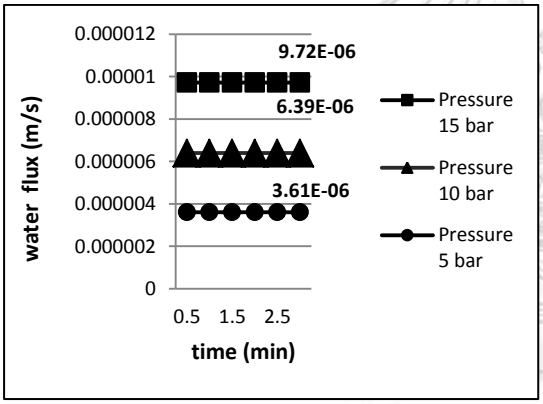


Figure B.12 Water flux rate for SWRO (Lactic acid 10 g/l)

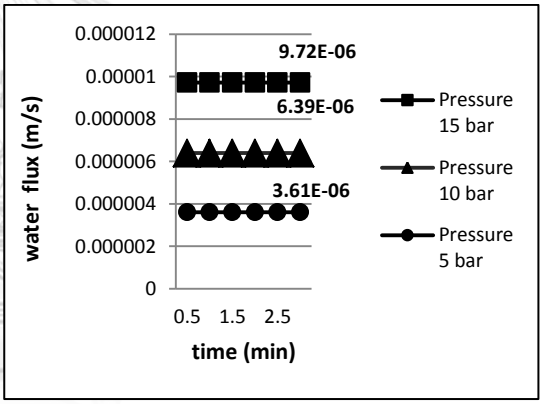


Figure B.13 Water flux rate for SWRO (Sodium lactate 5 g/l)

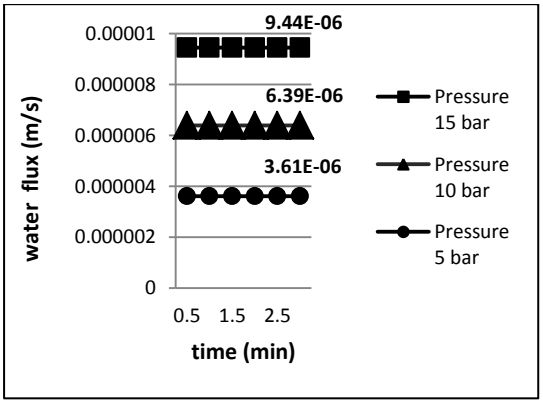


Figure B.14 Water flux rate for SWRO (Ammonium lactate 5 g/l)

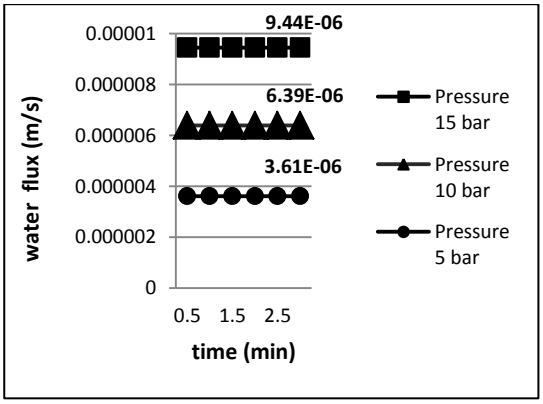


Figure B.15 Water flux rate for SWRO (Calcium lactate 5 g/l)

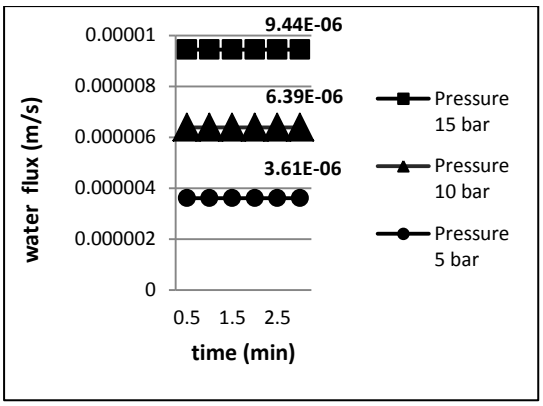


Figure B.16 Water flux rate for SWRO (NaLAC Broth 5 g/l)

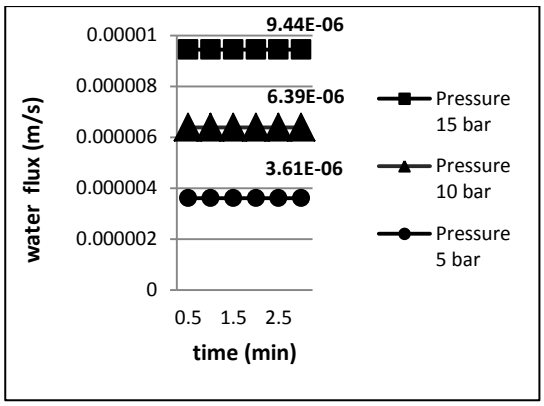


Figure B.17 Water flux rate for SWRO (NH_4LAC Broth 5 g/l)

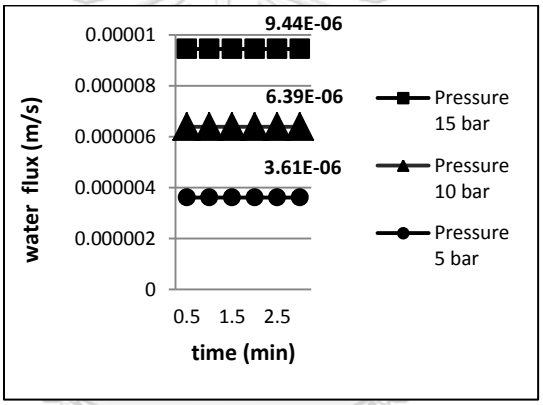


Figure B.18 Water flux rate for SWRO (CaLAC Broth 5 g/l)

APPENDIX C: GRAPHICAL PROPERTIES ON BWRO MEMBRANE

Experimental materials were tested as the model feed solution for separation lactic acid consisting of lactic acid, ammonium lactate (NH_4LAC), calcium lactate (CaLAC) and sodium lactate (NaLAC). The graphical properties of three materials when applying operating pressure higher than osmotic pressure during the experiment were presented in Figure C.1.

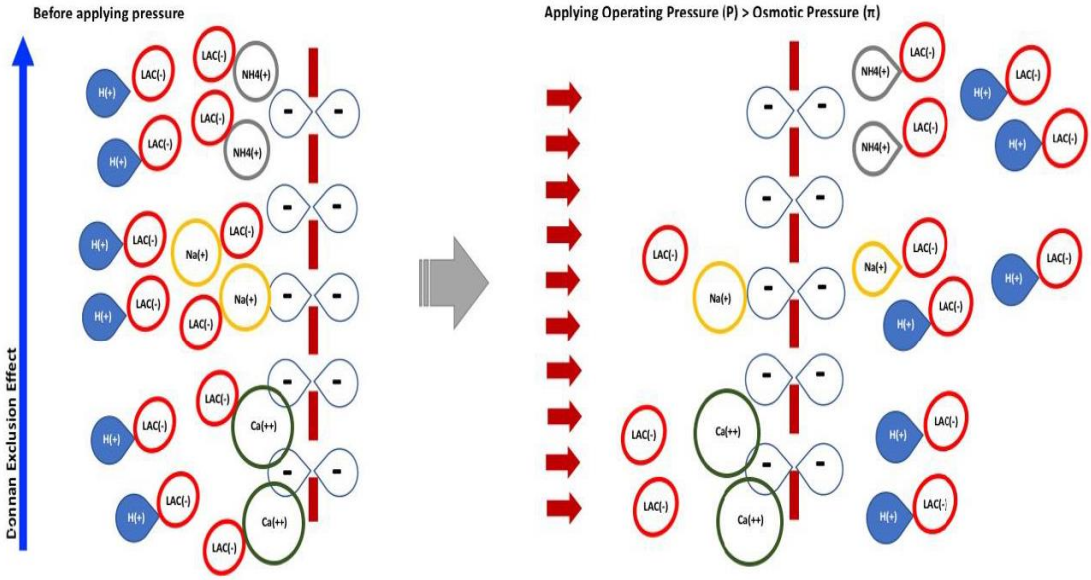


Figure C Graphical properties.

APPENDIX D: RAW DATA OF LACTATE SEPARATION OF LACTATE MODEL SOLUTION AT BWRO MEMBRANE UNIT

Table D.1 Lactate separation of lactate model solution at BWRO membrane

Operating condition	Retentate			Permeate		
	LA (g/l)	LA (g)	% rejection	LA (g/l)	LA (g)	% separation
1. CaLAC						
pH 4 , 4 bar	6.88	8.99	**89.9**	1.31	1.01	**10**
pH 4 , 4 bar	6.79	8.88	**88.8**	1.43	1.12	**11**
pH 4 , 6 bar	6.41	4.94	**49.4**	3.16	5.06	**51**
pH 4 , 6 bar	6.32	4.86	**48.6**	3.21	5.14	**51**
pH 6 , 4 bar	5.48	9.96	**99.6**	0.15	0.04	**0.4**
pH 6 , 4 bar	5.51	9.94	**99.4**	0.25	0.06	**0.6**
pH 6 , 6 bar	13.6	5.89	**58.9**	2.57	4.11	**41**
pH 6 , 6 bar	14.1	7.71	**77.1**	2.68	2.29	**43**
2. NaLAC						
pH 4 , 4 bar	7.24	8.66	**86.6**	1.6	1.34	**13**
pH 4 , 4 bar	7.62	8.66	**86.6**	1.58	1.34	**13**
pH 4 , 6 bar	6.55	3.15	**31.5**	4.28	6.85	**69**
pH 4 , 6 bar	6.52	3.1	**31.0**	4.31	6.9	**69**
pH 6 , 4 bar	5.15	9.89	**98.9**	0.35	0.11	**1**
pH 6 , 4 bar	5.26	9.89	**98.9**	0.38	0.11	**1**
pH 6 , 6 bar	9.6	4.24	**42.4**	3.6	5.76	**58**
pH 6 , 6 bar	9.69	4.38	**43.8**	3.51	5.62	**56**
3. NH_4LAC						
pH 4 , 4 bar	4.98	9.21	**92.1**	1.42	0.79	**8**
pH 4 , 4 bar	4.88	9.09	**90.9**	1.39	0.91	**9**
pH 4 , 6 bar	3.51	3.56	**35.6**	4.59	6.44	**65**
pH 4 , 6 bar	3.69	3.39	**33.9**	4.63	6.61	**66**
pH 6 , 4 bar	4.31	9.32	**93.2**	2.11	0.68	**7**
pH 6 , 4 bar	4.37	9.21	**92.1**	2.18	0.79	**8**
pH 6 , 6 bar	4.76	2.99	**29.9**	4.62	7.01	**70**
pH 6 , 6 bar	4.54	2.91	**29.1**	4.68	7.09	**71**

