การแยกกรดแลกติกจากน้ำหมักโดยเรซินแลกเปลี่ยนไอออน และอิเล็กโทรไดแอลิซิส

นางสาววาษิณี บุญคง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปีการศึกษา 2549 ISBN 974-14-2530-9 ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

SEPARATION OF LACTIC ACID FROM FERMENTATION BROTH BY ION - EXCHANGE RESIN AND ELECTRODIALYSIS

Miss Wasinee Boonkong

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science Program in Petrochemistry and Polymer Science Faculty of Science

Chulalongkorn University

Academic Year 2006 ISBN 974-14-2530-9 Copyrigth of Chulalongkorn University

Thesis Title	Separation of lactic acid from fermentation broth by ion-
	exchange resin and electrodialysis
Ву	Ms. Wasinee Boonkong
Field of Study	Petrochemical and Polymer science
Thesis Advisor	Associate Professor Polkit Sangvanich, Ph.D.
Thesis Co-advisor	Dr.Nuttha Thongchul

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

Callent Mero

(Professor Piamsak Menasveta, Ph.D.)

THESIS COMMITTEE

.Chairman

(Professor Pattarapan Prasassarakich, Ph.D.)

(Associate Professor Polkit Sangvanich, Ph.D.)

NUttha Mongella Thesis Co-advisor

(Dr.Nuttha Thongchul)

Member

(Associate Professor Amorn Petsom, Ph.D.)

(Assistant Professor Warinthorn Chavasiri, Ph.D.)

วาษิณี บุญคง: การแยกกรดแลกติกจากน้ำหมักโดยเรซินแลกเปลี่ยนไอออนและอิเล็กโทร ใดแอลิซิส (SEPARATION OF LACTIC ACID FROM FERMENTATION BROTH BY ION - EXCHANGE RESIN AND ELECTRODIALYSIS) อาจารย์ที่ปรึกษา: รศ.คร. พลกฤษณ์ แสงวณิช, อาจารย์ที่ปรึกษาร่วม: คร.ณัฏฐา ทอง จุล 92 หน้า. ISBN 974-14-2530-9

กรดแลกติกมีการนำมาใช้อย่างกว้างขวางในอุตสาหกรรมอาหาร อุตสาหกรรมยา และ อุสาหกรรมเครื่องสำอาง ในปัจจุบัน ปริมาณกรดแลกติกในตลาดโลกได้มีปริมาณเพิ่มขึ้น เนื่องมาจากมีการค้นพบพอลิแลกติกแอซิด ซึ่งเป็นพอลิเมอร์ที่สามารถย่อยสลายได้เองตาม ธรรมชาติ สำหรับการผลิตกรดแลกติกได้มาจากกระบวนการหมักผลิตผลทางการเกษตร โดยใช้ แบคทีเรีย หรือเชื้อราในการหมัก โดยการใช้เชื้อราในการหมักนั้นจะทำให้ได้กรดแอล(+)แลกติก เพียงไอโซเมอร์เดียว ซึ่งการผลิตพอลิแลกติกแอซิด จำเป็นต้องใช้กรดแอล(+)แลกติกในการผลิต

ดังนั้นในงานวิจัยนี้จึงทำการศึกษาและเปรียบเทียบเทคนิคการแยกกรดแลกติกจากน้ำหมักโดยวิธี เรซินแลกเปลี่ยนไอออนและวิธีอิเล็กโทรไดแอลิซิส โดยน้ำหมักที่นำมาแยกนั้นได้มาจากการหมัก โดยเชื้อรา ซึ่งในน้ำหมักประกอบไปด้วย กรดแลกติก กรดฟูมาริก เอทานอล และกลูโคส โดย Dowex Marathon WBA จะให้การแยกกรดแลกติกดีกว่าเมื่อเทียบกับ Amberlite IRA-400 และ Amberlite XAD-7 ซึ่งการดูดซับเป็นแบบ Langmuir isotherm ซึ่งสภาวะที่เหมาะสมสำหรับการ

ดูดซับกรดแลกติกจากน้ำหมักคือ ค่าความเป็นกรดค่างที่ 6.0 โดยใช้อัตราการไหลของน้ำหมัก 0.8 มิลลิลิตรต่อนาที ชะกรดแลกติกออกมาด้วยสารละลายผสมของกรดฟอสฟอริกเข้มข้น 1 โมลาร์ และ กรดซัลฟูริก 1 โมลาร์ ที่อัตราส่วน 70:30 อัตราไหล 0.3 มิลลิลิตรต่อนาที สำหรับ กระบวนการอิเล็กโทรไดแอลิซิส ได้ทำการสร้างโมดูลระดับห้องปฏิบัติการ มีพื้นที่การใช้งานของ เยื่อแผ่น 2.925 × 10⁻³ ตารางเมตร ปัจจัยที่นำมาศึกษาได้แก่ ก่าความเข้มข้นของสารป้อน ก่าความ เป็นกรดค่าง ศักย์ไฟฟ้า และอัตราการไหลของน้ำหมัก โดยภายใต้ภาวะที่เหมาะสมจะให้ มีค่าร้อย ละการคืนกลับเท่ากับ 92 ร้อยละความบริสุทธิ์เท่ากับ 100 พลังงานไฟฟ้าจำเพาะที่ใช้ในการแยก 0.6122 กิโลวัตต์.ชั่วโมงต่อกิโลกรัม

 # # 477 25883 23: MAJOR PETROCHEMISTRY AND POLYMER SCIENCE KEYWORDS: LACTIC ACID/ SEPARATION/ ELECTRODIALYSIS ION-EXCHANGE/ FERMENTATION BROTH.

WASINEE BOONKONG: SEPARATION OF LACTIC ACID FROM FERMENTATION BROTH BY ION - EXCHANGE RESIN AND ELECTRODIALYSIS. THESIS ADVISOR: ASSOC.PROF. POLKIT SANGVANICH, Ph.D., THESIS CO-ADVISOR: NUTTHA THONGCHUL, Ph.D 92 pp. ISBN 974-14-2530-9

Lactic acid has long been widely used in food, pharmaceutical, and cosmetic industries. Currently, the worldwide market is increased due to the discovery of the biodegradable polymer (polylactic acid). Lactic acid is commercially produced by fermentation of agricultural products by lactic acid bacteria or filamentous fungi. Fermentation by filamentous fungi yields optically pure L-lactic acid which is strictly required for polylactic acid synthesis. In this work, we studied and compared L-lactic acid separation from filamentous fungal fermentation broth, containing L-lactic acid, byproducts including fumaric acid, and ethanol, and the remaining glucose, using ionexchange chromatography and electrodialysis. Compared with Amberlite IRA-400 and Amberlite XAD-7, Dowex Marathon WBA gave better lactic acid separation. The adsorption equilibrium followed Langmuir isotherm. The optimal condition of lactic acid adsorption was at pH 6.0, and 0.8 mL/min while the optimal condition of the desorption process was at 0.3 mL/min using mixture of 1.0 M phosphoric acid and 1.0 M sulfuric acid at the ratio of 70:30 as an eluant. The final lactic acid recovery was 76% with 90% purity. A laboratory scale single-stage electrodialysis apparatus was constructed with effective membrane area of 2.925×10^{-3} m². The effects of feed solution concentration, flow rate, pH of the fermentation broth, and applicable voltage were studied. Under the optimal condition, lactic acid recovery was 92% with 100% purity and the specific energy consumption of 0.6122 kWh/kg

ACKNOWLEDGEMENTS

I would like to express my appreciation to my advisor Assoc. Prof. Dr. Polkit Sangvanich for his kind supervision, instruction and encouragement during my research. I would also like to thank my co-advisor Dr.Nuttha Thongchul for her help and advise. I would like to thank Prof. Dr. Pattarapan Prasassarakich, Assoc. Prof. Dr. Amorn Petsom, Assist. Prof. Dr Warinthorn Chavasiri for their valuable suggessions and comments as committee members.

In addition, I wish to thank the Institute of Biotechnology and Genetic Engineering for all facilities and grant. Appreciation is also extended to the Graduate School of Chulalongkorn University for partial granting support to conduct this research.

I appreciate the assisitance from Miss Sitanan Thitiprasert for the fermentation broth, the National Center for Genetic Engineering and Biotechnology for YSI equipment and the electrodialysis apparatus from Mr. Sakchai Chanhong.

Special thanks also give to all staffs of Institute of Biotechnology and Genetic Engineering for valuable friendship and help as well as all of my friends, especially Miss Kesada, Miss Paweena, Miss Supansa, Miss Udomporn, Miss Parichart and my younger sisters in laboratory for their encouragement.

Finally, I wish to express my deep sense of appreciation to my beloved family for their inspiration, understanding, great support and encouragement throughout my study.

CONTENTS

ABSTRACT (IN THAI) ir		
ABSTRACT (IN ENGLISH)		v
ACK	NOWLEDGEMENTS	vi
CON	TENTS	vii
LIST	OF TABLES	х
LIST	OF FIGURES	xii
LIST	OF SCHEMES	XV
LIST	OF ABBREVIATIONS AND SYMBOLS	xvi
CHA	PTER I INTRODUCTION	1
1.1	Lactic acid	1
1.2	Objectives and scope of Research	3
CHA	PTER II THEORETICAL AND LITERATURE REVIEWS	5
2.1	Ion exchange chromatography	5
	2.1.1 Equilibrium Relations for Adsorbents	6
	2.1.2 Adsorption isotherm can be classified in to 4 types	7
	2.1.3 Adsorption process	8
	2.1.4 Breakthrough curve	10
	2.1.5 Mass transfer zone	11
2.2	Electrodialysis	12
	2.2.1 Ion Transport	14
	⁹ 2.2.2 Restriction of electrodialysis	15
	2.2.3 Mode of operating	16
	2.2.4 The performance of an electrodialysis	18
	2.2.5 Flux of solute (J _s)	19
	2.2.6 Specific energy consumption (E)	19

Page

CHAPTER III EXPERIMENTAL		21
3.1	Materials	21
3.2	Equipments	21
3.3	Methodology	22
	3.3.1 L-Lactic acid fermentation from D-glucose	22
	3.3.2 Shake flask culture	23
	3.3.3 Adsorption of L-lactic acid by anion-exchange resin	23
	3.3.3.1 Batch adsorption	23
	3.3.3.2 Fixed-bed adsorption	24
	3.3.3 Desorption	25
	3.3.3.4 L-lactic acid recovery in fixed-bed column	26
	3.3.4 Electrodialysis	26
	3.3.4.1 Apparatus	26
	3.3.4.2 Operating conditions	28
	3.3.5 Analytical method	29
	3.3.5.1 High performance liquid chromatography	29
	3.3.5.2 Glucose-lactate analyzer	29
	3.3.5.3 Titration	29
CHAI	PTER IV RESULTS AND DISCUSSION	30
4.1	Lactic acid fermentation broth	30
4.2	Ion exchange chromatography	30
	4.2.1 Adsorption process	30
	4.2.1.1 Batch adsorption	30
	4.2.1.1.1 Equilibrium time	30
	4.2.1.1.2 Equilibrium time of Dowex Marathon WBA	35
	4.2.1.1.3 Adsorption isotherm	36
	4.2.1.2 Fixed – bed adsorption	38
	4.2.1.2.1 Maximum flow rate	38
	4.2.1.2.2 Breakthrough curve	38
	4.2.2 Desorption process	39

4.2.2.1 The effect of eluants to lactic acid recovery	39
4.2.2.2 L-lactic acid recovery in fixed-bed column	40
4.3 Electrodialysis	41
4.3.1 Effects of voltage	41
4.3.2 Effects of ratio of lactic acid to sodium lactate	41
4.3.3 Effect of flow rate	42
4.3.4 Effect of pH	43
4.3.5 Specific Energy consumption	44
4.3.6 Comparison of specific energy consumption with previous works	45
CHAPTER V CONCLUSIONS AND RECOMMENDATIONS	46
5.1 Conclusions	46
5.1.1 Ion exchange resin	46
5.1.2 Electrodialysis	47
5.2 Recommendations	47
REFERENCES	48
APPENDICES	51
VITA	92

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

LIST OF TABLES

Table

3.1	Operating conditions	28
4.1	Concentration of lactic acid and by products at pH 5.41	29
4.2	L-lactic acid recovery in shake flask	39
4.3	L-lactic acid recovery in fixed-bed column	40
4.4	Amount of separation, J _s and specific energy consumption of condition 1-3	41
4.5	Amount of separation, J _s and specific energy consumption of condition 4-6	42
4.6	Amount of separation, J _s and specific energy consumption of condition 7-9	42
4.7	Amount of separation, J _s and specific energy consumption of condition 10-12	43
4.8	Amount of separation and specific energy consumption of condition 1-9.	44
4.9	Comparison of specific energy consumption with previous works	45
C1	Lactic acid adsorbed on resin Amberlite XAD-7 : pH 3 ;Ratio resin to starting solution : 1:1	58
C2	Lactic acid adsorbed on resin Amberlite XAD-7 : pH 3 ;Ratio resin to starting solution : 2:1	58
C3	Lactic acid adsorbed on resin Amberlite XAD-7 : pH 3 ;Ratio resin to starting solution : 2:2	58
C4	Lactic acid adsorbed on resin Amberlite XAD-7 : pH 4.5 ;Ratio resin to starting solution : 1:1	58

Page

Table	3	Page
C5	Lactic acid adsorbed on resin Amberlite XAD-7 : pH 4.5 ;Ratio resin to starting solution : 2:1	59
C6	Lactic acid adsorbed on resin Amberlite XAD-7 : pH 4.5 ;Ratio resin to starting solution : 2:2	59
C7	Lactic acid adsorbed on resin Amberlite XAD-7 : pH 6.0 ;Ratio resin to starting solution : 1:1	60
C8	Lactic acid adsorbed on resin Amberlite XAD-7 : pH 6.0 ;Ratio resin to starting solution : 2:1	60
C9	Lactic acid adsorbed on resin Amberlite XAD-7 : pH 6.0 ;Ratio resin to starting solution : 2:2	60
C10	Lactic acid adsorbed on resin Amberlite IRA-400 : pH 3;Ratio resin to starting solution : 1:1	61
C11	Lactic acid adsorbed on resin Amberlite IRA-400 : pH 3;Ratio resin to starting solution : 2:1	61
C12	Lactic acid adsorbed on resin Amberlite IRA-400 : pH 3;Ratio resin to starting solution : 2:2	61
C13	Lactic acid adsorbed on resin Amberlite IRA-400 : pH 4.5;Ratio resin to starting solution : 1:1	62
C14	Lactic acid adsorbed on resin Amberlite IRA-400 : pH 4.5;Ratio resin to starting solution : 2:1	62
C15	Lactic acid adsorbed on resin Amberlite IRA-400 : pH 4.5;Ratio resin to starting solution : 2:2	62
C16	Lactic acid adsorbed on resin Amberlite IRA-400 : pH 6.0;Ratio resin to starting solution : 1:1	63