APPENDIX E: RAW DATA OF IONS SEPARATION OF LACTATE MODEL SOLUTION AT BWRO MEMBRANE UNIT

Table E.1 Ions separation of lactate model solution at BWRO membrane

Operating condition	Ca^{2+}		Na^{+}		NH_4^{+}	
	Mass (g)	**% rejection**	**Mass (g)**	**% rejection**	**Mass (g)**	**% rejection**
pH 4 , 4 bar	0.01	**1**	0.04	**3**	0.05	**5**
pH 4 , 4 bar	0.01	**1**	0.09	**6**	0.05	**5**
pH 4 , 6 bar	0.03	**3**	0.71	**49**	0.64	**60**
pH 4 , 6 bar	0.04	**3**	0.67	**47**	0.64	**60**
pH 6 , 4 bar	0.01	**1**	0.05	**1**	0.09	**9**
pH 6 , 4 bar	0.01	**1**	0.05	**1**	0.09	**9**
pH 6 , 6 bar	0.02	**2**	1.4	**36**	0.79	**75**
pH 6 , 6 bar	0.02	**2**	1.55	**39**	0.79	**75**

APPENDIX F: RAW DATA OF LACTIC ACID AND IONS SEPARATION OF FERMENTATION BROTH (5 g/L) AT BWRO MEMBRANE UNIT

Table F.1 Lactic acid and ions separation of fermentation at BWRO membrane

Ion	CaLAC		NaLAC		NH4LAC	
	Mass (g)	**% Separation**	**Mass (g)**	**% Separation**	**Mass (g)**	**% Separation**
LA	5.42	**54.2**	6.69	**66.9**	7.20	**72.0**
Cl^-	0.16	**46.8**	0.27	**57.0**	0.37	**61.7**
SO_4	0.03	**30.6**	0.02	**20.7**	0.02	**24.9**
NH_4^+	1.28	**62.7**	0.39	**65.4**	1.12	**69.6**
P	0.01	**3.8**	0.01	**8.4**	0.01	**5.0**
K^+	0.05	**63.1**	0.08	**48.8**	0.04	**32.6**
Mg^{2+}	0.01	**8.9**	0.01	**8.6**	0.01	**2.3**
Mn^{2+}	0.01	**15.2**	0.01	**18.0**	0.01	**16.5**
Fe^{2+}	0.01	**31.2**	0.01	**37.8**	0.01	**41.3**
Na^+	0.02	**57.6**	2.58	**57.7**	0.02	**55.2**
Ca^{2+}	0.16	**5.9**	0.01	**3.6**	0.01	**4.9**

APPENDIX G: GRAPHICAL ABSTRACT FOR RECOVERY PROCESS

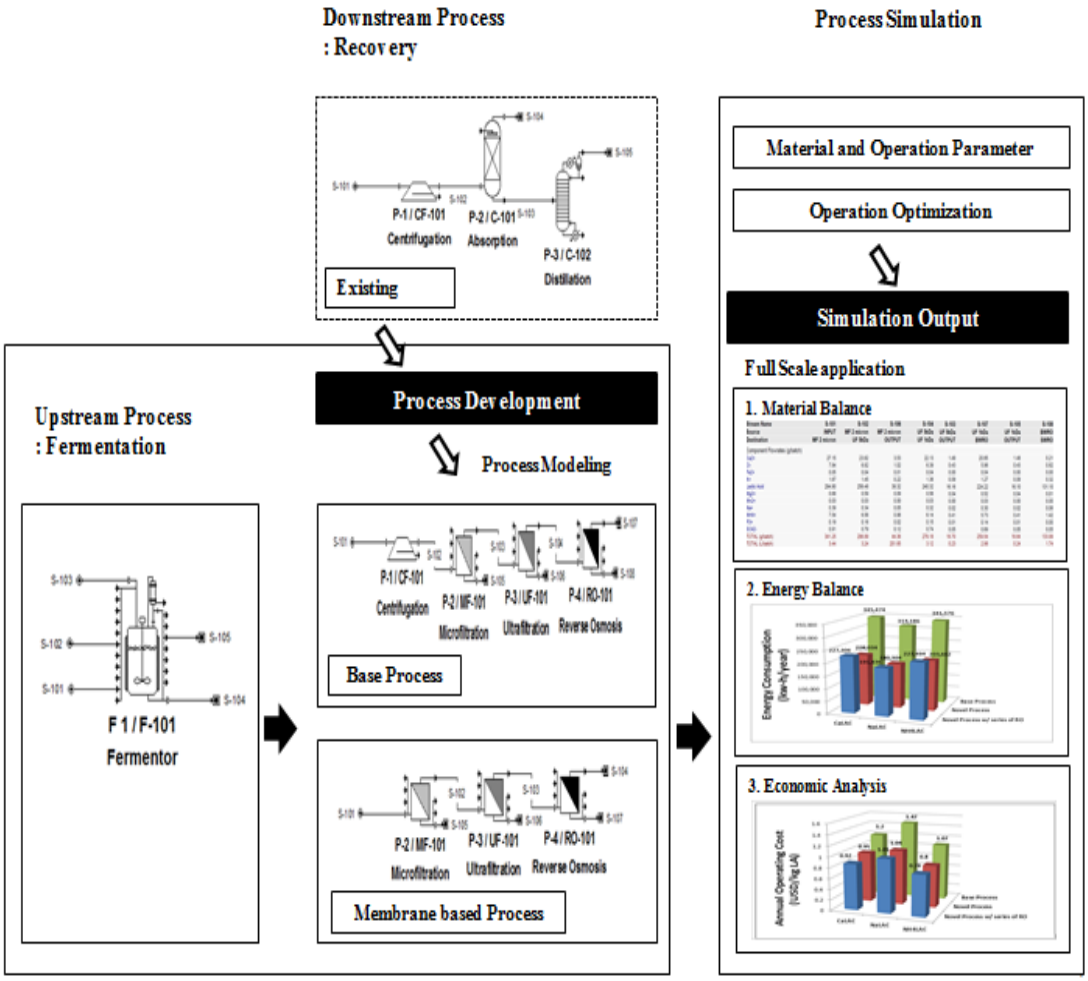


Figure G Graphical Abstract

APPENDIX H: SIMULATED MASS BALANCED

Table H.1 Calculated flowrates and compositions of ions/species of the intermediate and output streams for the based case process to recovery and preconcentrate lactic acid from (A) CaLAC broth; (B) NaLAC broth; and (C) NH4LAC broth.

(A)

Stream Name	S-101	S-102	S-103	S-104	S-105	S-106	S-107	S-108
Source	INPUT	Centrifuge	Centrifuge	MF 2 micron	MF 2 micron	UF 30 kDa	UF 30 kDa	UF 5 kDa
Destination	Centrifuge	MF 2 micron	OUTPUT	UF 30 kDa	OUTPUT	UF 5 kDa	OUTPUT	UF 1 kDa
Component Flowrates (g/batch)								
Bacteria	8.16	0.01	8.15	0.00	0.01	0.00	0.00	0.00
Ca2+	28.39	26.49	1.90	23.60	2.89	21.57	2.03	19.83
Cl-	7.97	7.43	0.54	6.62	0.81	6.05	0.57	5.56
Fe2+	0.40	0.37	0.03	0.33	0.04	0.30	0.03	0.28
K+	2.27	2.12	0.16	1.89	0.23	1.72	0.16	1.58
Lactic Acid	293.39	283.42	9.98	252.52	30.89	230.81	21.72	212.11
Mg2+	0.55	0.51	0.04	0.45	0.06	0.41	0.04	0.38
Mn2+	0.03	0.03	0.00	0.02	0.00	0.02	0.00	0.02
Na+	0.50	0.40	0.10	0.36	0.04	0.32	0.03	0.30
NH4+	7.43	6.89	0.54	6.14	0.75	5.61	0.53	5.16
P3+	0.20	0.16	0.04	0.14	0.02	0.13	0.01	0.12
SO42-	0.96	0.88	0.07	0.79	0.10	0.72	0.07	0.66
TOTAL (g/batch)	350.27	328.71	21.56	292.88	35.83	267.69	25.19	246.01
TOTAL (L/batch)	3.40	3.22	0.02	3.09	0.03	2.94	0.02	2.79

Stream Name	S-109	S-110	S-111	S-112	S-113	S-114	S-115
Source	UF 5 kDa	UF 1 kDa	UF 1 kDa	BWRO	BWRO	SWRO	SWRO
Destination	OUTPUT	BWRO	OUTPUT	SWRO	OUTPUT	OUTPUT	OUTPUT
Component Flowrates (g/batch)							
Bacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ca2+	1.75	18.16	1.67	0.18	17.98	0.01	0.18
Cl-	0.49	5.09	0.47	0.84	4.25	0.02	0.82
Fe2+	0.02	0.26	0.02	0.03	0.23	0.00	0.03
K+	0.14	1.45	0.13	0.33	1.12	0.01	0.32
Lactic Acid	18.70	194.29	17.82	113.44	80.86	0.00	113.49
Mg2+	0.03	0.35	0.03	0.01	0.34	0.00	0.01
Mn2+	0.00	0.02	0.00	0.00	0.02	0.00	0.00
Na+	0.03	0.27	0.03	0.06	0.21	0.00	0.06
NH4+	0.45	4.72	0.43	1.06	3.64	0.03	1.06
P3+	0.01	0.11	0.01	0.00	0.11	0.00	0.00
SO42-	0.06	0.60	0.06	0.04	0.57	0.00	0.04
TOTAL (g/batch)	21.68	225.34	20.66	116.02	109.32	0.07	116.01
TOTAL (L/batch)	0.02	2.61	0.02	1.64	0.09	7.08	0.10

(B)

Stream Name	S-101	S-102	S-103	S-104	S-105	S-106	S-107	S-108
Source	INPUT	Centrifuge	Centrifuge	MF 2 micron	MF 2 micron	UF 30 kDa	UF 30 kDa	UF 5 kDa
Destination	Centrifuge	MF 2 micron	OUTPUT	UF 30 kDa	OUTPUT	UF 5 kDa	OUTPUT	UF 1 kDa
Component Flowrates (g/batch)								
Bacteria	8.93	0.01	8.92	0.00	0.01	0.00	0.00	0.00
Ca2+	1.31	1.21	0.10	1.10	0.11	1.03	0.07	0.96
Cl-	10.17	9.52	0.65	8.62	0.90	8.08	0.53	7.55
Fe2+	0.22	0.21	0.01	0.19	0.02	0.18	0.01	0.17
K+	3.20	3.01	0.19	2.72	0.29	2.55	0.17	2.38
Lactic Acid	313.41	293.98	19.43	266.05	27.93	249.56	16.50	233.09
Mg2+	0.24	0.22	0.02	0.20	0.02	0.18	0.01	0.17
Mn2+	0.03	0.03	0.00	0.03	0.00	0.03	0.00	0.03
Na+	84.93	80.09	4.84	72.48	7.61	67.98	4.49	63.50
NH4+	4.80	4.19	0.60	3.79	0.40	3.56	0.24	3.32
P3+	0.19	0.18	0.01	0.16	0.02	0.15	0.01	0.14
SO42-	0.93	0.82	0.11	0.74	0.08	0.70	0.05	0.65
TOTAL (g/batch)	428.36	393.47	34.89	356.09	37.38	334.01	22.08	311.97
TOTAL (L/batch)	3.72	3.54	0.03	3.35	0.03	3.23	0.02	3.10

Stream Name	S-109	S-110	S-111	S-112	S-113	S-114	S-115
Source	UF 5 kDa	UF 1 kDa	UF 1 kDa	BWRO	BWRO	SWRO	SWRO
Destination	OUTPUT	BWRO	OUTPUT	SWRO	OUTPUT	OUTPUT	OUTPUT
Component Flowrates (g/batch)							
Bacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ca2+	0.07	0.89	0.07	0.01	0.88	0.00	0.01
Cl-	0.53	7.00	0.55	1.79	5.21	0.08	1.71
Fe2+	0.01	0.16	0.01	0.02	0.13	0.00	0.02
K+	0.17	2.21	0.17	0.50	1.71	0.02	0.48
Lactic Acid	16.47	216.07	17.02	178.39	37.69	2.95	175.44
Mg2+	0.01	0.16	0.01	0.00	0.16	0.00	0.00
Mn2+	0.00	0.02	0.00	0.00	0.02	0.00	0.00
Na+	4.49	58.86	4.64	17.31	41.55	0.78	16.53
NH4+	0.23	3.08	0.24	1.01	2.07	0.05	0.96
P3+	0.01	0.13	0.01	0.00	0.13	0.00	0.00
SO42-	0.05	0.60	0.05	0.04	0.56	0.00	0.04
TOTAL (g/batch)	22.04	289.19	22.77	199.09	90.10	3.88	195.21
TOTAL (L/batch)	0.02	3.00	0.02	1.72	0.09	35.30	0.18

(C)

Stream Name	S-101	S-102	S-103	S-104	S-105	S-106	S-107	S-108
Source	INPUT	Centrifuge	Centrifuge	MF 2 micron	MF 2 micron	UF 30 kDa	UF 30 kDa	UF 5 kDa
Destination	Centrifuge	MF 2 micron	OUTPUT	UF 30 kDa	OUTPUT	UF 5 kDa	OUTPUT	UF 1 kDa
Component Flowrates (g/batch)								
Bacteria	8.74	0.01	8.73	0.00	0.01	0.00	0.00	0.00
Ca2+	1.37	1.26	0.11	1.15	0.11	1.09	0.07	1.02
Cl-	9.17	8.60	0.57	7.88	0.72	7.41	0.46	6.97
Fe2+	0.03	0.02	0.00	0.02	0.00	0.02	0.00	0.02
K+	2.96	2.82	0.14	2.58	0.24	2.43	0.15	2.29
Lactic Acid	308.31	277.17	31.14	253.89	23.28	238.91	14.98	224.57
Mg2+	0.27	0.25	0.01	0.23	0.02	0.22	0.01	0.21
Mn2+	0.04	0.03	0.01	0.03	0.00	0.03	0.00	0.03
Na+	0.23	0.21	0.02	0.19	0.02	0.18	0.01	0.17
NH4+	56.72	53.83	2.89	49.30	4.52	46.40	2.91	43.61
P3+	0.18	0.17	0.01	0.16	0.01	0.15	0.01	0.14
SO42-	0.94	0.87	0.06	0.80	0.07	0.75	0.05	0.71
TOTAL (g/batch)	388.93	345.24	43.69	316.24	29.00	297.58	18.66	279.73
TOTAL (L/batch)	3.64	3.48	0.04	3.29	0.02	3.18	0.01	3.08

Stream Name	S-109	S-110	S-111	S-112	S-113	S-114	S-115
Source	UF 5 kDa	UF 1 kDa	UF 1 kDa	BWRO	BWRO	SWRO	SWRO
Destination	OUTPUT	BWRO	OUTPUT	SWRO	OUTPUT	OUTPUT	OUTPUT
Component Flowrates (g/batch)							
Bacteria	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ca2+	0.07	0.95	0.07	0.01	0.94	0.00	0.01
Cl-	0.44	6.49	0.47	2.78	3.72	0.05	2.72
Fe2+	0.00	0.02	0.00	0.00	0.01	0.00	0.00
K+	0.15	2.13	0.16	0.27	1.86	0.01	0.27
Lactic Acid	14.33	209.30	15.27	160.41	48.89	0.58	159.83
Mg2+	0.01	0.19	0.01	0.00	0.19	0.00	0.00
Mn2+	0.00	0.03	0.00	0.00	0.02	0.00	0.00
Na+	0.01	0.15	0.01	0.04	0.11	0.00	0.04
NH4+	2.78	40.65	2.97	19.81	20.83	0.38	19.43
P3+	0.01	0.13	0.01	0.00	0.13	0.00	0.00
SO42-	0.05	0.66	0.05	0.07	0.59	0.00	0.06
TOTAL (g/batch)	17.85	260.71	19.02	183.41	77.29	1.02	182.39
TOTAL (L/batch)	0.01	3.31	0.01	1.60	0.06	51.05	0.16

Table H.2 Calculated flowrates and compositions of ions/species of the intermediate and output streams for the membrane based process to recovery and preconcentrate lactic acid from (A) CaLAC broth; (B) NaLAC broth; and (C) NH4LAC broth.

(A)

Stream Name	S-101	S-102	S-106	S-104	S-103	S-107	S-105	S-108
Source	INPUT	MF 2 micron	MF 2 micron	UF 5kDa	UF 5kDa	UF 1kDa	UF 1kDa	BWRO
Destination	MF 2 micron	UF 5kDa	OUTPUT	UF 1kDa	OUTPUT	BWRO	OUTPUT	SWRO
Component Flowrates (g/batch)								
Bacteria	8.26	0.02	8.24	0.00	0.02	0.00	0.00	0.00
Ca2+	27.15	24.31	2.84	22.78	1.53	21.26	1.53	0.21
Cl-	7.84	7.02	0.82	6.58	0.44	6.14	0.44	0.84
Fe2+	0.05	0.04	0.00	0.04	0.00	0.04	0.00	0.00
K+	1.67	1.49	0.17	1.40	0.09	1.31	0.09	0.33
Lactic Acid	294.80	264.01	30.80	247.39	16.62	230.81	16.58	134.95
Mg2+	0.68	0.61	0.07	0.57	0.04	0.53	0.04	0.01
Mn2+	0.03	0.03	0.00	0.03	0.00	0.03	0.00	0.00
Na+	0.39	0.35	0.04	0.33	0.02	0.31	0.02	0.06
NH4+	7.54	6.75	0.79	6.32	0.42	5.90	0.42	1.46
P3+	0.19	0.17	0.02	0.16	0.01	0.15	0.01	0.00
SO42-	0.91	0.81	0.09	0.76	0.05	0.71	0.05	0.05
TOTAL (g/batch)	349.50	305.62	43.89	286.36	19.25	267.18	19.19	137.94
TOTAL (L/batch)	3.44	3.34	199.50	3.21	0.24	3.07	0.24	1.79

Stream Name	S-109	S-110	S-111
Source	BWRO	SWRO	SWRO
Destination	OUTPUT	OUTPUT	OUTPUT
Component Flowrates (g/batch)			
Bacteria	0.00	0.00	0.00
Ca2+	21.04	0.00	0.21
Cl-	5.29	0.00	0.84
Fe2+	0.03	0.00	0.00
K+	0.98	0.00	0.33
Lactic Acid	95.86	0.00	134.95
Mg2+	0.52	0.00	0.01
Mn2+	0.02	0.00	0.00
Na+	0.24	0.00	0.06
NH4+	4.44	0.00	1.46
P3+	0.15	0.00	0.00
SO42-	0.66	0.00	0.05
TOTAL (g/batch)	129.24	0.00	137.93
TOTAL (L/batch)	0.11	0.14	0.12

(B)