Table	e	Page
C17	Lactic acid adsorbed on resin Amberlite IRA-400 : pH 6.0;Ratio resin to starting solution : 2:1	63
C18	Lactic acid adsorbed on resin Amberlite IRA-400 : pH 6.0;Ratio resin to starting solution : 2:2	63
C19	Lactic acid adsorbed on resin Dowex Marathon WBA : pH 3;Ratio resin to starting solution : 1:1	. 64
C20	Lactic acid adsorbed on resin Dowex Marathon WBA : pH 3;Ratio resin to starting solution : 2:1	. 64
C21	Lactic acid adsorbed on resin Dowex Marathon WBA : pH 3;Ratio resin to starting solution : 2:2	. 64
C22	Lactic acid adsorbed on resin Dowex Marathon WBA : pH 4.5;Ratio resin to starting solution : 1:1	65
C23	Lactic acid adsorbed on resin Dowex Marathon WBA : pH 4.5;Ratio resin to starting solution : 2:1	65
C24	Lactic acid adsorbed on resin Dowex Marathon WBA : pH 4.5;Ratio resin to starting solution :2:2	. 65
C25	Lactic acid adsorbed on resin Dowex Marathon WBA : pH 6.0;Ratio resin to starting solution : 1:1	. 66
C26	Lactic acid adsorbed on resin Dowex Marathon WBA : pH 6.0;Ratio resin to starting solution : 2:1	. 66
C27	Lactic acid adsorbed on resin Dowex Marathon WBA : pH 6.0;Ratio resin to starting solution : 2:2	66
C28	Amount of lactic acid desorption in shack flask	67

Table Page 201		Page
C29	Amount of lactic acid adsorption and desorption with 1.0 M NaCl	68
C30	Amount of lactic acid adsorption and desorption with 1.0 M HCl	69
C31	Amount of lactic acid adsorption and desorption with 1.0 M H ₃ PO ₄	70
C32	Amount of lactic acid adsorption and desorption with 1.0 M H ₂ SO ₄	71
C33	Amount of lactic acid adsorption and desorption with $1.0 \text{ M H}_3\text{PO}_4 + 1.0 \text{ M H}_2\text{SO}_4$:70:30	72
D1	Electrodialysis results: Operating condition 1	75
D2	Electrodialysis results: Operating condition 2	76
D3	Electrodialysis results: Operating condition 3	77
D4	Electrodialysis results: Operating condition 4	78
D5	Electrodialysis results: Operating condition 5	79
D6	Electrodialysis results: Operating condition 6	80
D7	Electrodialysis results: Operating condition 7	81
D8	Electrodialysis results: Operating condition 8	82
D9	Electrodialysis results: Operating condition 9	83
D10	Electrodialysis results: Operating condition 10	84
D11	Electrodialysis results: Operating condition 11	85
D12	Electrodialysis results: Operating condition 12	86
E1	Amount of adsorption and desorption of fixed-bed adsorption with 1.0 M	
	$H_3PO_4 + 1.0 \text{ M } H_2SO_4 : 70:30$	89

LIST OF FIGURES

FigurePa		Page
1.1	Scope of research	4
2.1	Mechanism of ion exchange	6
2.2	Three common types of adsorption isotherms	7
2.3	Concentration profiles for adsorption in fixed bed (a) profiles at various positions and times in the bed, (b) breakthrough concentration profile in fluid at the outlet of bed	10
2.4	Diagram of a typical ED cell	13
2.5	Ion transport	14
2.6	Concentration polarization	15
2.7	Batch mode	16
2.8	Continuous mode	17
2.9	Feed and bleed mode	18
3.1	Concentration of residual resin	24
3.2	Breakthrough curve	25
3.3	Auto collecting sample from column	26
3.4	The laboratory scale electrodialysis unit	27
3.5	Schematic of electrodialysis	27
4.1	Adsorbed lactic acid on Amberlite XAD-7 at pH 3.0, 4.5, and 6.0 with different ratios (a) 0.1g:2.5 mL ; (b) 0.2 g:2.5 mL ; (c) 0.2 g:5 mL	31
4.2	Adsorbed lactic acid on Dowex Marathon WBA at pH 3.0, 4.5, and 6.0 with different ratios (a) 0.1g:2.5 mL ; (b) 0.2 g:2.5 mL ; (c) 0.2 g:5 mL	32
4.3	Adsorbed lactic acid on Amberlite IRA-400 at pH 3.0, 4.5, and 6.0 with different ratios (a) 0.1g:2.5 mL ; (b) 0.2 g:2.5 mL ; (c) 0.2 g:5 mL	33

Figure

4.4	Concentration of residual lactic acid on Dowex Marathon WBA	35
4.5	Adsorption isotherm of lactic acid by Dowex Marathon WBA at pH 6.0	36
4.6	Relation of 1/q and 1/c plot of lactic acid by Dowex marathon WBA at	
	рН 6.0	37
4.7	Breakthrough curve of lactic acid in Dowex Marathon WBA resin column	38
B1	The HPLC chromatogram of standard solution of glucose, lactic acid,	
	fumaric acid, and ethanol	56
C1	The HPLC chromatogram of lactic acid from ion-exchange resin	
	purification	73
D1	The HPLC chromatogram of lactic acid from electrically sis purification	87



Page

LIST OF ABBREVIATIONS AND SYMBOLS

°C	celsius degree
kg	kilogram
mm	millimeter
m ³	qubic meter
cm	centimeter
cm^2	square meter
М	molar
μL	micro-liter
mL	milli-liter
L	liter
V	volt
W	watt
t	time
Κ	dissociation constant
q	adsorption capacity
q_0	maximum adsorption capacity
LA	lactic acid
DC	direct current
ED	electrodialysis
CS	concentrating Stream
DS	diluting Stream
CE	current efficiency
i	current density
F	Faraday's constant
N	molar flux
V_c	volume of concentrate solution
C _c	salt (sodium lactate) concentration in the concentrate solution
J_s	flux of lactate
Δm_s	mass of lactate through the membrane
A _m	area of membrane
Δt	time

- ε specific energy consum
- Φ voltage
- I current
- rpm round per minute
- HPLC high performance liquid chromatography
- RI refractive index
- t_B breakthrough time
- t_E exhaustion time



สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER I

INTRODUCTION

1.1 Lactic acid

Lactic acid is widely used in the food industry as an acidulant in food and beverages manufacturing. It is also used as preservative in the production of beer, jelly, cheese, dried egg white and other food products. It is also used in plastic, leather tanning woolen dyeing and pharmaceutical formulation as a mild acidulant. To date, many attemps have focused on biodegradable. Polylactic acids have a wide range application. For example, it can be used for making clothes, wipes, carpet tiles, diapers, feminine hygiene products, upholstery, interior and exterior furniture, filtration, and agricultural products.

Currently, lactic acid market depends on not only the existing applications in food and pharmaceutical. Each year, world wide market for biodegradable plastics grows by more than 20 percent; thus, substantially increasing the demand for lactic acid. The worldwide consumption rates of lactic acid are approximately 130,000 to 150, 000 tons per year in 1999. By the end of 2011, the global demand for lactic acid is expected to reach 200,000 tons [www.chemsystem.com].

Lactic acid can be produced by chemical synthesis or by fermentation. Commercially, chemical synthesis of lactic acid is produced by the hydrolysis of lactonitrile. Lactonitrile is obtained by hydrogen cyanide reacted with acetaldehyde in the presence of an alkali. The chemical synthesis produces a racemic mixture of lactic acid. Two companies, Musashino, Japan and Sterling Chemicals Inc., USA, are using this technology to produce lactic acid (Narayanan *et al.* 2004).

Lactic acid can be also produced by bacterial and fungal fermentation. Lactic bacteria, *Lactobacilli*, have been extensively used in lactic acid fermentation because they can synthesize the optical isomers of lactic acid at a high production rate. The filamentous fungus, *Rhizopus* is an obligate aerobe that is often used for industrial production of optically pure L-(+)-lactic acid. The advantages of using

Rhizopus species as an alternative to lactic acid bacteria include use of inexpensive raw materials and production of optically pure L(+) lactic acid. Since pure stereo isomer of lactic acid is strictly required for biodegradable polylactic acid production, production of lactic acid by fungal fermentation eases the purification process by omitting the separation of stereo isomers compared to lactic acid obtained fermentation

Lactic acid can be separated and substantially purified from fermentation broth by various separation techniques such as reactive extraction (Jarvinen *et al.* 2000), membrane separation (Persson *et al.* 2001), ion exchange (Sosa *et al.* 2000), electrodialysis (Lee *et al.* 1998), chemical reaction distillation (Choi and Hong 1999), and reverse osmosis (Timmer *et al.* 1993). Depending on the nature of fermentation broth and the use of lactic acid, particular technique provides different advantages and disadvantages. The disadvantages of reactive extraction are use of toxic solvent and contaminated recovery products. The membrane separation has a major problem in fouling of particles from fermentation broth. The chemical reaction distillation is a time consuming process and uses high energy consumption for separation.

Ion exchange technique has been widely used in bioseparation (Houwing *et al.* 2002; Tong *et al.* 2001). It has many advantages; it provides single step separation at high recovery and purity percentage (Tong *et al.* 2004). Ion exchange processes are the very low running costs. Very little energy is required, the regenerant chemicals are cheap and if well maintained resin beds can last for many years before replacement is needed.

Electrodialysis is one of very promising and perspective methods provide by the rapid development of membrane process. With the technique, high concentration of fermentation broth can be fed into the process to obtain high recovery and purity percentage, while normally in typical membrane separation, feeding highly concentrated solution leads to concentration polarization while eventually (Thang *et al.* 2004).

In this study, lactic acid was separated and purification from fermentation broth produced by filamentous fungus *Rhizopus*. In fact, the fermentation broth of *Rhizopus* consist of lactic acid and byproducts including fumaric acid, and ethanol, and the remaining glucose as the raw material depending on the fermentation condition. 2 techniques were selected to purity lactic acid from broth; those were ion exchange and electrodialysis according to the advantages discussed prior. One-step separation processes using these 2 techniques were compared in term of recovery and purity percentages, cost of materials, and energy consumption. The preliminary results obtained to be used for a large scale separation process of L-lactic acid.

1.2 Objectives and scope of Research

The aim of this study was to improve the efficient of separation and purification L(+)-lactic acid from the fermentation broth. Two separation techniques, anion exchange and electrodialysis were conducted in this study to determine the feasible technique for L-lactic separation. The scope of this work is summarized in Figure 1.1.





Figure 1.1 Scope of research

CHAPTER II

THEORETICAL AND LITERATURE REVIEWS

2.1 Ion exchange chromatography

Separation in ion exchange chromatography depends upon the reversible adsorption of charged solute molecules to immobilized ion exchange groups of opposite charge.

Ion exchangers are cross-linking polymeric resins. It consists of am insoluble matrix to which charged groups have been covalently bound. The charged groups are associated with mobile counter ions. These counter ions can be reversibly exchanged with other ions of the same charge without altering the matrix. It is possible to have both positively and negatively charged exchangers. Positively charged exchangers have negatively and charged counter ions (anions) available for exchange, so called anion exchangers. Negatively charged exchangers have positively charged counter ion (cations), so called cation exchanger. Ion exchange occurs at the active sites where the charged groups are covalently bound and this phenomenon depends on the relative concentration and favorability of the counter ions in solution and the affinity and the number of the active sites. The number of active sites can be tropically increased by increasing the surface area of the resins.

Cross-linking, usually on the order of 0.5 to 15 percent of weight, comes from the addition of divinyl benzene to the reaction mixture during resin production step. The size of the particles also plays a role in the utility of the resin. Small particles are usually more effective because of the increase in surface area but causing large head losses which drives up pump working load equipment and energy consumption. Temperature and pH also affect the effectiveness of ion exchange. pH is inherently correlated to the number of ions available for exchange. Where temperature governs the kinetics of the process; however, the role of the temperature and the rate-limiting step has yet been clearly determined.

Regeneration is also an important feature of ion exchanger. The resin is flushed with another newly exchanged ion in solution in order to remove and substitute the previous occupied ions on the exchanger. Regeneration is usually performed after most of the active sites have been occupied and the ion exchange is no longer effective. By regeneration, the same resin beads can be reused over the period of time, and the desired ions can be obtained from the back wash effluent simultaneously.



Figure 2.1 Mechanism of ion exchange

2.1.1 Equilibrium Relations for Adsorbents

The equilibrium between the concentration of solute in the fluid phase and its concentration on the solid resemble somewhat the equilibrium solubility of gas in a liquid. Data are plotted as **adsorption isotherm** as shown in Figure 2.2. The concentration in the solid phase is expressed as q, kg adsorbate (solute)/ kg adsorbent (solid), and in the fluid phase (gas or liquid) as c, kg adsorbate/m³ fluid.





Figure 2.2 Three common types of adsorption isotherms.

2.1.2 Adsorption isotherm can be classified in to 4 types

1. Linear isotherm

Data that follow a linear law can be expressed by an equation similar to Henry's law;

$$q = Kc$$

where *K* is a constant determined experimentally, m^3/kg adsorbent. This linear isotherm is not common, but in the dilute region it can be used to approximate data for many system.

2. Langmuir isotherm

The Langmuir isotherm had a theoretical basis and is given by following, where q_0 and K are empirical constants:

$$q = \frac{q_0 c}{K + c}$$

Where q_0 is kg adsorbate/kg solid and K is kg/m³. The equation was derived assuming that there are only a fixed number of active sites available for adsorption, that only a monolayer is formed, and that the adsorption is reversible and reaches an

equilibrium condition. By ploting 1/q versus 1/c, the slope is K/q_0 and intercept is $1/q_0$.

3. Freundlich isotherm

The Freundlich isotherm equation, which is empirical, often approximates data for many physical adsorption systems and is particularly usedful for liquid;

$$q = Kc^n$$

Where K and n are constants and must be determined experimentally. If a log-log plot is made for q versus c, the slope is the dimensionless exponent n. The dimensions of K depend on the value of n (Geankoplis 1993). This equation is sometimes used to correlate data for hydrocarbon gases on activated carbon.

4. Radke-Prausnitz

Data that follow Radke-Prausnitz isotherm is expressed by the following

$$1/q = 1/aC + K/bC^{\beta}$$
 $\beta < 1$

Where a low concentration of C; q = aC; and the adsorption is linear isotherm ;

a high concentration of C; $q = b C^{\beta}$; and the adsorption is Freundlich isotherm ;

at $\beta = 0$, $1/q = 1/aC + K/b C^{\beta}$; and the adsorption is Langmuir isotherm

Almost all adsorption system show that as temperature is in creased, the amount adsorbed by the adsorbent decreases strongly. This is useful since adsorption is normally at room temperatures and desorption can be attained by raising the temperature.

2.1.3 Adsorption process

1. Batch adsorption

Batch adsorption is often used to adsorb solutes from liquid solutions when the quantities treated are small in amount, as in the pharmaceutical or other industries. As with many other processes, an equilibrium relation such as the Freundlich or Langmuir isotherm and a material balance are needed. The initial feed concentration is c_F and the final equilibrium concentration is c. Also, the initial concentration of solute adsorbed on solid is q_F and the final equilibrium value is q. The material balance on adsorbate is

$$q_FM + c_FS = qM + cS$$

where M is the amount of adsorbent, kg; and S is the volume of feed solution, m^3 .

When the variable q in equation is plotted versus c, the result is a straight line. If the equilibrium isotherm is also plotted on the same graph, the intersection of both lines gives the final equilibrium values of q and c.

2. Fixed -bed adsorption column

The concentrations of the solute in the fluid phase and of the solid adsorbent phase change with time and also with position in fixed bed as adsorption proceeds. At the inlet to the bed, the solid assumed to contain no solute at the start of the process. As the fluid first contacts the inlet of the bed, most of the mass transfer and adsorption takes place here. As the fluid passes to the bed, the concentration in this fluid drops very rapidly with distance in the bed and reached to zero well before the end of the bed is reached. The concentration profile at the start at time t_1 is shown in figure 2.3 (a), where the concentration ratio c/c_0 is plotted versus bed length. The fluid concentration c_0 is the feed concentration and c is the fluid concentration at a point in the bed.

After a short time, the solid near the entrance to the tower is almost saturated, and most of the mass transfer and adsorption now takes place at a point slightly far from the inlet. At a later time t₂, the profile or mass-transfer zone where most of the concentration change takes place has moved farther down the bed. The concentration profiles shown are for the fluid phase. Concentration profiles for the concentration of adsorbates on the solid would be similar. The solid at the entrance would be nearly saturated and this concentration would remain almost constant down to the mass-transfer zone, where it would drop off rapidly to almost zero. The dashed line for time



Figure 2.3 Concentration profiles for adsorption in fixed bed (a) profiles at various positions and times in the bed, (b) breakthrough concentration profile in the fluid at the outlet of bed

2.1.4 Breakthrough curve

The breakthrough curve can be defined as the "S" shaped curve that typically results when the effluent adsorbate concentration is plotted against time or volume. The *breakthrough point* is the point on the breakthrough curve where the effluent adsorbate concentration reaches its maximum allowable concentration, which often corresponds to the treatment goal. The treatment goal is usually based on regulatory or risk based numbers.

2.1.5 Mass Transfer Zone

The mass transfer zone (MTZ) is the area within the adsorbate bed where adsorbate is actually being adsorbed on the adsorbent. As the concentration wave moves through the bed, most of the mass transfer is occurring in a fairly small region. This *mass transfer zone* moves down the bed until it "breaks through". The shape of the mass transfer zone depends on the adsorption isotherm (equilibrium expression), flow rate, and the diffusion characteristics. Usually, the shape must be determined experimentally.

The current uses of ion exchange are long and numerous. An application that can hit close to home is in the treatment of water for drinking, use (commercial, industrial, and residential), and wastewater treatment. Ion exchangers can soften the water, deionize it, and even be used in desalination. In industrial uses, pure water is often crucial for the successful development of a product. Preparation of various acids, bases, salts, and solutions is also aided by ion exchange. Analytical chemistry uses ion exchange in chromatography. The recovery of valuable metals is also possible with resins. Industrial drying of treatment of gases is accomplished often with ion exchange. The food industry uses ion exchange in a variety of ways, ranging from wine-making to sugar manufacture. In the medical world, dozens of important manifestations of the benefits of ion exchange can be found, from development and preparation of key drugs and antibiotics, such as streptomycin and quinine, to treatments for ulcers, TB, kidneys, and much more. Ion exchange is used to prevent coagulation in blood stores and in dextrose, as well. An ion exchange is also useful in death, as it plays a role in the treatment of formaldehyde.

Ion exchange can be used recovered many organic acids such as carboxylic acid. Carboxylic acid was separated by using a polymer adsorbent of pyridine skeletal structure and a cross-linked structure. The polymer adsorbent showed good selectivity and high adsorption capacity for carboxylic acids even in the presence of inorganic salts. The selected eluants were aliphatic alcohol, aliphatic ketone, and carboxylic ester (Kawabata *et al.*1982)

Ion exchange was applied for the recovery of L-(+)-lactic acid from fermentation broth. In 2001 Cao et al., recovered L-(+)-lactic acid from fermentation broth using amberlite IRA-400 anion exchange resin. Adsorption isotherm and breakthrough curves for the separation were obtained at pH 5.0 and 2.0, respectively. The isotherm was found to be a Langmuir type at pH 5.0, whereas the isotherm was type II (multilayer adsorption) at pH 2.0. At pH 5.0, the maximum adsorption capacity of the resin, q_m and dissociation constant, K_d were 222.46 mg/g wet resin and 60.7 mg/ml, respectively. Breakthrough curve for the separation of lactic acid was also obtained. The maximum adsorption capacity (197.09 mg/g wet resin) at pH 5.0 was much larger than that at pH 2.0 (106 mg/g wet resin). 1.0M H₂SO₄ could be used for the elution of lactic acid at pH 5.0 with high recovery. However, the total yield was 92.11% when the column separation was performed at pH 2.0 and water was used as eluant (Cao *et al.* 2002).

Tong et al. (2004) investigated the separation of lactic acid from the fermentation broth with paper sludge as a cellulosic feedstock using weak anion exchanger Amberlite IRA-92. Some factors such as flow rate, sample volume loaded, pH, and column were systematically examined to improve the purity, yield, and productivity in lactic acid purification. Adsorption isotherm of standard lactic acid and lactic acid in the fermentation broth by anion exchanger IRA-92 were also investigated. The results show that increasing pH of fermentation broth from 5.0 to 6.0 can significantly enhance lactic acid recovery yield, purity, and productivity. The decrease in flow rate and sample volume loaded can also improve the recovery yield and purity but apparently reduce the productivity. In addition, scaling up of purification process in laboratory scale slightly affected the recovery yield and purity. After optimization, the yield, purity, and productivity were approximately 82.6%, 96.2% and 1.16 g LA/ (g-resin dry), respectively.

2.2 Electrodialysis

Electrodialysis was modified from a conventional dialysis in the early 1900 by the addition of electrodes and direct current to increase the rate of dialysis in electrolyte solutions. Since 1940 membrane separation process has been introduced electrodialysis. Nowadays, Electrodialysis refers to an electrochemical separation process in which mineral salts and other ionic species are transported through ion selective membranes from one solution into another under the driving force of a direct current (DC) electrical potential.

The ion selective membranes play important roles in ED. The membranes which allow passage of positively charged ions (such as sodium and potassium) are called cation membranes. Whereas the membranes that allow passage of negatively charged ions (such as chloride and phosphate) are called anion membranes.

Cation membranes contain negatively charged groups such as sulfonate (SO₃⁻) which are chemically bonded to the polystyrene base. This produces a negatively charged grid which attracts positively charged ions and repels negatively charged ions. Anion membranes contain positively charged bonded groups such as a quaternary ammonium (NH₃⁺) compound. The resulting positively charged grid attracts anions and repels cations. Membrane is 0.2-0.5 mm in thickness and reinforced with screen to provide mechanical stability. Flat sheet membranes, containing 30 to 50% water, consists of a network of small pores in which water cannot transport.



Figure 2.4 Diagram of a typical ED cell

Figure 2.4 shows a schematic diagram of a typical ED cell consisting of series of anion and cation exchange membranes arranged in an alternating pattern between anode and cathode. A cell consists of feed and permeated compartments with two adjacent membranes. When an ionic feed solution such as an aqueous salt solution, is pumped through the cell pairs, ion transport does not occur as long as no direct is applied. When an ionic solution is pumped through cells which the electrical potential between anode and cathode the negatively charged ions migrate toward the anode. While the positively charged ions move toward the cathode, resulting in the ionic concentration increases in alternating compartments accompanied by a simultaneous decrease in ionic concentration in the other compartments. Consequently alternating diluted and concentrated solutions are obtained.



CS is Concentrating Stream DS is Diluting Stream

Figure 2.5 Ion transport

2.2.1 Ion transport

Ion transport

The total transport of ions through ion exchange membranes can be described by three phenomena, including convection, diffusion, and migration (Krol, 1997).

Convection results from fluid motion caused by an external such as mechanical driving force, density and temperature of solution.

Diffusion occurs in all solutions and arises from local uneven concentrations of reagents.

Migration occurs from electrostatic force which arises due the application of a voltage on the electrodes.

2.2.2 Restriction of electrodialysis

1. Concentration polarization

In electrodialysis it is desirable to operate at the highest practical current density in order to get the maximum ion flux per unit membrane area. Operating current level is, however, restricted by concentration polarization. This polarization results from the difference in the transport numbers in the solution, where cations and anions carry roughly equal amounts of current, and in the highly selective ion exchange membrane, where virtually all the current is carried by the counter ions. The difference in transport number leads to a situation in which the solution close to the membrane surface on the diluate side becomes depleted of salt ions. At the same time the concentration near the membrane on the concentrate side increases. Severe concentration polarization is highly undesired in electrodialysis since it drastically decreases the process efficiency due to the increasing electrical resistance in the solution (Krol 1997; รัตนาจิระวัตนานนท์ 2543).



2. Kinetic polarization

Reduction and oxidation occurred at the surface of electrode are conducting gases, causing of kinetic polarization. The forming gases surrounded by the electrode surface leads to the decrease in electrode efficiency (Crow 1996).

2.2.3 Mode of operating

1. Batch mode

The simple batch mode requires the least membrane area but the largest feed tank. The tank is first filled with the entire quantity of feed to be concentrated, then, as feed is circulated through the membranes, concentrate flows back into the tank. The feed is concentrated by small amounts with each pass through the membranes, and the overall concentration in the feed tank increases as water is removed by permeation through the membrane. Batch system is straightforward in operation and design.



Figure 2.7 Batch mode

2. Continuous Mode.

The single-stage continuous operation mode requires the smallest feed tank but requires larger membrane area than other modes. In this mode, the feed tank is always containing a solution at initial concentration, whereas the recirculation loop is always at final concentration. Because flux is generally low at high concentration, this membrane system runs at a consistent but low flux, rather than over a range of fluxes, as in batch systems. Feed rate from the tank is dependent on the permeate rate and the desired final concentration. Flow ratio control is used to adjust the retentate (concentrate) outlet from the system. This design is suitable for diluted waste streams where achieving high solids concentration in the retentate is not a primary economic driver.

After passing out from the membrane systems, most of the concentrate (retentate) solution is recirculated into the membrane system whereas some of the solution in the left the ED unit. While the permeate solution will be drained out or recirculated into the ED unit depending on specific separation process.



Figure 2.8 Continuous mode

3. Feed and bleed modes

In feed-and-bleed modified batch operation, the process fluid is pumped from the process tank and through the membrane system. At this point, the process fluid is divided into two streams: one that returns to the process tank, and the other that is recirculated through the membrane system. Permeate is directed to drain, the next processing step, or it is reused, depending on the application. A relatively small volume of bleed returns to the process tank, but most remains at membrane outlet pressure and returns to the suction of the recirculation pump.

The advantages of feed-and-bleed modified batch are many, such as lower energy costs than those for modified batch. In addition, the piping to and from the process tank is much smaller because of lower flow to and from the tank. Consequently, the turnover of the process tank occurs at a much slower rate. The smaller pipe size has the obvious advantage of being considerably less expensive and results in a more compact system. The slower rate of turnover in the process.



Figure 2.9 Feed and bleed mode

2.2.4 The performance of an electrodialysis

The performance of an electrodialysis process can be determined by determines the so called current efficiency (CE). Current efficiency the efficiency of current utilization in transporting salts from the diluted stream to the concentrated stream and is usually given as a percentage. Current efficiency can be calculated by on the following relation:

Current efficiency (%) = $100 \times N \times F/i$

Where i is the current density (A m⁻²) F is the Faraday's constant (96485.3 as mol⁻¹) N is the molar flux (mols⁻¹ m⁻²)

$$N = \frac{V_c dC_c}{A_m dt}$$

Where V_c is the volume of concentrate solution, A_m is the total effective membrane area installed, C_c is the salt (sodium lactate) concentration in the concentrate solution and *t* is the time of operation.

2.2.5 Flux of solute (J_s)

Flux of solute is a concentration stream of solute per area of the membrane and time.

$$J_{s} = \frac{\Delta m_{s}}{A_{m}\Delta t}$$

2.2.6 Specific energy consumption (E)

Specific energy consumption (E) is the energy to require for separation per unit mass.

$$\epsilon = \frac{\Phi \int I dt}{\Delta m_s}$$

$$\epsilon = Specific energy consumption$$

$$\Phi = voltage (V)$$

$$I = current$$

$$t = time$$

$$\Delta m_s = mass of solute through the membrane$$

Solute flux represents rate of the separation. Fast separation requires high solute flux. Specific energy consumption determines the efficiency of the separation. The high efficiency separation uses low specific energy consumption. (Moresi and Sappino 1998)

Electrodialysis is capable of transporting ionic compounds from one solution to another. Its application includes the recovery of metals such as nickel, gold, copper, cadmium, chromium, platinum, silver, zinc, tin-lead, and other metals from a variety
of plating baths; desalination of seawater; recovery of salts, acids, and alkali from industrial rinsed waters; and concentrate ion of diluted salts, acid, or base solutions. Large scale ED has been used in the food and pharmaceutical industry (Andres *et al.* 1995; Chen *et al.* 1995; Lopez 1988), chemical, textile, and water treatment industries for purposes ranging from recovery, concentration, and purification, to brackish water desalination (Strathmann 1991), and ground water denitrification (Hell *et al.* 1998). It is also useful in removing unwanted total dissolved solids that can build up in product streams.

Thang *et al.* (2004) separated lactic acid from grass silage juice by electrodialysis. The separation was carried out in two steps. In the first step, lactate together with other charged compounds were separated from the neutral ones, such as sugars and non-ionic amino acids, at pH 6.7. In the second step, lactic acid was recovered from the inorganic salts at pH 2.0. Each step was divided into different phases (time periods). For each phase, the performance in electrodialytic separation of lactic acid was investigated and discussed. This performance is characterized in terms of mass transfer, current efficiency, energy consumption, and water transfer. The results data show that the first electrodialysis (ED) step at pH 6.7 is significantly less effective than the second ED step at pH 2.0. Higher energy consumption and lower current efficiency as well as longer operation time were observed during the first ED step. High concentration of uncharged compounds, such as sugars and non-ionic amino acids likely caused the negative effects during the separation of salts as well as lactate.

Habova *et al.* (2003) used two-stage electrodialysis (ED) to recover lactic acid from model solution and real fermentation broth. In the first step sodium lactate was concentrated with desalting electrodialysis using ion exchange membranes Ralex (Mega, Czech Republic). The second step was the electroconversion of sodium lactate to lactic acid by electrodialysis with bipolar membranes (EDBM) Neosepta (Tokuyama Corp., Japan). The optimal conditions of ED were determined. The fermentation broth from lactic acid fermentation had to be pretreated before electrodialysis experiments. The pretreatment consisted of ultrafiltration, decolourisation and removing of multivalent metal ions. In the first ED step the final lactate concentration of 175 g/1 was obtained and afterwards the final lactic acid concentration of 151 g/l was reached in the second ED step. (Habova *et al.* 2004)

Chapter III

Experimental

3.1 <u>Materials</u>

Chemical	Company	Country	Grade
Fumaric acid	Fluka	Switzerland	Lab
Hydrocloric acid	Merck	Germany	Lab
Lactic acid	ADM company	USA.	Commercial
Phosforic acid	Carlo Erba	Italy	Commercial
Sulfuric acid	Merck	Germany	Lab
Sodium hydroxide	Merck	Germany	Lab
95% Ethanol	Carlo Erba	Italy	Commercial
Dextrose (glucose) Sian	nchai chemical import	Thailand	Commercial
Amberlite IRA-400	Sigma	Germany	Lab
Amberlite XAD-7	Sigma	Germany	Lab
Dowex Marathone WB	A Sigma	Germany	Lab

3.2 Equipments

Equipment / Instrument	Company
Centrifuge (KR-20000T)	Kubota Corporation, Japan
Electronic balance (FX-180)	A&D, Japan
Electronic balance (FX-3000)	A&D, Japan
High Performance Liquid Chromatography (HPL	C) with
Pump (LC-8A)	Shimadzu, Japan
Detector (RID-6A)	Shimadzu, Japan
Analytical Column (Aminex HPX-87H)	
Controller (SLC-8A)	Shimadzu, Japan
Peristaltic pump (7554-20)	Masterflex,Cole Parmer
	Instrument Co.,Ltd.U.S.A.
Peristaltic pump (CH2A#459)	Amicon Co., Ltd., U.S.A
Oven (UL-80)	Memmert, Germany

Equipment / Instrument	Company
pH meter (F-13)	Japan
Rotary incubator shaker (G-25)	New Brunswick Scientific
	Co., Inc. U.S.A.
Sonicator (Ultrasonic Cleaner D 200)	Delta, D.S.C. Group, China
Glucose lactate analyzer (YSI 7100)	Yellow Spring Instrument, U.S.A.