Stream Name	S-101	S-102	S-106	S-104	S-103	S-107	S-105	S-108
Source	INPUT	MF 2 micron	MF 2 micron	UF 5kDa	UF 5kDa	UF 1kDa	UF 1kDa	BWRO
Destination	MF 2 micron	UF 5kDa	OUTPUT	UF 1kDa	OUTPUT	BWRO	OUTPUT	SWRO
Component Flowrates (g/batch)								
Bacteria	9.07	0.03	9.04	0.00	0.03	0.00	0.00	0.00
Ca2+	1.39	1.37	0.02	1.28	0.09	1.20	0.08	0.01
Cl-	8.01	7.87	0.14	7.38	0.50	6.90	0.47	1.84
Fe2+	0.02	0.02	0.00	0.02	0.00	0.02	0.00	0.00
K+	2.72	2.67	0.05	2.50	0.17	2.34	0.16	0.47
Lactic Acid	315.66	310.03	5.63	290.52	19.51	271.93	18.59	208.30
Mg2+	0.21	0.21	0.00	0.20	0.01	0.18	0.01	0.00
Mn2+	0.03	0.03	0.00	0.03	0.00	0.03	0.00	0.00
Na+	81.65	80.19	1.46	75.15	5.05	70.34	4.81	19.18
NH4+	5.07	4.98	0.09	4.67	0.31	4.37	0.30	1.56
P3+	0.19	0.19	0.00	0.17	0.01	0.16	0.01	0.00
SO42-	0.84	0.82	0.01	0.77	0.05	0.72	0.05	0.04
TOTAL (g/batch)	424.87	408.41	16.46	382.68	25.73	358.19	24.49	231.42
TOTAL (L/batch)	3.78	3.76	149.66	3.62	0.31	3.46	0.32	1.73

Stream Name	S-109	S-110	S-111
Source	BWRO	SWRO	SWRO
Destination	OUTPUT	OUTPUT	OUTPUT
Component Flowrates (g/batch)			
Bacteria	0.00	0.00	0.00
Ca2+	1.19	0.00	0.01
Cl-	5.06	0.00	1.84
Fe2+	0.01	0.00	0.00
K+	1.88	0.00	0.47
Lactic Acid	63.63	0.00	208.29
Mg2+	0.18	0.00	0.00
Mn2+	0.02	0.00	0.00
Na+	51.16	0.00	19.18
NH4+	2.81	0.00	1.56
P3+	0.16	0.00	0.00
SO42-	0.68	0.00	0.04
TOTAL (g/batch)	126.78	0.00	231.41
TOTAL (L/batch)	0.12	0.23	0.20

(C)

Stream Name	S-101	S-102	S-106	S-104	S-103	S-107	S-105	S-108
Source	INPUT	MF 2 micron	MF 2 micron	UF 5kDa	UF 5kDa	UF 1kDa	UF 1kDa	BWRO
Destination	MF 2 micron	UF 5kDa	OUTPUT	UF 1kDa	OUTPUT	BWRO	OUTPUT	SWRO
Component Flowrates (g/batch)								
Bacteria	8.88	0.02	8.86	0.00	0.02	0.00	0.00	0.00
Ca2+	1.11	1.07	0.05	0.99	0.07	0.94	0.05	0.01
Cl-	8.35	7.98	0.36	7.42	0.56	7.03	0.39	2.54
Fe2+	0.02	0.02	0.00	0.02	0.00	0.02	0.00	0.01
K+	2.68	2.56	0.12	2.38	0.18	2.25	0.13	0.28
Lactic Acid	305.26	291.93	13.33	271.52	20.41	257.13	14.39	191.47
Mg2+	0.28	0.27	0.01	0.25	0.02	0.24	0.01	0.00
Mn2+	0.44	0.42	0.02	0.39	0.03	0.37	0.02	0.05
Na+	0.28	0.27	0.01	0.25	0.02	0.24	0.01	0.07
NH4+	48.35	46.24	2.11	43.01	3.23	40.73	2.28	19.17
P3+	0.15	0.14	0.01	0.13	0.01	0.12	0.01	0.00
SO42-	0.90	0.86	0.04	0.80	0.06	0.75	0.04	0.07
TOTAL (g/batch)	376.70	351.78	24.91	327.17	24.62	309.83	17.34	213.68
TOTAL (L/batch)	3.70	3.67	131.12	3.52	0.37	3.44	0.25	1.64

Stream Name	S-109	S-110	S-111
Source	BWRO	SWRO	SWRO
Destination	OUTPUT	OUTPUT	OUTPUT
Component Flowrates (g/batch)			
Bacteria	0.00	0.00	0.00
Ca2+	0.92	0.00	0.01
Cl-	4.49	0.00	2.54
Fe2+	0.01	0.00	0.01
K+	1.97	0.00	0.28
Lactic Acid	65.66	0.00	191.47
Mg2+	0.23	0.00	0.00
Mn2+	0.32	0.00	0.05
Na+	0.17	0.00	0.07
NH4+	21.56	0.00	19.17
P3+	0.12	0.00	0.00
SO42-	0.68	0.00	0.07
TOTAL (g/batch)	96.15	0.00	213.68
TOTAL (L/batch)	0.08	0.23	0.18

Table H.3 Calculated flowrates and compositions of ions/species of the intermediate and output streams for the in-parallel membrane based process to recovery and preconcentrate lactic acid from (A) CaLAC broth; (B) NaLAC broth; and (C) NH4LAC broth.

(A)

Stream Name	S-101	S-102	S-106	S-104	S-103	S-105	S-107	S-109
Source	INPUT	MF 2 micron	MF 2 micron	UF 5kDa	UF 5kDa	UF 1kDa	UF 1kDa	BWRO
Destination	MF 2 micron	UF 5kDa	OUTPUT	UF 1kDa	OUTPUT	OUTPUT	BWRO	BWRO_2
Component Flowrates (g/batch)								
Bacteria	8.26	0.02	8.24	0.00	0.02	0.00	0.00	0.00
Ca2+	27.15	24.31	2.84	22.78	1.53	1.53	21.26	21.04
Cl-	7.84	7.02	0.82	6.58	0.44	0.44	6.14	5.29
Fe2+	0.05	0.04	0.00	0.04	0.00	0.00	0.04	0.03
K+	1.67	1.49	0.17	1.40	0.09	0.09	1.31	0.98
Lactic Acid	294.80	264.01	30.80	247.39	16.62	16.58	230.81	95.86
Mg2+	0.68	0.61	0.07	0.57	0.04	0.04	0.53	0.52
Mn2+	0.03	0.03	0.00	0.03	0.00	0.00	0.03	0.02
Na+	0.39	0.35	0.04	0.33	0.02	0.02	0.31	0.24
NH4+	7.54	6.75	0.79	6.32	0.42	0.42	5.90	4.44
P3+	0.19	0.17	0.02	0.16	0.01	0.01	0.15	0.15
SO42-	0.91	0.81	0.09	0.76	0.05	0.05	0.71	0.66
TOTAL (g/batch)	349.50	305.62	43.89	286.36	19.25	19.19	267.18	129.24
TOTAL (L/batch)	3.44	3.34	199.50	3.21	0.24	0.24	3.07	0.11

Stream Name	S-108	S-114	S-110	S-111	S-112	S-113
Source	BWRO_2	BWRO_2	BWRO	MX	SWRO	SWRO
Destination	MX	OUTPUT	MX	SWRO	OUTPUT	OUTPUT
Component Flowrates (g/batch)						
Bacteria	0.00	0.00	0.00	0.00	0.00	0.00
Ca2+	0.21	20.83	0.21	0.42	0.00	0.42
Cl-	0.73	4.56	0.84	1.57	0.00	1.57
Fe2+	0.00	0.03	0.00	0.01	0.00	0.01
K+	0.25	0.73	0.33	0.57	0.00	0.57
Lactic Acid	65.17	30.69	134.95	200.13	0.00	200.13
Mg2+	0.01	0.52	0.01	0.02	0.00	0.02
Mn2+	0.00	0.02	0.00	0.00	0.00	0.00
Na+	0.05	0.19	0.06	0.11	0.00	0.11
NH4+	1.10	3.34	1.46	2.56	0.00	2.56
P3+	0.00	0.14	0.00	0.01	0.00	0.01
SO42-	0.05	0.61	0.05	0.10	0.00	0.10
TOTAL (g/batch)	67.57	61.67	137.94	205.51	0.00	205.51
TOTAL (L/batch)	0.06	0.05	0.11	0.17	0.00	0.17

(B)

Stream Name	S-101	S-102	S-106	S-104	S-103	S-107	S-105	S-109
Source	INPUT	MF 2 micron	MF 2 micron	UF 5kDa	UF 5kDa	UF 1kDa	UF 1kDa	BWRO
Destination	MF 2 micron	UF 5kDa	OUTPUT	UF 1kDa	OUTPUT	BWRO	OUTPUT	BWRO_2
Component Flowrates (g/batch)								
Bacteria	9.07	0.03	9.04	0.00	0.03	0.00	0.00	0.00
Ca2+	1.39	1.37	0.02	1.28	0.09	1.20	0.08	1.19
Cl-	8.01	7.87	0.14	7.38	0.50	6.90	0.47	5.06
Fe2+	0.02	0.02	0.00	0.02	0.00	0.02	0.00	0.01
K+	2.72	2.67	0.05	2.50	0.17	2.34	0.16	1.88
Lactic Acid	315.66	310.03	5.63	290.52	19.51	271.93	18.59	63.63
Mg2+	0.21	0.21	0.00	0.20	0.01	0.18	0.01	0.18
Mn2+	0.03	0.03	0.00	0.03	0.00	0.03	0.00	0.02
Na+	81.65	80.19	1.46	75.15	5.05	70.34	4.81	51.16
NH4+	5.07	4.98	0.09	4.67	0.31	4.37	0.30	2.81
P3+	0.19	0.19	0.00	0.17	0.01	0.16	0.01	0.16
SO42-	0.84	0.82	0.01	0.77	0.05	0.72	0.05	0.68
TOTAL (g/batch)	424.87	408.41	16.46	382.68	25.73	358.19	24.49	126.78
TOTAL (L/batch)	3.78	3.76	149.66	3.62	0.31	3.46	0.32	0.12