3.3 <u>Methodology</u>

3.3.1 L-Lactic acid fermentation from D-glucose

Microorganism

Rhizopus oryzae NRRL 395, a filamentous fungus producing L(+)lactic acid, was obtained from the Northern Regional Research Center, Peroria, IL.,U.S.A.. The stock culture was maintained on potato dextrose agar (PDA) plate and sub cultured every month to maintained fungal activity.

Inoculum Preparation

The sporangiospores were collected from the 7-day culture on PDA plates by shaving and extracting the spores with sterile water. The spore concentration was determined by spore counting using a haemacytometer and then was adjusted to 10^6 /mL by dilution with sterile water. 0.5 mL of 10^6 /mL of *Rhizopus oryzae* was used for inoculating each shaken flask.

Medium Compositions

Growth medium

The growth medium consisted of 50 g/L D-glucose and 5 g/L yeast extract. Yeast extract was autoclaved separately from D-glucose to prevent any undesirable reaction due to heat sterilization.

Production medium

The medium for enhancing lactic acid production consisted of 70 g/L D-glucose, 0.6 g/L KH₂PO₄, 0.25 g/L MgSO₄, 0.088 g/L ZnSO₄, and 2250.3 g/L urea. ZnSO₄ and urea were autoclaved separately from the other compositions to prevent undesirable reaction.

3.3.2 Shake flask culture

0.5 mL *Rhizopus oryzae* spore suspension (10^6 /mL) was inoculated into 50 mL growth medium. After 48 hours of incubation at 30 °C with rotational speed of 200 rpm, the growth medium was replaced with the production medium and further incubated at 30 °C with rotational speed of 200 rpm for 72 hours. After that, the fermentation broth was filtered through the filter paper to remove cellular materials and insoluble particles.

3.3.3 Adsorption of L-lactic acid by anion-exchange resin

Strong- base, weak- base, and neutral anion exchange resin (Amberlite IRA –400, Dowex Marathone WBA, and Amberlite XAD-7, purchased from Sigma Chemical Co.) were used in this study.

3.3.3.1 Batch adsorption

Equilibrium time and adsorption isotherm were determined. The effects of ratio of resin to starting solution, and pH on L-lactic acid adsorption on each resin were observed. To determine the equilibrium time, resin was mixed with 40 g/L starting solution (0.1 g: 2.5 mL, 0.2 g: 2.5 mL, and 0.2 g: 5 mL) and then incubated in an incubator shaker at 30° C, 150 rpm. The solution sample was collected at every 5 min in the 1st h, then at every 10 min in the 2nd h. After that at every 30 min until reaching 6 h. The remaining L- lactic acid in the solution was determined by YSI/ HPLC. The amount of L- lactic acid adsorbed on resin was calculated. The



adsorbed L- lactic acid on resin was plotted versus time. The equilibrium time was then determined as explained in Figure 3.1.

Figure 3.1 Concentration of residual on resin

Composite adsorption isotherms were determined by using 1:2 (wt/vol) ratio of wet resins to starting solution with equilibrium method. Lactic acid solution with concentrations ranging from 5-100 g/L at pH 6.0 was used as the starting solution. The flasks containing the adsorbents and lactic solution were mixed and incubated at 30 $^{\circ}$ C in the incubator-shaker and allowed to equilibrate for least 24 hours. The lactate concentration remaining in solution was determined by HPLC.

3.3.3.2 Fixed-bed adsorption

Breakthrough curve

The activated resins were transferred into column $(0.8 \times 46 \text{ cm})$ equipped with adjustable plungers. The feed solution, containing 40 g/L of lactate at a pH 6.0, was introduced into the column by a peristaltic pump at different flow rates (0.35 mL/min, 0.65 mL/min and

0.80 mL/min). Samples were collected from the column, and the lactic acid concentration was analyzed to the breakthrough point. The resins were considered saturated when the lactic acid concentration in the effluent was at least 95% of that in the feed. The lactic acid concentration of each fraction was determined by HPLC. The adsorption capacity was expressed in g L-lactate adsorbed/ g resin. The example of breakthrough curve in shown in Figure 3.2.



Figure 3.2 Breakthrough curve

3.3.3.3. Desorption

The adsorbed L-lactic acid was separated from adsorption resins using various eluants at different concentrations including HCl, H₂SO₄, NH₃, H₃PO₄, and NaCl. 0.5 g adsorbed resin was mixed with 10 mL eluant and incubated at 200 rpm in the incubator shaker. After 12 h, the concentration of L-lactic acid eluted into the solution was determined by HPLC.

3.3.3.4 L-lactic acid recovery in fixed-bed column

Fermentation broth with lactic acid concentration of 40 g/L at pH 6.0 was applied on the Dowex Marathon WBA ion exchange resin column at a flow rate of 0.8 ml/min until the resins were saturated. The saturated resins were washed with ethanol to remove excess L-lactic acid. Later, adsorbed lactic acid on the resin was eluted by various eluants at a flow rate of 0.3 ml/min. The eluants used in this study included HCl, H₃PO₄, NaCl, and the mixture of H₃PO₄ and H₂SO₄.



Figure 3.3 Auto collecting sample from column

3.3.4 Electrodialysis

3.3.4.1 Apparatus

The laboratory scale electrodialysis unit (Figure 3.4) consisted of a power supply (adjustable voltage outputs from 0 to 15 V and 3 independent circuits with peristaltic pumps and storage containers (for the diluate, the concentrate and the electrode solution). The anode and cathode were made of graphite and stainless steel, respectively. The membrane stack ED with a pair of anion and cation exchange membranes L-lactic

acid recovery. The effective membrane area was 29.25 cm^2 , the distance between the membranes was 1 mm.



Figure 3.4 The laboratory scale electrodialysis unit



Figure 3.5 Schematic of electrodialysis

3.3.4.2 Operating conditions

Single- stack electrodialysis experiments were carried out in a batch mode. The electrode solution (0.1 M NaOH), the concentrate (L-lactate, initial concentration 40 g/L) were circulated through the compartment of ED stack. The effects of concentrations of electrode solution, concentrate solution, diluate solution, pH, voltage, and flow rate was studied. The experimental conditions determined are shown in Table 3.1.

	Pa	Parameters		Concentration of solutions			
Conditions	Flow rate (ml/min)	Hq	Voltage (V)	Lactic acid solution(g/L)	Lactate solution(g/L)	NaOH (M)	Parameters
1	5	6	6	20	40	0.1	
2	8	6	6	20	40	0.1	Voltage
3	10	6	6	20	40	0.1	
4	8	6	6	40	40	0.1	
5	8	6	6	0	40	0.1	Ratio
2	8	6	6	20	40	0.1	
6	8	6	4	20	40	0.1	
2	8	6	6	20	40	0.1	Flow rate
7	8	6	8	20	40	0.1	
8	8	3	6	20	40	0.1	
2	8	6	6	20	40	0.1	рН
9	8	9	6	20	40	0.1	

Table 3.1 Operating conditions

3.3.5 Analytical method

3.3.5.1 High performance liquid chromatography

HPLC was used to analyze the organic compounds (glucose, Llactic acid, fumaric acid, and ethanol) present in the samples. Samples were centrifuged, diluted, and filtered through cellulose acetate μ membrane. Aliquot of 20 μ L diluted particles-free samples were injected into an organic acid analysis column (Biorad, Aminex HPX-87H ion exclusion organic acid column; 300 mm x 7.8 mm) maintained at 45 °C in a column oven (Shimadzum, CTO-6A) and 0.005 M H₂SO₄ was used as an eluant at 0.6 mL/min flow rate. An RI detector (Shimadzu, RID-6A) was set to detect the organic compounds. A standard containing 2 g/L of each component was injected as a reference to determine the sample concentration.

3.3.5.2Glucose-lactate analyzer

Glucose and L-lactic acid were also analyzed by YSI 7100 (Yellow Spring Instrument).

3.3.5.3 Titration

Amount of NaOH in the electrode solution from ED experiment was determined by titration using 0.1 M HCl. Phenolphthalene was used as an end point indicator.

Chapter IV

Results and discussion

4.1 Lactic acid fermentation broth

A shaken flask culture was performed at 30° C 200 rpm for 5 days. The fermentaion broth produced by filamentous fungus *Rhizopus oryzae* NRRL 395 consists of lactic acid, products including fumaric acid, and ethanol, and the remaining glucose substrate (Table 4.1).

Table 4.1 Concentration of *R. oryzae* fermentation broth at pH 5.41

Broth composition	Concentration (g/L)
Glucose	0.69
Lactic acid	38.13
Fumaric acid	0.55
Ethanol	3.85

4.2 Ion exchange chromatography

Ion exchange chromatography was studied in two processes, adsorption and desorption process.

4.2.1 Adsorption process

Adsorption process is the method of lactic acid adsorption on resin, which was studied in batch adsorption and fixed-bed adsorption.

4.2.1.1 Batch adsorption

4.2.1.1.1 Equilibrium time



Figures 4.1-4.3 show the profiles of the amount of lactic acid adsorbed on different resins at various pH.

Figure 4.1 Adsorbed lactic acid on Amberlite XAD-7 at pH 3.0, 4.5, and 6.0 with different ratios of resin to starting solution (a) 0.1g:2.5 mL;(b) 0.2 g:2.5 mL; (c) 0.2 g:5 mL



Figure 4.2 Adsorbed lactic acid on Dowex Marathon WBA at pH 3.0, 4.5, and 6.0 with different ratios of resin to starting solution (a) 0.1g:2.5 mL;
(b) 0.2 g:2.5 mL; (c) 0.2 g:5 mL



Figure 4.3 Adsorbed lactic acid on Amberlite IRA-400 at pH 3.0, 4.5, and 6.0 with different ratios of resin to starting solution (a) 0.1g:2.5 mL ;
(b) 0.2 g:2.5 mL ; (c) 0.2 g:5 mL

The adsorption rate of lactic acid on 3 types of resin, Amberlite IRA –400, Dowex Marathon WBA, and Amberlite XAD-7, rapidly increased from the beginning (during the first 5-10 minutes) and then became constant later after reaching the equilibrium.

This could be explained by reversible and desorption which occurred simultaneously. Considering the resin containing certain amount of active sites. Lactate ions bound with the counter ions at the active sites while at the same time some lactate ions left the active site. Since, adsorption took place faster than desorption; therefore, at the beginning of the process, more lactate ions bound at the active sites than those left the sites as it could be observed from dramatic increase in the amount of lactate adsorbed on the resin at the beginning when freshly empty active site resin available until it reached equilibrium.

The results showed that among three resins studied in this work, Dowex Marathon WBA had the maximum adsorption capacity of lactic acid, which was 0.3 g / g resin at pH 6.0 (Figure 4.2) while Amberlite XAD-7 and Amberlite IRA-400 had the maximum adsorption capacity of lactic acid of approximately 25 mg / g resin at pH 6.0 (Figure 4.1) and 100 mg / g resin at pH 6.0 (Figure 4.3) respectively. Therefore, Dowex marathon WBA was selected for further studies in this work.

• The effect of pH on adsorption process

The effects of pH on the lactic acid adsorption on ion exchange resin were observed (Figures 4.1-4.3). The amount of adsorption at pH 6.0 was better than those at pH 3.0 and 4.5. This can be explained that when the pH in the solution increased, it increased the negative charges to induce the exchanger group causing more lactic acid bound at the active site. Therefore, pH 6.0 would be used for further studies in this work.

• The effect of ratio of resin to lactic acid solution

The results showed that (Figures 4.1-4.3), all of the ratio had a similar maximum adsorption capacity. Accordingly, the ratio had no effect on lactic acid adsorption. Therefore to minimize the amount of resin used, the ratio of 1:1 would be used in further studies.

4.2.1.1.2 Equilibrium time of Dowex Marathon WBA

Equilibrium time of Dowex Marathon WBA was determined according to section 3.3.3.1. The concentration of residual lactic acid was plotted versus time. The contact lines of the curve were drawn (Figure 4.4). At the intersection, the parallel line to Y-axis was drawn and the time at the intersection was the equilibrium time which was 5 minutes. The equilibrium time obtained from the plot was then used to determine the maximum flow rate for fixed bed adsorption.



Figure 4.4 Concentration of residual lactic acid on Dowex Marathon WBA

4.2.1.1.3 Adsorption isotherm

Adsorption isotherm of Dowex Marathon WBA was determined. The result showed that the adsorption of equilibrium of lactic acid on Dowex Marathon WBA followed Langmuir isotherm which could be determined by equation 4.2, where K was the dissociation constant, q_0 was the maximum adsorption capacity, c was the remaining lactic acid in solution, and q was the amount of lactic acid adsorbed on the resin. Eq 4.3 was derived from eq 4.2. From eq 4.3, the plot of $\frac{1}{q}$ versus $\frac{1}{c}$ could be used to determine K and q_0

where the slope of the plot was $\frac{\mathbf{K}}{\mathbf{q}_0}$ and Y- intercept was $\frac{1}{\mathbf{q}_0}$.

$$q = \frac{q_0 c}{K + c} \qquad \text{eq.4.2}$$

$$\frac{1}{q} = \frac{1}{q_0} + \left(\frac{K}{q_0}\right) \cdot \left(\frac{1}{c}\right)$$
 eq.4.3





Figure 4.5 Adsorption isotherm of lactic acid by Dowex Marathon WBA at pH 6.0



Figure 4.6 Relation of 1/q and 1/c plot of lactic acid by Dowex marathon WBA at pH 6.0

From linear equation;	y = 426.29x + 0.7097
Y- intercept was $\frac{1}{q_0}$;	$\frac{1}{q_0} = 0.7097$
	$q_0 = 1.4090$ g lactic acid / g resin
Slope was $\frac{\mathbf{K}}{\mathbf{q}_0}$;	$\frac{\mathbf{K}}{\mathbf{q}_0} = 426.29$
	$\frac{K}{1.4090} = 426.29$
	K = 600.64

At pH 6.0, the dissociation constant (*K*) was 600.64 g/L and the maximum amount of lactate adsorbed on the resin (q_0) 1.41 g/g wet resin.

4.2.1.2 Fixed – bed adsorption

Fixed-bed adsorption was studied in the optimal flow rate for lactic acid purification in fixed-bed separation.

4.2.1.2.1 Maximum flow rate

The maximum flow rate could be calculated from eq 4.1.

Maximum flow rate (ml/min) = $\frac{\text{Void volume(ml)}}{\text{Contacte quilibrium time(min)}}$ eq 4.1 = $\frac{4 \text{ mL}}{5 \text{ min}}$ = 0.8 mL/min



4.2.1.2.2 Breakthrough curve

Figure 4.7 Breakthrough curve of lactic acid in Dowex Marathon WBA resin column

The breakthrough curves of the different flow rates are shown was in Figure 4.7. At the minimum flow rate of 0.35 mL/min has a t_B (breakthrough time) at 32.5

minute and t_E (exhaustion time) at 71.5 minute. At the flow rate of 0.65 mL/min, t_B and t_E at 18.36 minute and 48.96 minute, respectively. At the maximum flow rate of 0.8 mL/min, t_B and t_E at 20 minute and 45 minute, respectively. Good separation performance required steeper and narrower breakthrough curve. Therefore, according to the results shown in figure 4.7, the flow rate of 0.8 mL/min provided the best adsorption performance and would be their used in further studies.