Stream Name	S-114	S-112	S-108	S-113	S-110	S-111
Source	BWRO_2	BWRO_2	BWRO	MX	SWRO	SWRO
Destination	MX	OUTPUT	MX	SWRO	OUTPUT	OUTPUT
Component Flowrates (g/batch)						
Bacteria	0.00	0.00	0.00	0.00	0.00	0.00
Ca2+	0.01	1.17	0.01	0.02	0.00	0.02
Cl-	1.35	3.71	1.84	3.19	0.00	3.19
Fe2+	0.00	0.01	0.00	0.01	0.00	0.01
K+	0.37	1.50	0.47	0.84	0.00	0.84
Lactic Acid	63.63	0.00	208.30	271.93	0.00	271.92
Mg2+	0.00	0.17	0.00	0.01	0.00	0.01
Mn2+	0.00	0.02	0.00	0.01	0.00	0.01
Na+	13.95	37.21	19.18	33.13	0.00	33.13
NH4+	1.00	1.81	1.56	2.56	0.00	2.56
P3+	0.00	0.15	0.00	0.01	0.00	0.01
SO42-	0.04	0.64	0.04	0.08	0.00	0.08
TOTAL (g/batch)	80.38	46.40	231.42	311.79	0.00	311.79
TOTAL (L/batch)	0.07	0.05	0.19	0.26	0.31	0.26

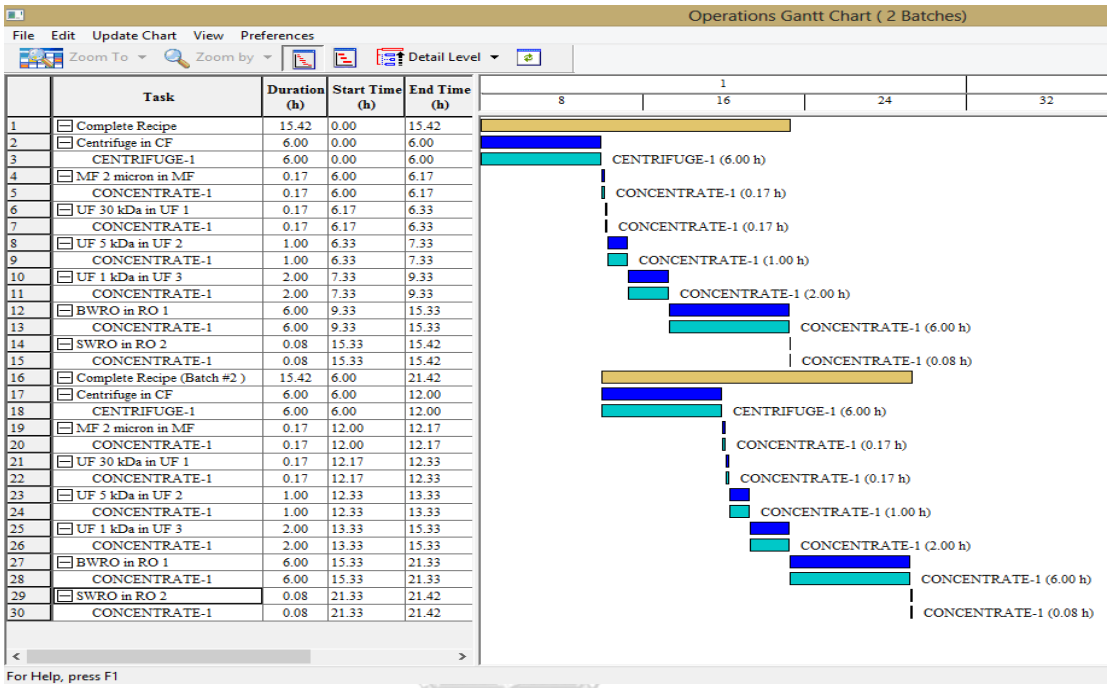
(C)

Stream Name	S-101	S-102	S-106	S-104	S-103	S-107	S-105	S-112
Source	INPUT	MF 2 micron	MF 2 micron	UF 5kDa	UF 5kDa	UF 1kDa	UF 1kDa	BWRO
Destination	MF 2 micron	UF 5kDa	OUTPUT	UF 1kDa	OUTPUT	BWRO	OUTPUT	BWRO_2
Component Flowrates (g/batch)								
Bacteria	8.88	0.02	8.86	0.00	0.02	0.00	0.00	0.00
Ca2+	1.11	1.07	0.05	0.99	0.07	0.94	0.05	0.92
Cl-	8.35	7.98	0.36	7.42	0.56	7.03	0.39	4.49
Fe2+	0.02	0.02	0.00	0.02	0.00	0.02	0.00	0.01
K+	2.68	2.56	0.12	2.38	0.18	2.25	0.13	1.97
Lactic Acid	305.65	292.30	13.35	271.86	20.44	257.45	14.41	65.79
Mg2+	0.28	0.27	0.01	0.25	0.02	0.24	0.01	0.23
Mn2+	0.04	0.04	0.00	0.04	0.00	0.04	0.00	0.03
Na+	0.28	0.27	0.01	0.25	0.02	0.24	0.01	0.17
NH4+	48.35	46.24	2.11	43.01	3.23	40.73	2.28	21.56
P3+	0.15	0.14	0.01	0.13	0.01	0.12	0.01	0.12
SO42-	0.90	0.86	0.04	0.80	0.06	0.75	0.04	0.68
TOTAL (g/batch)	376.70	351.77	24.92	327.16	24.62	309.82	17.34	95.99
TOTAL (L/batch)	3.70	3.67	131.17	3.52	0.37	3.44	0.25	0.07

Stream Name	S-113	S-114	S-108	S-109	S-110	S-111
Source	BWRO_2	BWRO_2	BWRO	MX	SWRO	SWRO
Destination	MX	OUTPUT	MX	SWRO	OUTPUT	OUTPUT
Component Flowrates (g/batch)						
Bacteria	0.00	0.00	0.00	0.00	0.00	0.00
Ca2+	0.01	0.91	0.01	0.03	0.00	0.03
Cl-	1.62	2.87	2.54	4.16	0.00	4.16
Fe2+	0.00	0.01	0.01	0.01	0.00	0.01
K+	0.25	1.72	0.28	0.53	0.00	0.53
Lactic Acid	53.23	12.56	191.67	244.90	0.00	244.89
Mg2+	0.00	0.23	0.00	0.01	0.00	0.01
Mn2+	0.00	0.03	0.00	0.01	0.00	0.01
Na+	0.05	0.12	0.07	0.12	0.00	0.12
NH4+	10.15	11.41	19.17	29.32	0.00	29.32
P3+	0.00	0.12	0.00	0.01	0.00	0.01
SO42-	0.06	0.62	0.07	0.13	0.00	0.13
TOTAL (g/batch)	65.39	30.59	213.83	279.22	0.00	279.22
TOTAL (L/batch)	0.05	0.02	0.17	0.22	0.28	0.22

APPENDIX I: RECIPE GRANT CHART

(A)



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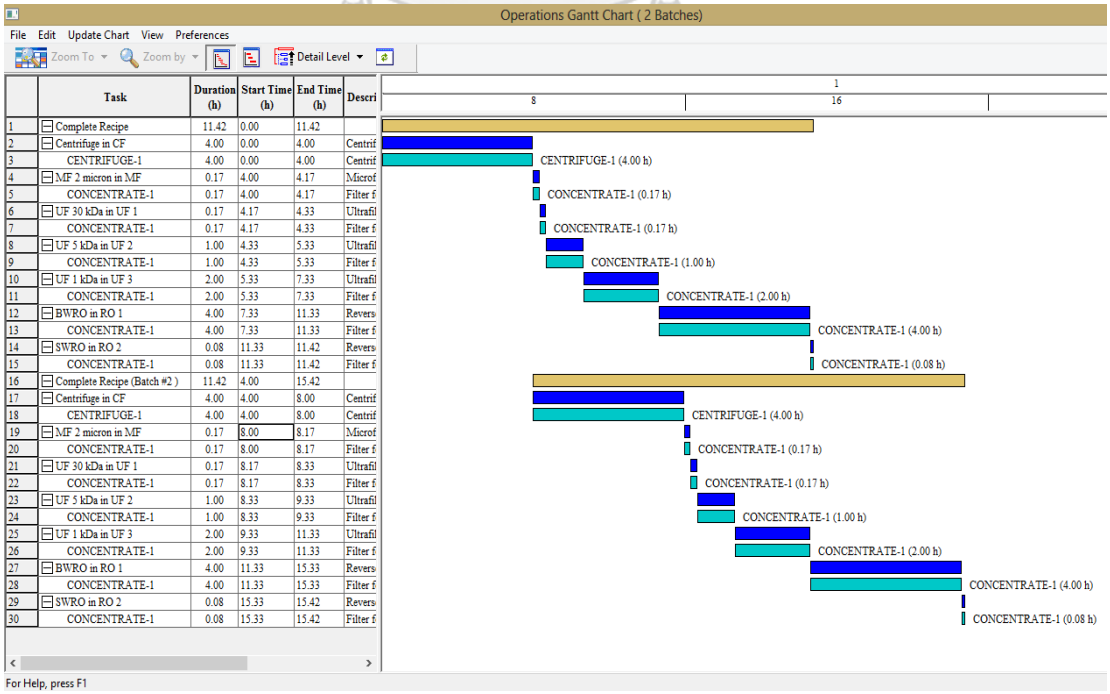


(C)