4.2.2 Desorption process

4.2.2.1 The effect of eluants to lactic acid recovery

The amount of lactate adsorbed on Dowex Marathon WBA, 0.0628 g lactate/ 0.5 g resin was calculated (Appendix D28). The concentration of L-lactic acid eluted with various eluants into the solution was determined by HPLC. The results of L-lactic acid recovery in shake flasks are shown in Table 4.1.

Fluents	Lactic recovery (%)			
Eluants –	1.0 M	2.0 M		
H_2SO_4	38	35		
HCl 🔍	40	38		
H ₃ PO ₄	41	34		
NH ₄ OH	22	20		
1.0 M NaCl	35	<u> </u>		

Table 4.2 L-lactic acid recovery in shake flask

From Table 4.1, the results of different eluants, almost of the eluants have similar recovery, about 0.02 g. lactic acid, accept NH_4OH both of the concentrations, 1.0 M and 2.0 M were present lower recovery than other elution agents about 2 times. The concentration of the eluants had no effect for the recovery. According to the results, 1.0M H₂SO₄, 1.0M HCl, 1.0M H₃PO₄, and 1.0 M NaCl would be used to eluants L-lactic acid in the fixed-bed column.

4.2.2.2 L-lactic acid recovery in fixed-bed column

From the methodology section 3.3.3.4, fermentation broth was adjusted to pH 6.0 were applied on the Dowex Marathon WBA ion exchange resin column at a flow rate of 0.8 mL/min until the resins were saturated. Saturated resins were washed with ethanol to remove excess L-lactic acid with ethanol. Adsorbed L- lactic acid on the resin was eluted by 1.0M H₂SO₄, 1.0M HCl, 1.0M H₃PO₄, 1.0M NaCl, and the mixture of H₃PO₄ and H₂SO₄ at a flow rate of 0.3 ml/min. The concentration of lactic acid in eluate was determined by HPLC. The results are shown in Table 4.3.

 Table 4.3 L-lactic recovery in fixed-bed column

Eluents	Recovery (%)	Purity (%)
1.0 M H ₂ SO ₄	42	94
1.0 M HCl	50	98
1.0 M H ₃ PO ₄	65	94
1.0 M NaCl	53	90
1.0M H ₃ PO ₄ +1.0M H ₂ SO ₄ : 70:30	76	90

The highest elution recovery of 76 % L-lactic acid was obtained with 1.0 M $H_3PO_4+1.0$ M H_2SO_4 : 70:30. Moreover, the mixture of 1.0 M H_3PO_4 and 1.0 M H_2SO_4 was obtained 90 % purity. However, the highest purity, 98% was shown when used 1.0 M HCl for elution of L-lactic acid, but has low recovery, 50% recovery.

4.3 Electrodialysis

The parameter of study in electrodialysis including effects of voltage, effects of ratio of lactic acid to sodium lactate, effects of flow rate and effect of pH and considered the flux of lactate (J_s) and the specific energy consumption(ϵ).

4.3.1 Effects of voltage

The results was shown in Table 4.3 and appendices D1-D3.

Table 4.4 Amount of separation, J_s and specific energy consumptionof operating condition with parameter studied of voltage

Voltage(V)	Amount of separation(g)	Flux, J _s (mol/m ² h)	Specific Energy consumption, E (kWh/kg)
5	0.59	0.74	0.9096
8	2.12	2.69	0.6122
10	0.79	1.00	0.4228

The maximum amount of separation and maximum flux, 2.12 g and 2.69 mol/m²h was to be apparent with the applied voltage, 8 V. This result can be explained that at the low applied voltage, there was the low separation indicated by lower flux. As the voltage was increased, concentration polarization becomes more pronounced. Therefore, the applied voltage 8 V would be used for further studies.

4.3.2 Effects of ratio of lactic acid to sodium lactate

The results are shown in Table 4.4 and all results of conditions 4-6 have shown in appendices D4-D6.



Ratio	Amount of separation(g)	Flux, J _s (mol/m ² h)	Specific Energy consumption, E (kWh/kg)
1:1	0.71	0.88	0.6518
1:0	0.47	1.21	0.3154
2:1	2.12	2.69	0.6122

Table 4.5 Amount of separation, J_s and specific energy consumption

 of operating conditions with parameter studied of ratio

The maximum amount of separation and maximum flux, 2.12 g and 2.69 mol/m²h was found with the ratio 2:1. This result can be explained that concentration gradient was one of the driving forces for separation. At low concentration gradient there was the low separation, and when concentration gradient increased, the separation was better. Therefore, the ratio 2:1 would be used for further studies.

4.3.3 Effects of flow rate

The results are shown in Table 4.5 and appendices D7-D9.

Table 4.6 Amount of separation, J_s and specific energy consumptionof operating conditions with parameter studied of flow rate

Flow rate (mL/min)	Amount of separation(g)	Flux, J _s (mol/m ² h)	Specific Energy consumption, & (kWh/kg)
4	0.45	0.57	2.3565
6	2.12	2.69	0.6122
8	0.53	0.68	0.1632

The maximum amount of separation and maximum flux, 2.12 g and 2.69 mol/m²h was found with the flow rate 6 mL/min. This result can be explained that at the low flow rate, there was low separation and has high energy

consumption. At the higher flow rate there was more separation but when the flow rate was too high, the amount of separation was less than those at optimal flow rate because the solution flow was too high so that the ions could not have sufficient time to separate before they was flushed away from the chamber. Therefore, the flow rate 6 ml/min would be used for further studies.

4.3.4 Effects of pH

The results are shown in Table 4.6 and appendices D10-D12.

рН	Amount of separation(g)	Flux, J _s (mol/m ² h)	Specific Energy consumption, & (kWh/kg)
3	0.45	0.80	0.3760
6	2.12	2.69	0.6122
9	2.34	2.97	0.7890

 Table 4.7 Amount of separation, J_s and specific energy consumption of operating conditions with parameter studied of pH

The maximum amount of separation and maximum flux, 2.34 g and 2.97 mol/m²h was found at pH 9. This result can be explained that at pH 3, which was lower than pK_a of lactic acid ($pK_a = 3.86$), lactic acid still in non- ionized form. At pH 6 and 9, which were higher than pK_a of lactic acid, lactic acid could be ionized to lactate ion form and transported through anion exchange membrane. This is in agreement with the results showing that more amount of ion separation and higher flux at higher pH than pK_a . Since, pHof the fermentation broth fed into the ED apparatus was 6, to simplify the separation process, pH 6 would be used for further study

4.3.5 Specific Energy consumption

Table 4.8 Amount of separation and specific energy consumption

	conditions			Concentration			
Conditions	Flowrate (ml/min)	Hq	Voltage (V)	Lactate solution(g/L)	Lactic solution(g/L)	Amount of separation (g)	Energy consumption (kWh/kg)
1	5	6	6	20	40	0.59	0.9096
2	8	6	6	20	40	2.12	0.6122
3	10	6	6	20	40	0.79	0.4228
4	8	6	6	40	40	0.71	0.6518
5	8	6	6	0	40	0.47	0.3154
6	8	6	4	20	40	0.45	2.3565
7	8	6	8	20	40	0.53	0.1632
8	8	3	6	20	40	0.63	0.376
9	8	6	6	20	40	2.12	0.6122

of conditions 1-9

Table 4.8 shows that the amount of separation and specific energy consumption of all of the operating condition. The specific energy consumption could be used to represent the separation efficiency. The best separation should condition provide minimal specific energy consumption. Under the optimal condition were concentration ratio of lactic acid to sodium lactate, 1:2 with 20 g/L lactic acid and 40 g/L sodium lactate broth at pH 6.0, electrical voltage 8.0 V, and the flow rate 6 ml/min that has amount of separation 2.12 g and specific energy consumption 0.6122 kWh/kg while compared with worst condition were concentration ratio of lactic acid to sodium lactate, 1:2 with 20 g/L lactic acid and 40 g/L sodium lactate broth at pH 6.0, electrical voltage 4.0 V, and the flow rate 6 ml/min. has the amount of separation 0.45 g and has the highest specific energy consumption at 2.3565 kWh/kg.

4.3.6 Comparison of specific energy consumption with previous works

The specific energy consumption represented the energy required for separation. It was found that with the optimal condition obtained in this work, lactic acid separation required less energy than the previous work done by Habova *et. al* (2003) and Thang *et. al* (2004) indicating lower separation cost required in this process.

 Table 4.9 Comparison of specific energy consumption with previous works

	Specific energy consumption (kWh/kg)		
This work (condition 2)	AZA	0.6122	
Habova <i>et.al</i> (2003)		1.5000	
Thang <i>et.al</i> (2004)		3.1900	

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

Chapter V

Conclusions and Recommendations

5.1 Conclusions

In this work the purification of lactic acid from fermentation broth, produced by filamentous fungus *R.oryzae* with ion exchange resin and electrodialysis was studied.

5.1.1 Ion exchange resin

• Types of anion exchange resin: Dowex Marathone WBA Amberlite IRA – 400, Dowex Marathon WBA, and Amberlite XAD-7, were used for lactic acid separation. It was found that Dowex Marathon WBA had the maximum lactic acid adsorption capacity among three types of resin studied. Therefore, Dowex Marathone WBA was used in further lactic acid purification.

• The adsorption isotherm of Dowex Marathone WBA followed Langmuir isotherm, with the maximum adsorption capacity of (q_0) 1.41 g lactic acid / g resin and dissociation constant (K) of 600.64 g/L.

• The effects of pH, flow rate, and ratio of the resin to starting solution on lactic acid separation were studied. The optimal condition for lactic acid adsorption was at pH 6.0 with flow rate 0.8 ml/min.

• Lactic acid was eluted from fixed-bed column using $1.0 \text{ M H}_3\text{PO}_4$ and $1.0 \text{ M H}_2\text{SO}_4$ at the ratio 7:3 at 0.3mL/min, has yielding 76% lactic acid recovery and 90% purity.

5.1.1 Electrodialysis

• Laboratory scale module with effective membrane area of $2.925 \times 10^{-3} \text{m}^2$ with distance between the membranes of 1mm was constructed. Graphite and stainless steel were used as an anode and cathode, respectively

• The effects of concentration ratio of lactic acid to sodium lactate, pH, electrical voltage, and the flow rate of feed solution on lactic acid recovery was studied.

• The optimal condition obtained was at the concentration ratio of lactic acid to sodium lactate of 2:1 using 40 g/L lactic acid and 20 g/L sodium lactate broth at pH 6.0, electrical voltage 8.0 V, and the flow rate 6 ml/min.

• This optimal condition gave 2.69 mole of lactate separated per a unit square meter of membrane area in an hour with the specific energy consumption 0.6122 kWh/kg.

5.2 Recommendations

• To further improve the lactic acid recovery by ion exchange chromatography, the appropriate eluant beyond those studied in this research should be determined.

• It was found that the graphite electrode in electrodialysis apparatus was eroded in long term use. It is suggested that platinum can be used as the anode instead of graphite to prevent electrode corrosion

• Multiple-stage separation using both techniques will be investigated in order to improve the recovery percentage of lactic acid.

• Process scaling up of both separation process should be studied in order to accommodate the fermentation broth from a large scale fermentation.

REFERENCES

<u>Thai</u>

รัตนา จิระรัตนานนท์.(2543). กระบวนการอิเล็กโตร ใดอะ ไลซิส. กระบวนการแยกด้วยเยื่อแผ่น สังเคราะห์. กรุงเทพมหานคร: โรงพิมพ์ไทยเส็ง

<u>English</u>

- Andres, L. J., Riera, F. A., Alvarez, R. (1995). Skimmed-Milk Demineralization by Electrodialysis - Conventional Versus Selective Membranes. *Journal of Food Engineering* 26, 57-66.
- Cao, X. J., Yun, H. S., Koo, Y. M. (2002). Recovery of L-(+)-lactic acid by anion exchange resin Amberlite IRA-400. *Biochemical Engineering Journal* 11, 189-196.
- Chen, D. H., Wang, S. S., Huang, T. C. (1995). Separation of Phenylacetic Acid, 6-Aminopenicillanic Acid and Penicillin-G with Electrodialysis Under Constant-Current. Journal of Chemical Technology and Biotechnology 64, 284-292.
- Choi, J. I., Hong, W. H. (1999). Recovery of lactic acid by batch distillation with chemical reactions using ion exchange resin. *Journal of Chemical Engineering of Japan* **32**, 184-189.
- Crow, D.R. (1996). *Principles and applications of electrochemistry*. Cornwall UK: Blackie Academic and Professional.
- Geankoplis, C.J. (1993). Transport processes and unit operations 3rd ed. New Jersey:
 Prentice Hall.Hamissa, F.A. and Abou-Zeid, A. 1981. Fermentative production of citric acid by yeasts. *Agricultural wastes* 3, 21-33

- Habova, V., Melzoch, K., Rychtera, M., Sekavova, B. (2004). Electrodialysis as a useful technique for lactic acid separation from a model solution and a fermentation broth. *Desalination* **162**, 361-372.
- Hell, F., Lahnsteiner, J., Frischherz, H., Baumgartner, G. (1998). Experience with full-scale electrodialysis for nitrate and hardness removal. *Desalination* 117, 173-180.
- Houwing, J., Billiet, H. A. H., van der Wielen, L. A. M. (2002). Optimization of azeotropic protein separations in gradient and isocratic ion-exchange simulated moving bed chromatography. *Journal of Chromatography A* 944, 189-201.
- Jarvinen, M., Myllykoski, L., Keiski, R., Sohlo, J. (2000). Separation of lactic acid from fermented broth by reactive extraction. *Bioseparation* **9**, 163-166.
- Kawabata, N., Yasuda, S., Yamazaki, T. (1982). Process for recovering a carboxylic acid. *US patent* **4323702**.
- Krol, J.J. (1997). Ionexchange membrane. Mass transport limitations. The Netherlands: J.J.Krol Print. Available from:www.membrane.nl/ serve/these/john_krol THESIS_john_k.pdf [2002, March 3]
- Lee, E. G., Moon, S. H., Chang, Y. K., Yoo, I. K., Chang, H. N. (1998). Lactic acid recovery using two-stage electrodialysis and its modelling. *Journal of Membrane Science* 145, 53-66.
- Lopez, L., M.H., (1988). The use of electrodialysis in food processing. Part 2: Review of practical applications. *Lebensm. Wiss. Technol.* **21**, 177–182.
- Moresi, M., Sappino, F. (1998). Effect of some operating variables on citrate recovery from model solutions by electrodialysis. *Biotechnology and Bioengineering* **59**, 344-350.

- Narayanan, N., Roychoudhury, P. K., Srivastava, A. (2004). L (+)lactic acid fermentation and its product polymerization. *Electronic Journal of Biotechnology* 7, 167-182.
- Persson, A., Jonsson, A. S., Zacchi, G. (2001). Separation of lactic acid-producing bacteria from fermentation broth using a ceramic microfiltration membrane with constant permeate flow. *Biotechnology and Bioengineering* **72**, 269-277.
- Sosa, A. V., Ochoa, J., Perotti, N. I. (2000). Modeling of direct recovery of lactic acid from whole broths by ion exchange adsorption. *Bioseparation* **9**, 283-289.
- Strathmann, H., (1991). Electrodialysis. In: Baker, R.W. (Ed.), Membrane Separation Systems, Recent Developments and Future Directions. Noyes Data Corp, New Jersey.
- Thang, V. H., Koschuh, W., Kulbe, K. D., Kromus, S., Krotscheck, C., Novalin, S. (2004). Desalination of high salt content mixture by two-stage electrodialysis as the first step of separating valuable substances from grass silage. *Desalination* 162, 343-353.
- Timmer, J. M. K., Vanderhorst, H. C., Robbertsen, T. (1993). Transport of Lactic-Acid Through Reverse-Osmosis and Nanofiltration Membranes. *Journal of Membrane Science* 85, 205-216.
- Tong, W. Y., Fu, X. Y., Lee, S. M., Yu, J., Liu, J. W., Wei, D. Z., Koo, Y. M. (2004). Purification of L(+)-lactic acid from fermentation broth with paper sludge as a cellulosic feedstock using weak anion exchanger Amberlite IRA-92. *Biochemical Engineering Journal* 18, 89-96.
- Tong, W. Y., Yao, S. J., Zhu, Z. Q. (2001). Separation characteristics of human epidermal growth factor in ion exchange chromatography with STREAMLINE DEAE resin. *Chemical Engineering Science* **56**, 6959-6965.

APPENDICES

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย



APPENDIX A

CULTURE, INOCULUM PREPARATION, AND MEDIUM COMPOSITIONS

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

A1 Culture

Rhizopus oryzae NRRL 395, a filamentous fungus producing L(+)-lactic acid was obtained from the Northern Regional Research Center, Peroria, IL. The stock culture was kept on potato dextrose agar (PDA) plate at 4°C. To maintain the viability, in every month the stock culture was transferred to the new PDA plate and incubated at 30°C for 7 days.

A2 Inoculum Preparation

The sporangiospores were collected from the 7-day culture on PDA plates by shaving and extracting the spores with sterile water. The spore concentration was determined by spore counting using a haemacytometer. The spore suspension was then adjusted to desired concentration by dilution with sterile water.

A3 Medium Compositions

Growth medium

The growth medium consisted of 50 g/L glucose and 5 g/L yeast extract. Yeast extract was autoclaved separately from the substrate to prevent any undesirable reaction due to heat sterilization. The control of pH was not necessary during the growth phase.

Production medium

Unless otherwise mentioned, the medium for enhancing lactic acid production consisted of 70 g/L glucose, 0.6 g/L KH₂PO₄, 0.25 g/L MgSO₄, 0.088 g/L ZnSO₄, and 225 0.3 g/L urea. ZnSO₄ and urea were autoclaved separately from the other compositions to prevent undesirable reaction.

จุฬาลงกรณมหาวทยาลย

APPENDIX B

ANALYTICAL MEDTHOD

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

B1 High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) was used to analyze the organic compounds (glucose, lactic acid, fumaric acid, and ethanol) present in the fermentation broth. Samples from this studies were diluted with distilled water. 20 μ L diluted particle-free samples were injected into an organic acid analysis column (Biorad, Aminex HPX-87H ion exclusion organic acid column; 300mm⁴7.8mm) maintained at 45°C in a column oven (Shimadzu-CTO-6A). 0.005 N H₂SO₄ was used as an eluant at 0.6 mL/min flow rate. An RI detector (Shimadzu-RID-6A) was set at the range of 200 to detect the organic compounds. A standard containing 2 g/L of each component was injected as a reference to determine the sample concentration. The peak height was used for the comparison basis. It is noted that HPLC can detect both L(+) and (D)-lactic acids.

B2 Glucose Analyzer

Glucose and L(+)-lactic acid were analyzed by YSI 7100 glucose analyzer(Yellow Spring Instrument Co., Inc.). This analytical instrument is accurate within the range of 0-2.5 g/L glucose and 0-0.5 g/L lactic acid. Before measurement, fermentation broth was centrifuged and diluted with distilled water. The calibrator standard contained 0.5 g/L L(+)-lactic acid, 2.5 g/L glucose, 1.0 g/L benzoic acid, and 2.0 g/L NaEDTA. The buffer powder used in this equipment was prepared by mixing 4.4 g K₂H₂EDTA, 0.05 g kanamycin sulfate, 7.3 g sodium benzoate, 12.0 g NaH₂PO₄, 54.7 g Na2PO₄, and 21.5 g NaCl together and grinded to powder-form. The buffer solution was prepared by dissolving 12.7 g buffer powder in 900 mL distilled water.


Figure B1 The HPLC chromatogram of standard solution of glucose, lactic acid, fumaric acid, and ethanol

APPENDIX C

ION-EXCHANGE RESIN

RESULTS

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

Amberlite XAD-7 : pH 3 :C1-C3

Weight of	Time	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
resin(g) (min)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
	0	32.43	31.26	31.85	0.0796	0.0000	-
0.1529	5	30.21	30.17	30.19	0.0755	0.0041	0.00014
0.1051	10	32.05	31.26	31.66	0.0791	0.0005	0.00001
0.1017	15	29.20	29.00	29.10	0.0728	0.0068	0.00023
0.1023	20	27.27	28.28	27.78	0.0694	0.0102	0.00037
0.1056	25	23.43	29.62	26.53	0.0663	0.0133	0.00057
0.1033	30	29.20	30.81	30.01	0.0750	0.0046	0.00016

C1: Ratio resin to starting solution : 1:1

C2: Ratio resin to starting solution : 2:1

Weight of resin(g)	Time	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	32.43	31. <mark>2</mark> 6	31.85	0.0796	0.0000	-
0.2022	5	28.88	30.15	29.52	0.0738	0.0058	0.00020
0.2073	10	30.03	29.51	29.77	0.0744	0.0052	0.00017
2.085	15	30.89	30.23	30.56	0.0764	0.0032	0.00010
0.2055	20	30.86	30.47	30.67	0.0767	0.0029	0.00010
0.2075	25	30.16	30.14	30.15	0.0754	0.0042	0.00014
0.2008	30	30.68	30.41	30.55	0.0764	0.0032	0.00011

C3: Ratio resin to starting solution : 2:2

Weight of	Time	Concentration Fime lacticacid (g/L)		ation (g/L)	Amount of lactic (g)	Lactic adsorbed(g)	a la la
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	32.43	31.26	31.85	0.1592	0.0000	-
0.2018	5	30.22	31.45	30.84	0.1542	0.0050	0.00017
0.2063	10	30.12	29.98	30.05	0.1503	0.0090	0.00030
0.2012	15	28.67	30.32	29.50	0.1475	0.0117	0.00041
0.2013	20	29.75	30.12	29.94	0.1497	0.0095	0.00032
0.2017	25	27.09	27.31	27.20	0.1360	0.0232	0.00086
0.2080	30	23.06	24.50	23.78	0.1189	0.0403	0.00175

Amberlite XAD-7 : pH 4.5 :C4-C6

Weight of Tin resin(g) (mi	Time	Concentration lacticacid (g/L)		Amount of lactic (g)	Lactic adsorbed(g)	q	
	(min)	1	2	average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	32.43	31.26	31.85	0.0796	0.0000	-
0.1038	5	30.91	30.57	30.74	0.0769	0.0028	0.00009
0.1011	10	29.05	29.56	29.31	0.0733	0.0063	0.00022
0.1036	15	30.23	30.52	30.38	0.0759	0.0037	0.00012
0.1008	20	30.11	31.00	30.56	0.0764	0.0032	0.00011
0.1086	25	31.37	31.00	31.19	0.0780	0.0016	0.00005
0.1058	30	30.94	31.14	31.04	0.0776	0.0020	0.00006

C4: Ratio resin to starting solution : 1:1

C5: Ratio resin to starting solution : 2:1

Weight of resin(g)	Time	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
	(min)	1	2	average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	32.43	31. <mark>2</mark> 6	31.85	0.0796	0.0000	-
0.2049	5	31.50	31.02	31.26	0.0782	0.0015	0.00005
0.2004	10	31.24	30.25	30.75	0.0769	0.0027	0.00009
0.204	15	32.43	30.12	31.28	0.0782	0.0014	0.00004
0.2031	20	31.99	30.01	31.00	0.0775	0.0021	0.00007
0.2054	25	32.14	30.12	31.13	0.0778	0.0018	0.00006
0.2007	30	32.17	31.24	31.71	0.0793	0.0003	0.00001

C6: Ratio resin to starting solution : 2:2

Weight of resin(g)	Time	Concentration 'ime lacticacid (g/L)		ation (g/L)	Amount of lactic (g)	Lactic adsorbed(g)	a el q
	(min)	1	2	average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	32.43	31.26	31.85	0.1592	0.0000	-
0.2095	5	31.02	29.51	30.27	0.1513	0.0079	0.00025
0.2063	10	31.3	29.72	30.51	0.1526	0.0067	0.00021
0.2015	15	30.35	29.77	30.06	0.1503	0.0089	0.00029
0.2024	20	29.8	29.78	29.79	0.1490	0.0103	0.00034
2.007	25	29.22	29.45	29.34	0.1467	0.0125	0.00043
0.2034	30	28.65	28.96	28.81	0.1440	0.0152	0.00053

Amberlite XAD-7 : pH 6 :C7-C9

C7: Ratio resin to starting solution : 1:1

Weight of Time resin(g) (min	Time	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	43.24	45.14	44.19	0.1105	0.0000	-
0.1020	5	43.66	43.12	43.39	0.1085	0.0020	0.00005
0.1033	10	42.96	42.24	42.60	0.1065	0.0040	0.00009
0.1086	15	43.04	44.34	43.69	0.1092	0.0013	0.00003
0.103	20	42.92	43.38	43.15	0.1079	0.0026	0.00006
0.1026	25	42.8	43.48	43.14	0.1079	0.0027	0.00006
0.1005	30	43.6	43.56	43.58	0.1090	0.0016	0.00004

C8: Ratio resin to starting solution : 2:1

Weight of	Time	e Concentration lacticacid (g/L)		Amount of lactic (g)	Lactic adsorbed(g)	q	
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	43.24	45. <mark>1</mark> 4	44.19	0.1105	0.0000	-
0.2038	5	39.2	39.9	39.55	0.0989	0.0116	0.0003
0.2049	10	39.98	40.46	40.22	0.1006	0.0100	0.00025
0.2084	15	39.54	38.8	39.17	0.0979	0.0126	0.0003
0.2082	20	40.08	39.7	39.89	0.0997	0.0108	0.0003
0.2088	25	42.5	41.8	42.15	0.1054	0.0051	0.0001
0.2088	30	39.28	39.52	39.40	0.0985	0.0120	0.0003

C9: Ratio resin to starting solution : 2:2

Weight of resin(g)	Time	Concentration Fime lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	43.24	45.14	44.19	0.2210	0.000	-
0.2000	5	38.5	39.1	38.80	0.1940	0.0270	0.0006
0.2085	10	36.2	36.6	36.40	0.1820	0.0390	0.00108
0.2070	15	36.8	39.5	38.15	0.1908	0.0303	0.0008
0.2027	20	40	39.1	39.55	0.1978	0.0233	0.0006
0.2045	25	40.5	40.3	40.40	0.2020	0.0190	0.0005
0.2023	30	37.6	36.9	37.25	0.1863	0.0348	0.0009

Amberlite IRA-400 : pH 3 :C10-C12

Weight of Time resin(g) (min)	Time	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin	
	0	30.96	31.35	31.1 <mark>6</mark>	0.0779	0.0000	-
0.1047	5	21.69	21.54	21.62	0.0540	0.0239	0.2279
0.1007	10	21.23	20.94	21.09	0.0527	0.0252	0.2501
0.1071	15	19.64	18.69	19.17	0.0479	0.0300	0.2800
0.1027	20	19.21	19.25	19.23	0.0481	0.0298	0.2904
0.1044	25	19.94	18.71	19.33	0.0483	0.0296	0.2834
0.1028	30	19.36	19	19.18	0.0480	0.0300	0.2913

C10: Ratio resin to starting solution : 1:1

C11: Ratio resin to starting solution : 2:1

Weight of	Time	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
resin(g) (min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin	
	0	30.96	31. <mark>35</mark>	31.16	0.0779	0.0000	-
0.2005	5	12.74	19.31	16.03	0.0401	0.0378	0.1887
0.2025	10	15.23	16.36	15.80	0.0395	0.0384	0.1897
0.2036	15	16.66	16.5	16.58	0.0415	0.0365	0.1790
0.2039	20	13.52	14.12	13.82	0.0346	0.0434	0.2126
0.2041	25	13.75	13.34	13.55	0.0339	0.0440	0.2158
0.2086	30	13.63	13.48	13.56	0.0339	0.0440	0.2110

C12: Ratio resin to starting solution : 2:2

Weight of	Time	Time Cone lactic		ation (g/L)	Amount of lactic (g)	Lactic adsorbed(g)	q q
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
	0	30.96	31.35	31.16	0.1558	0.0000	-
0.2050	5	23.22	22.06	22.64	0.1132	0.0426	0.2078
0.2004	10	22.26	20.9	21.58	0.1079	0.0479	0.2390
0.2049	15	21.37	21.31	21.34	0.1067	0.0491	0.2396
0.2081	20	19.81	19.64	19.73	0.0986	0.0572	0.2747
0.2013	25	20.29	20.22	20.26	0.1013	0.0545	0.2709
0.2063	30	19.42	19.31	19.37	0.0968	0.0590	0.2859

Amberlite IRA-400 : pH 4.5 :C13-C15

Weight of resin(g)	Time (min)	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
_		1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	36.49	36.43	36.46	0.0912	0.0000	-
0.1056	5	28.36	27.99	28.18	0.0704	0.0208	0.1966
0.1028	10	27.91	28.13	28.02	0.0701	0.0212	0.2057
0.1085	15	26.83	25.76	26.30	0.0657	0.0255	0.2347
0.1008	20	27.62	27.76	27.69	0.0692	0.0220	0.2180
0.1025	25	2 <mark>5.3</mark> 2	27.28	26.30	0.0658	0.0255	0.2483
0.105	30	27 <mark>.1</mark> 1	27.35	27.23	0.0681	0.0231	0.2202

C13: Ratio resin to starting solution : 1:1

C14: Ratio resin to starting solution : 2:1

Weight of resin(g)	Time (min)	C la	oncentra cticacid	ation (g/L)	Amount of lactic (g)	Lactic adsorbed(g)	q
		1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	36.49	36.43	36.46	0.0912	0.0000	-
0.2056	5	26.32	26.27	26.30	0.0657	0.0255	0.1238
0.204	10	25.83	25.69	25.76	0.0644	0.0268	0.1314
0.2007	15	25.53	25.61	25.57	0.0639	0.0273	0.1359
0.2057	20	24.84	24.97	24.91	0.0623	0.0289	0.1407
0.207	25	24.55	24.53	24.54	0.0614	0.0299	0.1442
0.2007	30	26.15	26.14	26.15	0.0654	0.0258	0.1287

C15: Ratio resin to starting solution : 2:2

Weight of	Time	Time Concentration la (g/L)		lacticacid	cticacid Amount of Lactic lactic (g) adsorbed(g)		Q E q
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	50.1	50.01	50.06	0.2503	0.0000	-
0.2019	5	36.14	33.98	35.06	0.1753	0.0750	0.3715
0.2053	10	37.23	37.57	37.40	0.1870	0.0633	0.3083
0.2012	15	36.92	37.88	37.40	0.1870	0.0633	0.3146
0.2044	20	36.45	36.82	36.64	0.1832	0.0671	0.3284
0.2016	25	36.81	35.41	36.11	0.1806	0.0698	0.3460
0.2017	30	36.95	36.6	36.78	0.1839	0.0664	0.3293