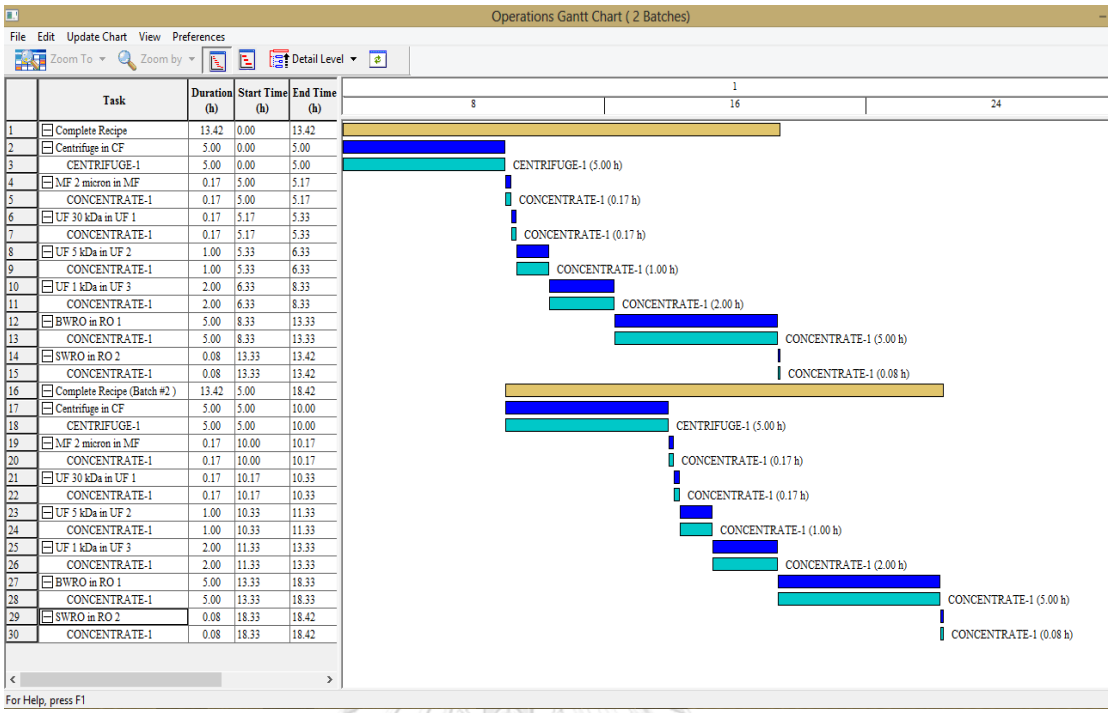
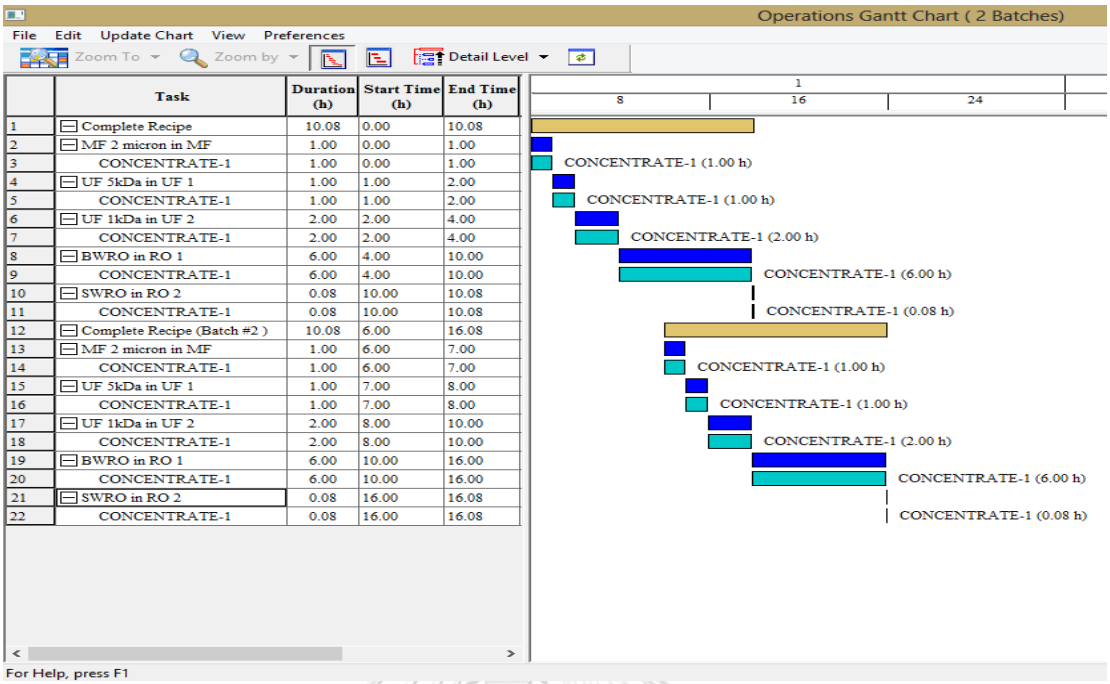
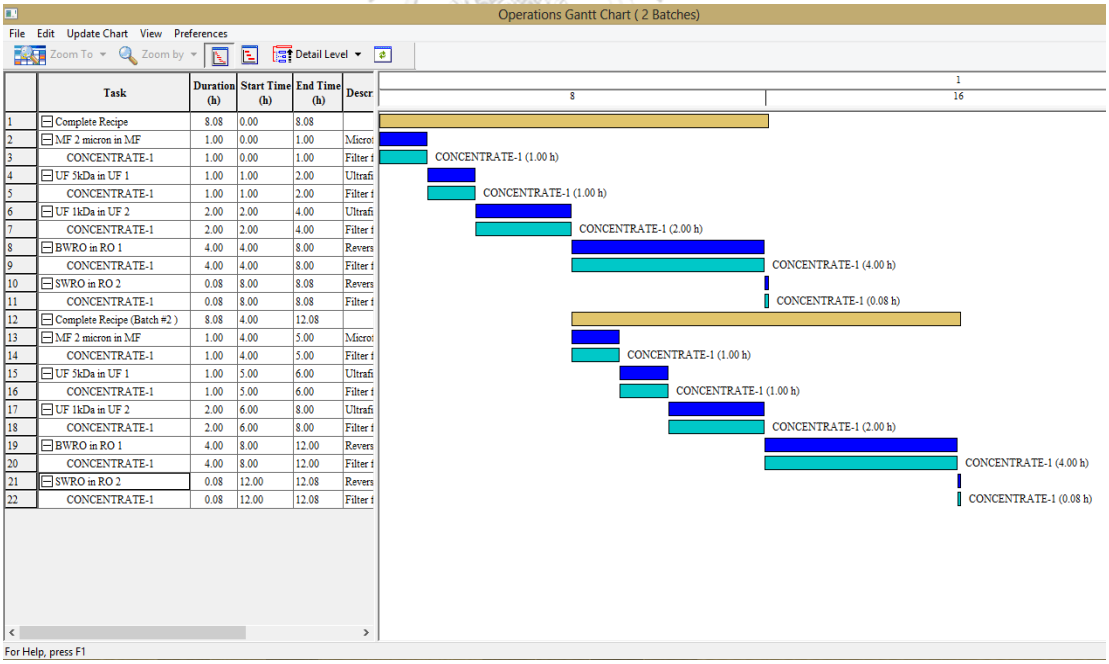


Figure I.1 Recipe Gantt chart for the base case process with different feed streams. (A) CaLAC broth; (B) NaLAC broth; and (C) NH4LAC broth.

(A)


Operations Gantt Chart (2 Batches)
File Edit Update Chart View Preferences
Zoom To Zoom by Detail Level
Task | Duration (h) | Start Time (h) | End Time (h)
1 Complete Recipe 10.08 0.00 10.08
2 MF 2 micron in MF 1.00 0.00 1.00
3 CONCENTRATE-1 1.00 0.00 1.00
4 UF 5kDa in UF 1 1.00 1.00 2.00
5 CONCENTRATE-1 1.00 1.00 2.00
6 UF 1kDa in UF 2 2.00 2.00 4.00
7 CONCENTRATE-1 2.00 2.00 4.00
8 BWRO in RO 1 6.00 4.00 10.00
9 CONCENTRATE-1 6.00 4.00 10.00
10 SWRO in RO 2 0.08 10.00 10.08
11 CONCENTRATE-1 0.08 10.00 10.08
12 Complete Recipe (Batch #2) 10.08 6.00 16.08
13 MF 2 micron in MF 1.00 6.00 7.00
14 CONCENTRATE-1 1.00 6.00 7.00
15 UF 5kDa in UF 1 1.00 7.00 8.00
16 CONCENTRATE-1 1.00 7.00 8.00
17 UF 1kDa in UF 2 2.00 8.00 10.00
18 CONCENTRATE-1 2.00 8.00 10.00
19 BWRO in RO 1 6.00 10.00 16.00
20 CONCENTRATE-1 6.00 10.00 16.00
21 SWRO in RO 2 0.08 16.00 16.08
22 CONCENTRATE-1 0.08 16.00 16.08
1
8
16
24
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (2.00 h)
CONCENTRATE-1 (6.00 h)
CONCENTRATE-1 (0.08 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (2.00 h)
CONCENTRATE-1 (6.00 h)
CONCENTRATE-1 (0.08 h)
For Help, press F1

(B)


Operations Gantt Chart (2 Batches)
File Edit Update Chart View Preferences
Zoom To Zoom by Detail Level
Task | Duration (h) | Start Time (h) | End Time (h) | Descr
1 Complete Recipe 8.08 0.00 8.08
2 MF 2 micron in MF 1.00 0.00 1.00 Micro
3 CONCENTRATE-1 1.00 0.00 1.00 Filter
4 UF 5kDa in UF 1 1.00 1.00 2.00 Ultrafi
5 CONCENTRATE-1 1.00 1.00 2.00 Filter
6 UF 1kDa in UF 2 2.00 2.00 4.00 Ultrafi
7 CONCENTRATE-1 2.00 2.00 4.00 Filter
8 BWRO in RO 1 4.00 4.00 8.00 Revers
9 CONCENTRATE-1 4.00 4.00 8.00 Filter
10 SWRO in RO 2 0.08 8.00 8.08 Revers
11 CONCENTRATE-1 0.08 8.00 8.08 Filter
12 Complete Recipe (Batch #2) 8.08 4.00 12.08
13 MF 2 micron in MF 1.00 4.00 5.00 Micro
14 CONCENTRATE-1 1.00 4.00 5.00 Filter
15 UF 5kDa in UF 1 1.00 5.00 6.00 Ultrafi
16 CONCENTRATE-1 1.00 5.00 6.00 Filter
17 UF 1kDa in UF 2 2.00 6.00 8.00 Ultrafi
18 CONCENTRATE-1 2.00 6.00 8.00 Filter
19 BWRO in RO 1 4.00 8.00 12.00 Revers
20 CONCENTRATE-1 4.00 8.00 12.00 Filter
21 SWRO in RO 2 0.08 12.00 12.08 Revers
22 CONCENTRATE-1 0.08 12.00 12.08 Filter
1
8
16
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (2.00 h)
CONCENTRATE-1 (4.00 h)
CONCENTRATE-1 (0.08 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (2.00 h)
CONCENTRATE-1 (4.00 h)
CONCENTRATE-1 (0.08 h)
For Help, press F1