Amberlite IRA-400 : pH 6 :C16-C18

Weight of	Time	me (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	50.1	50.01	50.06	0.1251	0.0000	-
0.1004	5	37.38	37. <mark>56</mark>	37.47	0.0937	0.0314	0.3130
0.1034	10	36.14	33.41	34.78	0.0869	0.0382	0.3691
0.1007	15	23.1	23.33	23.22	0.0580	0.0671	0.6660
0.1033	20	38.55	38.45	38.50	0.0963	0.0289	0.2793
0.102	25	36.57	36.83	36.70	0.0918	0.0334	0.3270
0.1045	30	26.36	26.36	26.36	0.0659	0.0592	0.5665

C16: Ratio resin to starting solution : 1:1

C17: Ratio resin to starting solution : 2:1

Weight of	Time	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	50.1	5 0.01	50.06	0.1251	0.0000	-
0.2010	5	25.68	24.98	25.33	0.0633	0.0618	0.3073
0.2032	10	25.51	27.34	26.43	0.0661	0.0590	0.2905
0.2015	15	33.23	33.02	33.13	0.0828	0.0423	0.2099
0.2076	20	32.97	33.57	33.27	0.0832	0.0419	0.2020
0.2078	25	33.63	33.65	33.64	0.0841	0.0410	0.1973
0.2047	30	33.04	33.64	33.34	0.0834	0.0418	0.2040

C18: Ratio resin to starting solution : 2:2

Weight of resin(g)	ight of Time sin(g) (min)		ntration (g/L)	lacticacid	Amount of lactic (g)	Lactic adsorbed(g)	ลีย ฯ
9		1	2	Average	C*V(g)	C0*V-C*V	(C0*V-C*V)/weight of resin
-	0	50.1	50.01	50.06	0.2503	0.0000	-
0.2084	5	36.87	36.99	36.93	0.1847	0.0657	0.3150
0.2034	10	36.46	39.04	37.75	0.1888	0.0616	0.3026
0.2039	15	36.59	37.62	37.11	0.1855	0.0648	0.3177
0.2036	20	36.77	34.41	35.59	0.1780	0.0724	0.3554
0.2041	25	36.77	36.52	36.65	0.1832	0.0671	0.3286
0.2047	30	37.23	37.38	37.31	0.1865	0.0638	0.3116

Dowex Marathon WBA: pH 3 :C19-C21

Weight of	Time	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	31.06	30.94	31.00	0.0775	0.0000	-
0.1022	5	26.49	26.75	26.62	0.0666	0.0110	0.1071
0.1057	10	26.89	26.84	26.87	0.0672	0.0103	0.0978
0.1079	15	27.43	27.18	27.31	0.0683	0.0092	0.0856
0.1008	20	27.07	27.7	27.39	0.0685	0.0090	0.0897
0.1042	25	27.48	27.38	27.43	0.0686	0.0089	0.0857
0.1036	30	27.51	27.39	27.45	0.0686	0.0089	0.0857

C19: Ratio resin to starting solution : 1:1

C20: Ratio resin to starting solution : 2:1

Weight of	Time	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	31.06	30.94	31.00	0.0775	0.0000	-
0.2011	5	30.9	30.78	30.84	0.0771	0.0004	0.0020
0.2048	10	25.43	26.64	26.04	0.0651	0.0124	0.0606
0.2019	15	25.95	26.08	26.02	0.0650	0.0125	0.0617
0.2084	20	25.55	25.57	25.56	0.0639	0.0136	0.0653
0.2034	25	25.93	25.99	25.96	0.0649	0.0126	0.0619
0.2040	30	25.6	25.58	25.59	0.0640	0.0135	0.0663

C21: Ratio resin to starting solution : 2:2

Weight of	Time	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	a f a
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	31.06	30.94	31.00	0.1550	0.0000	-
0.2008	5	30.62	30.34	30.48	0.1524	0.0026	0.0129
0.2028	10	30.14	30.12	30.13	0.1507	0.0043	0.0214
0.2061	15	29.67	29.92	29.80	0.1490	0.0060	0.0292
0.2022	20	29.74	30.21	29.98	0.1499	0.0051	0.0253
0.2013	25	28.95	29.9	29.43	0.1471	0.0079	0.0391
0.2026	30	29.22	27.05	28.14	0.1407	0.0143	0.0707

Dowex Marathon WBA: pH4.5 :C22-C24

Weight of	Time	ime (g/L)		Amount of lactic (g)	Lactic adsorbed(g)	q	
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	33.77	32.96	33.37	0.0834	0.0000	-
0.1014	5	29.53	30.17	29.85	0.0746	0.0088	0.0865
0.1018	10	30.01	30.16	30.09	0.0752	0.0082	0.0804
0.1007	15	29.89	27.63	28.76	0.0719	0.0115	0.1142
0.1026	20	30.22	28.88	29.55	0.0739	0.0095	0.0928
0.1018	25	30.73	30.37	30.55	0.0764	0.0070	0.0690
0.1048	30	29.2 <mark>2</mark>	27.6	28.41	0.0710	0.0124	0.1181

C22: Ratio resin to starting solution : 1:1

C23: Ratio resin to starting solution : 2:1

Weight of	Time	Concentration lacticacid (g/L)			Amount of lactic (g)	Lactic adsorbed(g)	q
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	33.77	32.96	33.37	0.0834	0.0000	-
0.2059	5	29.2	29.36	29.28	0.0732	0.0102	0.0495
0.2021	10	28.74	29.35	29.05	0.0726	0.0108	0.0534
0.2049	15	28.97	28.35	28.66	0.0717	0.0118	0.0573
0.2078	20	27.7	28.27	27.99	0.0700	0.0134	0.0647
0.2032	25	27.08	28.52	27.80	0.0695	0.0139	0.0684
0.2027	30	28.47	28.57	28.52	0.0713	0.0121	0.0597

C24: Ratio resin to starting solution : 2:2

Weight of	Time	Conce	ncentration lacticacid (g/L)		Amount of lactic (g)	Lactic adsorbed(g)	Q q
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	33.77	32.96	33.37	0.1668	0.0000	-
0.2000	5	33.2	33.31	33.26	0.1663	0.0005	0.0026
0.2002	10	30.42	33.47	31.95	0.1597	0.0071	0.0353
0.2070	15	32.86	32.62	32.74	0.1637	0.0031	0.0150
0.2068	20	33.19	31.7	32.45	0.1622	0.0046	0.0221
0.2067	25	32.8	32.64	32.72	0.1636	0.0032	0.0155
0.2037	30	33.2	32.88	33.04	0.1652	0.0016	0.0079

Dowex Marathon WBA: pH6:C25-C27

Weight of	Time	Conce	ntration (g/L)	lacticacid	Amount of lactic (g)	Lactic adsorbed(g)	q
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	50.69	49.82	50.26	0.1256	0.0000	-
0.1012	5	44.16	45.41	44.79	0.1120	0.0136	0.1348
0.1073	10	44.87	45.13	45.00	0.1125	0.0131	0.1221
0.1054	15	43.4	43.79	43.60	0.1090	0.0166	0.1576
0.1001	20	42.98	43.02	43.00	0.1075	0.0181	0.1808
0.1030	25	42.74	43.94	43.34	0.1084	0.0173	0.1675
0.1014	30	42.06	42.1	42.08	0.1052	0.0204	0.2012

C25: Ratio resin to starting solution : 1:1

C26: Ratio resin to starting solution : 2:1

Weight of	Time	Conce	ntration (g/L)	lacticacid	Amount of lactic (g)	Lactic adsorbed(g)	q
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	50.69	4 <mark>9</mark> .82	50.26	0.1256	0.0000	-
0.2006	5	44.58	43.69	44.14	0.1103	0.0153	0.0761
0.2038	10	41.9	43.3	42.60	0.1065	0.0191	0.0937
0.2035	15	43.65	44.91	44.28	0.1107	0.0149	0.0732
0.2021	20	44.26	43.9	44.08	0.1102	0.0154	0.0762
0.2041	25	43.25	40.25	41.75	0.1044	0.0212	0.1040
0.2008	30	44.04	43.91	43.98	0.1099	0.0157	0.0780

C27: Ratio resin to starting sol	ution :	

Weight of	Time (min)	Concer	ntration l (g/L)	acticacid	Amount of lactic (g)	Lactic adsorbed(g)	
resin(g)	(min)	1	2	Average	C*V(g)	C ₀ *V-C*V	(C ₀ *V-C*V)/weight of resin
-	0	50.69	49.82	50.26	0.2513	0.0000	-
0.2033	5	48	48.9	48.45	0.2423	0.0091	0.0445
0.2007	10	48.85	49.27	49.06	0.2453	0.0060	0.0299
0.2029	15	48.82	47.66	48.24	0.2412	0.0101	0.0498
0.2061	20	48.13	48.23	48.18	0.2409	0.0104	0.0505
0.2074	25	48.81	47.96	48.39	0.2419	0.0094	0.0452
0.2033	30	47.87	48.37	48.12	0.2406	0.0107	0.0526

C28: Amount of lactic acid desorption in shack flask

		Amo	ount (g)				Amount of	desorption(g)		% Lactic acid
Time (min)	Glucose	Lactic acid	Fumaric acid	Ethanol	Eluants	Glucose	Lactic acid	Fumaric acid	Ethanol	Recovery
Amount of lactiac loaded	0.0663	3.6608	0.0053	0.3698	$1M H_2SO_4$	0.0017	0.0237	0.0000	0.0000	38
20	0.0000	0.1191	0.0000	0.0063	$2M H_2SO_4$	0.0006	0.0222	0.0000	0.0000	35
40	0.0082	0.4103	0.0003	0.0307	1M HCl	0.0013	0.0250	0.0000	0.0000	40
60	0.0070	0.4257	0. <mark>00</mark> 06	0.0368	2M HCl	0.0016	0.0236	0.0000	0.0000	38
75	0.0132	0.3358	0.0005	0.0287	1M H ₃ PO ₄	0.0000	0.0256	0.0000	0.0000	41
90	0.0000	0.3213	0.0021	0.0241	2M H ₃ PO ₄	0.0000	0.0216	0.0000	0.0000	34
105	0.0000	0.2668	0.0000	0.0201	1M NH ₄ OH	0.0000	0.0140	0.0000	0.0000	22
120	0.0000	0.3240	0.0000	0.0245	2M NH ₄ OH	0.0000	0.0125	0.0000	0.0000	20
Total amount of	0.0294	2 2020	0.0025	0.1710	1M NaCl	0.0000	0.0222	0.0000	0.0000	35
non lactic adsorbed (g)	0.0284	2.2030	0.0033	0.1710						
Amount of lactiac	0.0270	1 4570	0.0018	0 1000						
acid adsorbed (g)	0.0379	1.43/0	0.0018	0.1900						

Amount of resin 11.6 g was adsorbed lactiac acid = 1.4578 g

Amount of resin 0.5 g in each flask was adsorbed lactic acid 0.0628 g

		Amou	nt (g)				Amount of	of desorption(g)	
Time (min)	Glucose	Lactic acid	Fumaric acid	Ethanol	Time (min)	Glucose	Lactic acid	Fumaric acid	Ethanol
Amount of lactiac					10				
loaded	0.5450	2.6172	0.5879	0.5864	10	0.0000	0.0319	0.0008	0.0031
10	0.0004	0.0000	0.0000	0.0000	20	0.0091	0.1280	0.0020	0.0007
20	0.0007	0.0948	0.0000	0.0029	30	0.0023	0.0313	0.0025	0.0000
30	0.0043	0.2796	0.0010	0.0216	40	0.0002	0.0031	0.0000	0.0000
40	0.0054	0.3182	0.0000	0.0216	50	0.0000	0.0000	0.0000	0.0000
50	0.0064	0.3203	0.0021	0.0244	60	0.0000	0.0000	0.0000	0.0000
60	0.0059	0.3388	0.0021	0.0204	70	0.0000	0.0000	0.0000	0.0000
70	0.0066	0.3076	0.0028	0.0195	80	0.0000	0.0000	0.0000	0.0000
80	0.0047	0.2851	0.0201	0.0200	90	0.0000	0.0000	0.0000	0.0000
90	0.0082	0.3052	0.0014	0.0199	100	0.0000	0.0000	0.0000	0.0000
Total amount of	0.0426	2 2405	0.0205	0 1502	110	0.0000	0.0000	0.0000	0.0000
non lactic adsorbed (g)	0.0420	2.2493	0.0293	0.1303	120	0.0000	0.0000	0.0000	0.0000
Amount of lactiac					Total amount				
acid adsorbed (g)	0.5024	0.3677	0.5585	0.4361	of desorption(g)	0.0116	0.1943	0.0052	0.0039

C29: Amount of lactic acid adsorption and desorption with 1.0 M NaCl



		Amo	ount (g)				Amount of	lesorption(g)	
Time (min)	Glucose	Lactic acid	Fumaric acid	Ethanol	Time (min)	Glucose	Lactic acid	Fumaric acid	Ethanol
Amount of lactiac loaded	0.0553	3.0507	0.0044	0.3081	10	0.0000	3.0507	0.0000	0.3081
10	0.0003	0.0018	0.0000	0.0000	20	0.0000	0.0018	0.0000	0.0000
20	0.0013	0.0593	0.0000	0.0037	30	0.0016	0.0593	0.0000	0.0037
30	0.0000	0.2812	0.0000	0.0000	40	0.0002	0.2812	0.0000	0.0000
40	0.0000	0.3269	0.0000	0.0000	50	0.0002	0.3269	0.0000	0.0000
50	0.0087	0.3420	0.0000	0.0000	60	0.0000	0.3420	0.0000	0.0000
60	0.0067	0.3631	0.0000	0.0321	70	0.0000	0.3631	0.0000	0.0321
70	0.0066	0.3309	0.0000	0.0257	80	0.0000	0.3309	0.0000	0.0257
80	0.0000	0.3269	0.0000	0.0000	90	0.0000	0.3269	0.0000	0.0000
90	0.0000	0.3436	0.0000	0.0000	100	0.0000	0.3436	0.0000	0.0000
100	0.0036	0.3379	0.0000	0.0000	110	0.0000	0.3379	0.0000	0.0000
Total amount of	0.0072	0 7129	0.0000	0.0(14	120	0.0000	2.7138	0.0000	0.0614
non lactic adsorbed (g)	0.0273	2.7138	0.0000	0.0614	Total amount				
Amount of lactiac	0.0280	0.3369	0.0044	0.2467	of desorption(g)	0.0020	0.3369	0.0000	0.2467
acid adsorbed (g)				- A - A - A - A - A - A - A - A - A - A					

C30: Amount of lactic acid adsorption and desorption with 1.0 M HCl



		Amo	ount (g)				Amount of	f desorption(g)	
Time (min)	Glucose	Lactic acid	Fumaric acid	Ethanol	Time (min)	Glucose	Lactic acid	Fumaric acid	Ethanol
Amount of lactiac loaded	0.0553	3.0507	0.0044	0.3081	10	0.0000	0.0067	0.0044	0.3081
10	0.0003	0.0018	0.0000	0.0000	20	0.0000	0.0042	0.0000	0.0000
20	0.0013	0.0593	0.0000	0.0037	30	0.0000	0.0034	0.0000	0.0037
30	0.0000	0.2812	0.0000	0.0000	40	0.0000	0.0033	0.0000	0.0000
40	0.0000	0.3269	0.0000	0.0000	50	0.0013	0.0488	0.0000	0.0000
50	0.0087	0.3420	0.0000	0.0000	60	0.0060	0.0923	0.0000	0.0000
60	0.0067	0.3631	0.0000	0.0321	70	0.0000	0.0408	0.0000	0.0321
70	0.0066	0.3309	0.0000	0.0257	80	0.0000	0.0130	0.0000	0.0257
80	0.0000	0.3269	0.0000	0.0000	90	0.0000	0.0035	0.0000	0.0000
90	0.0000	0.3436	0.0000	0.0000	100	0.0000	0.0018	0.0000	0.0000
100	0.0036	0.3379	0.0000	0.0000	110	0.0000	0.0004	0.0000	0.0000
Total amount of	0.0272	0 7120	0.0000	0.0(14	120	0.0000	0.0000	0.0000	0.0614
non lactic adsorbed (g)	0.0273	2.7138	0.0000	0.0614	Total amount	0.0053	0 0101	0.0044	0.04/7
Amount of lactiac	0.0290	0 2260	0.0044	0.2467	of desorption(g)	0.0073	0.2181	0.0044	0.2467
acid adsorbed (g)	0.0280	0.3369	0.0044	0.240/	20				