(C)

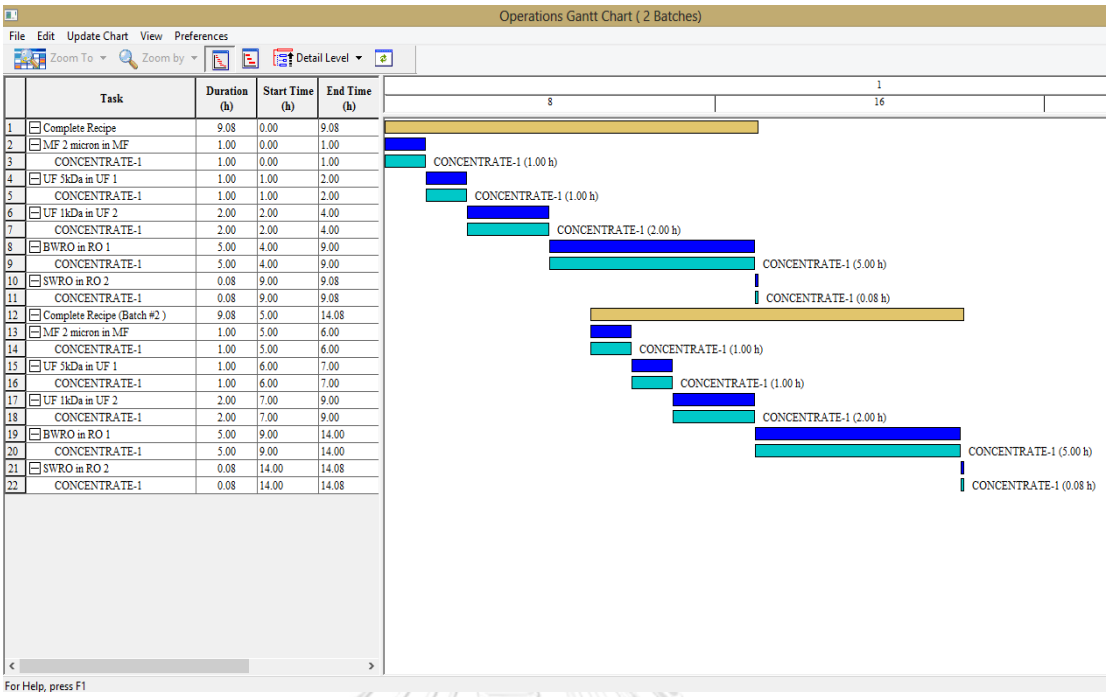
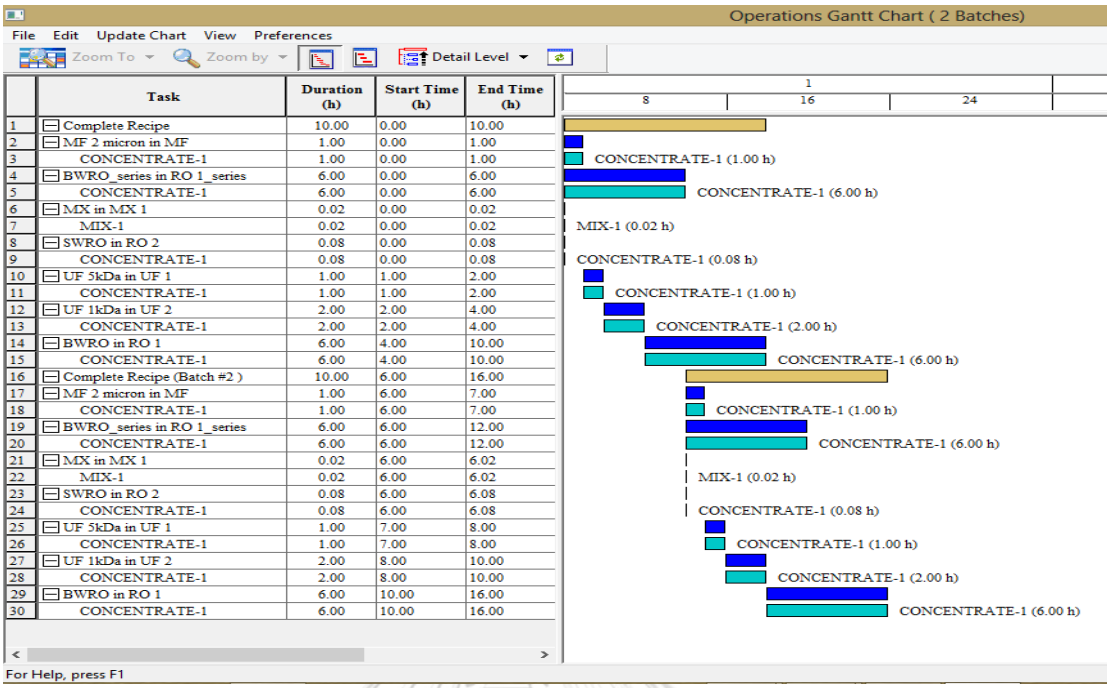
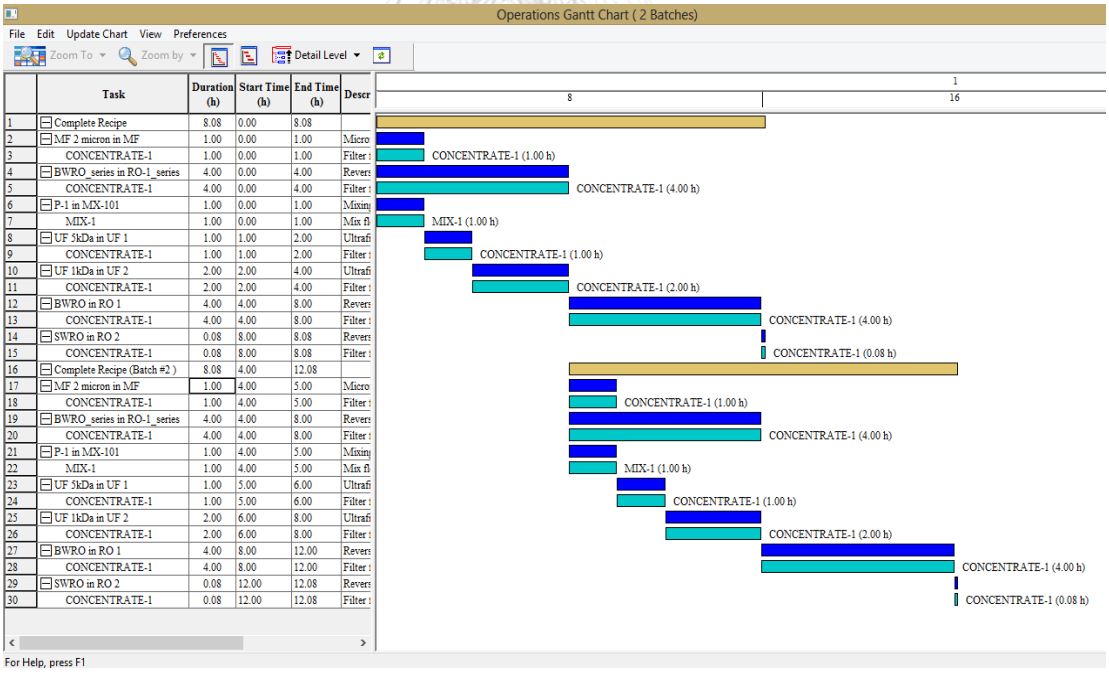


Figure I.2 Recipe Gantt chart for the membrane based process with different feed streams. (A) CaLAC broth; (B) NaLAC broth; and (C) NH4LAC broth.

(A)


Operations Gantt Chart (2 Batches)
File Edit Update Chart View Preferences
Zoom To Zoom by Detail Level
Task | Duration (h) | Start Time (h) | End Time (h)
1 Complete Recipe 10.00 0.00 10.00
2 MF 2 micron in MF 1.00 0.00 1.00
3 CONCENTRATE-1 1.00 0.00 1.00
4 BWRO_series in RO 1_series 6.00 0.00 6.00
5 CONCENTRATE-1 6.00 0.00 6.00
6 MX in MX 1 0.02 0.00 0.02
7 MIX-1 0.02 0.00 0.02
8 SWRO in RO 2 0.08 0.00 0.08
9 CONCENTRATE-1 0.08 0.00 0.08
10 UF 5kDa in UF 1 1.00 1.00 2.00
11 CONCENTRATE-1 1.00 1.00 2.00
12 UF 1kDa in UF 2 2.00 2.00 4.00
13 CONCENTRATE-1 2.00 2.00 4.00
14 BWRO in RO 1 6.00 4.00 10.00
15 CONCENTRATE-1 6.00 4.00 10.00
16 Complete Recipe (Batch #2) 10.00 6.00 16.00
17 MF 2 micron in MF 1.00 6.00 7.00
18 CONCENTRATE-1 1.00 6.00 7.00
19 BWRO_series in RO 1_series 6.00 6.00 12.00
20 CONCENTRATE-1 6.00 6.00 12.00
21 MX in MX 1 0.02 6.00 6.02
22 MIX-1 0.02 6.00 6.02
23 SWRO in RO 2 0.08 6.00 6.08
24 CONCENTRATE-1 0.08 6.00 6.08
25 UF 5kDa in UF 1 1.00 7.00 8.00
26 CONCENTRATE-1 1.00 7.00 8.00
27 UF 1kDa in UF 2 2.00 8.00 10.00
28 CONCENTRATE-1 2.00 8.00 10.00
29 BWRO in RO 1 6.00 10.00 16.00
30 CONCENTRATE-1 6.00 10.00 16.00
1
8
16
24
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (6.00 h)
MIX-1 (0.02 h)
CONCENTRATE-1 (0.08 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (2.00 h)
CONCENTRATE-1 (6.00 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (6.00 h)
MIX-1 (0.02 h)
CONCENTRATE-1 (0.08 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (2.00 h)
CONCENTRATE-1 (6.00 h)
For Help, press F1

(B)


Operations Gantt Chart (2 Batches)
File Edit Update Chart View Preferences
Zoom To Zoom by Detail Level
Task | Duration (h) | Start Time (h) | End Time (h) | Descr
1 Complete Recipe 8.08 0.00 8.08
2 MF 2 micron in MF 1.00 0.00 1.00 Micro
3 CONCENTRATE-1 1.00 0.00 1.00 Filter
4 BWRO_series in RO-1_series 4.00 0.00 4.00 Revers
5 CONCENTRATE-1 4.00 0.00 4.00 Filter
6 P-1 in MX-101 1.00 0.00 1.00 Mixin
7 MIX-1 1.00 0.00 1.00 Mix fl
8 UF 5kDa in UF 1 1.00 1.00 2.00 Ultrafi
9 CONCENTRATE-1 1.00 1.00 2.00 Filter
10 UF 1kDa in UF 2 2.00 2.00 4.00 Ultrafi
11 CONCENTRATE-1 2.00 2.00 4.00 Filter
12 BWRO in RO 1 4.00 4.00 8.00 Revers
13 CONCENTRATE-1 4.00 4.00 8.00 Filter
14 SWRO in RO 2 0.08 8.00 8.08 Revers
15 CONCENTRATE-1 0.08 8.00 8.08 Filter
16 Complete Recipe (Batch #2) 8.08 4.00 12.08
17 MF 2 micron in MF 1.00 4.00 5.00 Micro
18 CONCENTRATE-1 1.00 4.00 5.00 Filter
19 BWRO_series in RO-1_series 4.00 4.00 8.00 Revers
20 CONCENTRATE-1 4.00 4.00 8.00 Filter
21 P-1 in MX-101 1.00 4.00 5.00 Mixin
22 MIX-1 1.00 4.00 5.00 Mix fl
23 UF 5kDa in UF 1 1.00 5.00 6.00 Ultrafi
24 CONCENTRATE-1 1.00 5.00 6.00 Filter
25 UF 1kDa in UF 2 2.00 6.00 8.00 Ultrafi
26 CONCENTRATE-1 2.00 6.00 8.00 Filter
27 BWRO in RO 1 4.00 8.00 12.00 Revers
28 CONCENTRATE-1 4.00 8.00 12.00 Filter
29 SWRO in RO 2 0.08 12.00 12.08 Revers
30 CONCENTRATE-1 0.08 12.00 12.08 Filter
1
8
16
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (4.00 h)
MIX-1 (1.00 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (2.00 h)
CONCENTRATE-1 (4.00 h)
CONCENTRATE-1 (0.08 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (4.00 h)
MIX-1 (1.00 h)
CONCENTRATE-1 (1.00 h)
CONCENTRATE-1 (2.00 h)
CONCENTRATE-1 (4.00 h)
CONCENTRATE-1 (0.08 h)
For Help, press F1

(C)

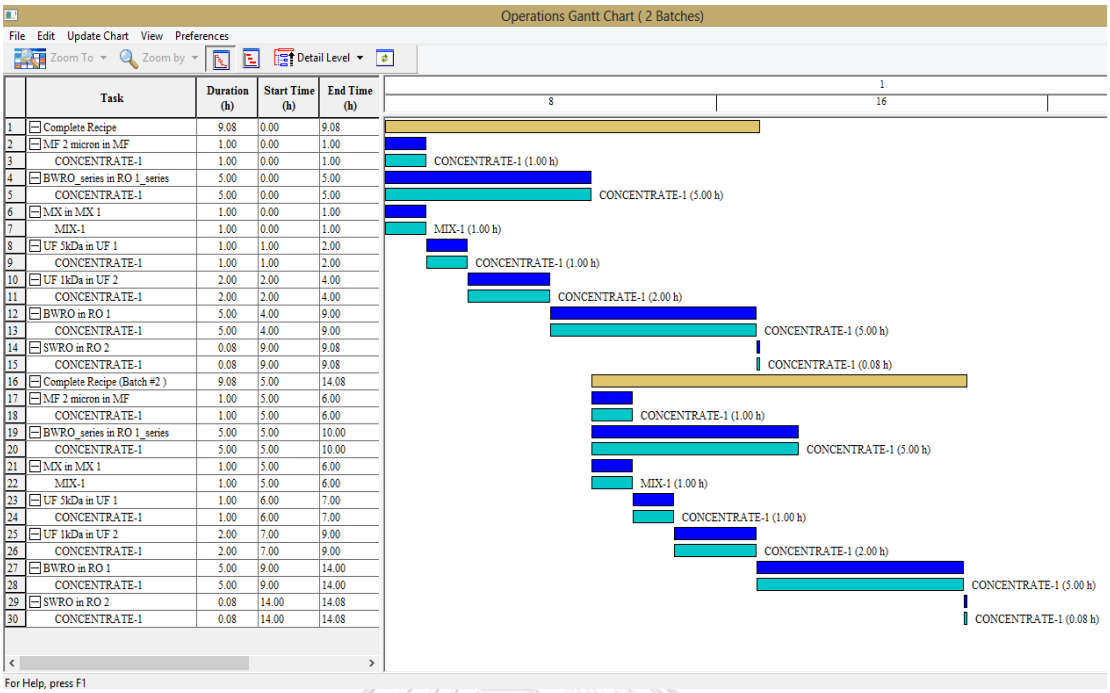


Figure I.3 Recipe Gantt chart for the in-parallel membrane based process with different feed streams. (A) CaLAC broth; (B) NaLAC broth; and (C) NH4LAC broth.

APPENDIX J: COST BREAKDROWN

Table J.1 Cost breakdown for CaLAC broth; (A) based case; (B) Membrane based; and (C) In-parallel membrane.

(A)

SUMMARY PER COST ITEM (Entire Process)				
Cost Item	**$/kg MP**	**$/batch**	**$/year**	**%**
Raw Materials	0.12	9	12,327	10.28
Labor	0.26	20	25,817	21.54
Consumables	0.47	36	47,176	39.36
Utilities	0.35	26	34,547	28.82
TOTAL	1.20	91	119,868	100.00

(B)

SUMMARY PER COST ITEM (Entire Process)				
Cost Item	**$/kg MP**	**$/batch**	**$/year**	**%**
Raw Materials	0.10	8	10,334	10.87
Labor	0.53	40	53,090	55.83
Consumables	0.20	15	20,282	21.33
Utilities	0.11	9	11,385	11.97
TOTAL	0.95	72	95,091	100.00

(C)

SUMMARY PER COST ITEM (Entire Process)				
Cost Item	**$/kg MP**	**$/batch**	**$/year**	**%**
Raw Materials	0.07	5	6,969	8.05
Labor	0.62	47	62,008	71.63
Consumables	0.11	8	10,675	12.33
Utilities	0.07	5	6,919	7.99
TOTAL	0.87	66	86,572	100.00

Table J.2 Cost breakdown for NaLAC broth; (A) based case; (B) Membrane based; and (C) In-parallel membrane.

(A)

SUMMARY PER COST ITEM (Entire Process)				
Cost Item	**$/kg MP**	**$/batch**	**$/year**	**%**
Raw Materials	0.28	14	27,697	18.90
Labor	0.39	20	38,745	26.44
Consumables	0.49	25	48,676	33.22
Utilities	0.31	16	31,417	21.44
TOTAL	1.47	74	146,536	100.00

(B)

SUMMARY PER COST ITEM (Entire Process)				
Cost Item	**$/kg MP**	**$/batch**	**$/year**	**%**
Raw Materials	0.22	11	22,306	21.43
Labor	0.58	29	58,118	55.84
Consumables	0.15	8	15,195	14.60
Utilities	0.08	4	8,451	8.12
TOTAL	1.04	53	104,071	100.00

(C)

SUMMARY PER COST ITEM (Entire Process)				
Cost Item	**$/kg MP**	**$/batch**	**$/year**	**%**
Raw Materials	0.17	9	17,090	16.95
Labor	0.58	29	58,118	57.65
Consumables	0.16	8	15,982	15.85
Utilities	0.10	5	9,617	9.54
TOTAL	1.01	51	100,807	100.00

Table J.3 Cost breakdown for NH_4LAC broth; (A) based case; (B) Membrane based; and (C) In-parallel membrane.

(A)

SUMMARY PER COST ITEM (Entire Process)				
Cost Item	**$/kg MP**	**$/batch**	**$/year**	**%**
Raw Materials	0.19	12	18,828	17.65
Labor	0.31	20	30,989	29.05
Consumables	0.33	21	32,703	30.66
Utilities	0.24	15	24,158	22.65
TOTAL	1.07	67	106,678	100.00

(B)

SUMMARY PER COST ITEM (Entire Process)				
Cost Item	**$/kg MP**	**$/batch**	**$/year**	**%**
Raw Materials	0.14	9	14,016	17.61
Labor	0.47	29	46,512	58.43
Consumables	0.12	8	12,166	15.28
Utilities	0.07	4	6,909	8.68
TOTAL	0.80	50	79,603	100.00

(C)

SUMMARY PER COST ITEM (Entire Process)				
Cost Item	**$/kg MP**	**$/batch**	**$/year**	**%**
Raw Materials	0.11	7	10,970	14.01
Labor	0.47	29	46,512	59.40
Consumables	0.13	8	12,821	16.37
Utilities	0.08	5	8,001	10.22
TOTAL	0.78	49	78,304	100.00

VITA

Miss Natnirin phanthumchinda was born on November 8th, 1984 in Prachinburi, Thailand. She graduated with the Bachelor's degree in food engineering from King Mongkut's Institute of Technology Ladkrabang and Master's degree in industrial engineering from Chulalongkorn University, Thailand in 2007 and 2009, respectively. Then, she was working for Incyam Company Limited as project engineer and technical researcher for 4 years. In 2012, she started Ph.D. in Biotechnology Program, Faculty of Science Chulalongkorn University.

Academic Publications

1. Phanthumchinda N, Rampai T, Prasirtsak B, Thitiprasert S, Tanasupawat S, Assabumrungrat S, Thongchul N. 2017. Alternative reverse osmosis to purify lactic acid from a fermentation broth. Chemical Industry & Chemical Engineering Quarterly 2017

2. Phanthumchinda N, Thitiprasert S, Tanasupawat S, Assabumrungrat S, Thongchul N. 2017. Process and cost modeling of lactic acid recovery from fermentation broths by membrane-based process. (Submitted)