C31: Amount of lactic acid adsorption and desorption with 1.0 M H₃PO₄

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

		Amo	ount (g)			0		Amount of	f desorption(g)	
Time (min)	Glucose	Lactic acid	Fumaric acid	Ethanol		Time (min)	Glucose	Lactic acid	Fumaric acid	Ethanol
Amount of lactiac loaded	0.0464	2.4819	0.0430	0.4684		10	0.0000	0.0072	0.0002	0.0003
10	0.0000	0.0013	0.0000	0.0000		20	0.0000	0.0030	0.0000	0.0000
20	0.0015	0.0742	0.0014	0.0107		30	0.0000	0.0018	0.0000	0.0000
30	0.0037	0.2489	0.0000	0.0434		40	0.0000	0.0015	0.0000	0.0000
40	0.0048	0.2989	0.0000	0.0519		50	0.0013	0.0230	0.0000	0.0000
50	0.0047	0.2866	0.0050	0.0503		60	0.0022	0.0368	0.0000	0.0000
60	0.0057	0.3156	0.0052	0.0548		70	0.0024	0.0348	0.0000	0.0000
70	0.0056	0.3005	0.0039	0.0507		80	0.0010	0.0111	0.0000	0.0000
80	0.0056	0.3162	0.0048	0.0532		90	0.0003	0.0027	0.0000	0.0000
90	0.0058	0.3493	0.00 <mark>5</mark> 3	0.0574		100	0.0000	0.0003	0.0000	0.0000
Total amount of	0.0270	0 1016	0.0050	0.2722		110	0.0000	0.0004	0.0000	0.0000
non lactic adsorbed (g)	0.0372	2.1916	0.0256	0.3723		120	0.0000	0.0000	0.0000	0.0000
Amount of lactiac acid adsorbed (g)	0.0092	0.2903	0.0174	0.0961	1525	Total amount of desorption(g)	0.0072	0.1224	0.0003	0.0003

C32: Amount of lactic acid adsorption and desorption with 1.0 M H_2SO_4



		Amo	ount (g)				Amount of	desorption(g)	
Time (min)	Glucose	Lactic acid	Fumaric acid	Ethanol	Time (min)	Glucose	Lactic acid	Fumaric acid	Ethanol
Amount of lactiac loaded	0.7467	1.7838	0.0000	0.9584	10	0.0058	0.0018	0.0000	0.0009
10	0.0172	0.0062	0.0000	0.0052	20	0.0107	0.0567	0.0000	0.0014
20	0.0500	0.0512	0.0000	0.0504	30	0.0000	0.1083	0.0000	0.0020
30	0.0662	0.1518	0.0000	0.1066	40	0.0000	0.0072	0.0000	0.0000
40	0.0740	0.1848	0.0000	0.1126	50	0.0000	0.0000	0.0000	0.0000
50	0.0710	0.1718	0.0000	0.0097	60	0.0000	0.0000	0.0000	0.0000
60	0.0729	0.1680	0.0000	0.0981	70	0.0000	0.0000	0.0000	0.0000
70	0.0732	0.1703	0.00 <mark>0</mark> 0	0.0962	80	0.0000	0.0000	0.0000	0.0000
80	0.0716	0.1659	0.0000	0.0939	90	0.0000	0.0000	0.0000	0.0000
90	0.0731	0.1607	0.0000	0.0895	100	0.0000	0.0000	0.0000	0.0000
100	0.0772	0.1628	0.0000	0.0918	110	0.0000	0.0000	0.0000	0.0000
110	0.0772	0.1625	0.0000	0.0930	120	0.0000	0.0000	0.0000	0.0000
Total amount of	0 7025	1 55(1	0.0000	0.0470	Total amount	0.01/5	0 1540	0.0000	0.0044
non lactic adsorbed (g)	0.7235	1.5561	0.0000	0.8470	of desorption(g)	0.0165	0.1740	0.0000	0.0044
Amount of lactiac	0.0232	0 2277	0.0000	0 1113	U.				
acid adsorbed (g)	0.0232	0.4477	0.0000	0.1113					

C33: Amount of lactic acid adsorption and desorption with 1.0 M H₃PO₄+1.0 M H₂SO₄:70:30

สถาบนวทยบรการ จุฬาลงกรณ์มหาวิทยาลัย





APPENDIX D

ELECTRODIALYSIS

RESULTS

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

D1: Operating condition 1	Voltage	5 V	
	Flow rate	6 ml/min	
	Lactic acid	40 g/L	
	Sodiumlactate	20 g/L	
	NaOH	0.1 M	
	рН	6	

Time	Current		Sodiumlac	tate		Lactic ad	cid	NaOH
(min)	(mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0	14.99	2.2 <mark>5</mark>		39.68	5.95		0.1102
20	47.6	16.08	2.41	0.16	36.11	5.42	0.54	0.1140
40	42.1	16.24	2.44	0.19	35.67	5.35	0.60	0.1300
60	36	16.51	2.48	0.23	34.45	5.17	0.79	0.1460
80	39.4	16.86	2.53	0.28	32.46	4.87	1.08	0.1620
100	42.7	17.87	2.68	0.43	32.13	4.82	1.13	0.1840
120	40.2	17.95	2.69	0.44	30.73	4.61	1.34	0.1900
140	44.1	18.33	2.75	0.50	31.14	4.67	1.28	0.2140
160	12.4	18.83	2.82	0.58	31.12	4.67	1.28	0.2220
180	32.6	18.91	2.84	0.59	29.32	4.40	1.55	0.2365

Total J _s	=	0.74 mol/m ² .h
Specific energy consumption (ε)	=	0.9096 KWh/Kg
% Recovery	=2	38 %

จฬาลงกรณมหาวทยาลย

D2 :Operating condition 2	Voltage	8 V
	Flow rate	6 ml/min
	Lactic acid	40 g/L
	Sodiumlactate	20 g/L
	NaOH	0.1 M
	рН	6

		Sodiumlactate				NaOH		
Time (min)	Current (mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0.00	29.27	4.39	1111111111	40.47	6.07	0.00	0.1110
20	82.03	33.12	4.97	0.58	38.61	5.79	0.28	0.1340
40	73.05	37.23	5.58	1.19	34.53	5.18	0.89	0.1700
60	70.00	37.44	5.62	1.23	30.45	4.57	1.50	0.1820
80	56.12	37.45	5.62	1.23	30.07	4.51	1.56	0.2100
100	54.38	37.64	5.65	1.26	28.21	4.23	1.84	0.2240
120	50.04	37.92	5.69	1.30	28.62	4.29	1.78	0.2600
140	44.09	38.26	5.74	1.35	24.40	3.66	2.41	0.2840
160	40.08	42.39	6.36	1.97	28.84	4.33	1.74	0.3120
180	35.09	43.42	6.51	2.12	25.11	3.77	2.30	0.3365

Total J_s Specific energy consumption (ε) % Recovery = 2.69 mol/m².h = 0.6122 KWh/Kg = 92 %

จุฬาลงกรณ์มหาวิทยาลัย

D3:Operating condition 3	Voltage	10 V
	Flow rate	6 ml/min
	Lactic acid	40 g/L
	Sodiumlactate	20 g/L
	NaOH	0.1 M
	pН	6

		Sodiumlactate		Lactic acid			NaOH	
Time (min)	Current (mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0	17.19	2.58	A STI COURT A	40.21	4.02		0.1110
20	10.5	18.48	2.77	0.19	40.03	4.00	0.02	0.0860
40	9.02	18.84	2.83	0.25	37.37	3.74	0.28	0.1340
60	8.7	19.02	2.85	0.28	35.72	3.57	0.45	0.1160
80	8.47	19.50	2.92	0.35	34.84	3.48	0.54	0.1160
100	8.79	19.62	2.94	0.37	33.78	3.38	0.64	0.1140
120	7.15	19.92	2.99	0.41	33.13	3.31	0.71	0.1160
140	4.01	20.79	3.12	0.54	32.52	3.25	0.77	0.1280
160	18.2	21.34	3.20	0.62	31.06	3.11	0.92	0.1320
180	16.97	22.44	3.37	0.79	30.80	3.08	0.94	0.1404

Total J _s		1.00	mol/m ² .h	
Specific energy consumption (ε)	=	0.4228	KWh/Kg	
% Recovery	<u>ุลฬาสงกร</u>	84	%	

D4 :Operating condition 4	Voltage	8 V
	Flow rate	6 ml/min
	Lactic acid	40 g/L
	Sodiumlactate	40 g/L
	NaOH	0.1 M
	pH	6

Time		Sodiumlactate			Lactic acid			
(min)	Current (mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0.00	34.03	5.10	sacab.	38.25	5.74		0.0900
20	12.50	35.69	5.35	0.25	35.29	5.29	0.44	0.0920
40	25.10	36.59	5.49	0.38	35.27	5.29	0.45	0.1000
60	37.40	36.90	5.53	0.43	34.96	5.24	0.49	0.1200
80	17.20	34.58	5.19	0.08	34.45	5.17	0.57	0.1260
100	20.80	36.57	5.49	0.38	34.06	5.11	0.63	0.1420
120	14.40	36.99	5.55	0.44	33.20	4.98	0.76	0.1440
140	18.00	38.13	5.72	0.61	32.67	4.90	0.84	0.1660
160	17.80	37.77	5.67	0.56	32.18	4.83	0.91	0.1540
180	12.20	38.65	5.80	0.69	32.05	4.81	0.93	0.1769

Total J_s

Specific energy consumption (ε) % Recovery 0.88 mol/m².h 0.6518 KWh/Kg 74 %

=

ฬาลงกรณมหาวทยาลย

D5 :Operating condition 5	Voltage	8V
	Flow rate	6 ml/min
	Lactic acid	40 g/L
	Sodiumlactate	0 g/L
	NaOH	0.1 M
	рН	6

	Sodiumlactate		Lactic acid			NaOH		
Time (min)	Current (mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0.00	0.00	0.00		35.92	5.39		0.1100
20	10.60	0.00	0.00	0.00	34.73	5.21	0.18	0.1200
40	7.60	0.52	0.08	0.08	33.91	5.09	0.30	0.1220
60	4.80	0.78	0.12	0.12	33.54	5.03	0.36	0.1400
80	5.10	1.03	0.15	0.15	33.13	4.97	0.42	0.1440
100	5.50	1.38	0.21	0.21	33.68	5.05	0.34	0.1500
120	6.50	1.81	0.27	0.27	31.89	4.78	0.60	0.1600
140	5.50	1.94	0.29	0.29	31.49	4.72	0.66	0.1720
160	7.60	3.22	0.48	0.48	30.70	4.60	0.78	0.1760
180	5.30	6.35	0.95	0.95	29.02	4.35	1.03	0.1769

Total J_s Specific energy consumption (ε) % Recovery



D6 :Operating condition 6	Voltage	8 V
	Flow rate	6 ml/min
	Lactic acid	40 g/L
	Sodiumlactate	20 g/L
	NaOH	0.1 M
	рН	6

T • (•)		Sodiumlactate			NaOH			
Time (min)	Current (mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0.00	29.27	4.39	Sacal.	44.54	6.68	6.68	0.1110
20	82.03	33.12	4.97	0.58	38.61	5.79	5.79	0.1340
40	73.05	37.23	5.58	1.19	34.53	5.18	5.18	0.1700
60	70.00	37.44	5.62	1.23	30.45	4.57	4.57	0.1820
80	56.12	37.45	5.62	1.23	30.07	4.51	4.51	0.2100
100	54.38	37.64	5.65	1.26	28.21	4.23	4.23	0.2240
120	50.04	37.92	5.69	1.30	28.62	4.29	4.29	0.2600
140	44.09	38.26	5.74	1.35	24.40	3.66	3.66	0.2840
160	40.08	42.39	6.36	1.97	28.84	4.33	4.33	0.3120
180	35.09	43.42	6.51	2.12	25.11	3.77	3.77	0.3365

Total J_s Specific energy consumption (ε) % Recovery

 $= 2.69 \text{ mol/m}^2.\text{h} = 0.6122 \text{ KWh/Kg} = 56 \%$

D7 :Operating condition 7	Voltage	8 V
	Flow rate	4 ml/min
	Lactic acid	40 g/L
	Sodiumlactate	20 g/L
	NaOH	0.1 M
	рН	6

		Sodiumlactate		etate		NaOH		
Time (min)	Current (mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0.00	19.81	2.97	Sala a	45.28	6.79		0.1102
20	44.00	20.18	3.03	0.06	45.07	6.76	0.03	0.1140
40	49.70	20.21	3.03	0.06	44.08	6.61	0.18	0.1300
60	53.80	20.44	3.07	0.09	43.97	6.60	0.20	0.1460
80	38.80	20.91	3.14	0.17	42.41	6.36	0.43	0.1620
100	52.40	21.26	3.19	0.22	41.25	6.19	0.60	0.1840
120	53.80	22.02	3.30	0.33	40.89	6.13	0.66	0.1900
140	44.00	22.15	3.32	0.35	40.39	6.06	0.73	0.2140
160	40.00	22.53	3.38	0.41	38.22	5.73	1.06	0.2220
180	35.80	22.79	3.42	0.45	37.76	5.66	1.13	0.2365

Total J_s Specific energy consumption (ϵ)

% Recovery

 $= 0.57 \text{ mol/m}^2.\text{h}$ = 2.3565 KWh/Kg = 40 %

D8 :Operating condition 8	Voltage	8 V
	Flow rate	6 ml/min
	Lactic acid	40 g/L
	Sodiumlactate	20 g/L
	NaOH	0.1 M
	рН	6

T • (•)			Sodiumlac	tate		NaOH		
Time (min)	Current (mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0.00	29.27	4.39		44.54	6.68	6.68	0.1110
20	82.03	33.12	4.97	0.58	38.61	5.79	5.79	0.1340
40	73.05	37.23	5.58	1.19	34.53	5.18	5.18	0.1700
60	70.00	37.44	5.62	1.23	30.45	4.57	4.57	0.1820
80	56.12	37.45	5.62	1.23	30.07	4.51	4.51	0.2100
100	54.38	37.64	5.65	1.26	28.21	4.23	4.23	0.2240
120	50.04	37.92	5.69	1.30	28.62	4.29	4.29	0.2600
140	44.09	38.26	5.74	1.35	24.40	3.66	3.66	0.2840
160	40.08	42.39	6.36	1.97	28.84	4.33	4.33	0.3120
180	35.09	43.42	6.51	2.12	25.11	3.77	3.77	0.3365

Total J_s Specific energy consumption (ε)

% Recovery



จุฬาลงกรณ์มหาวิทยาลัย

D9 :Operating condition 9	Voltage	8 V
	Flow rate	8 ml/min
	Lactic acid	40 g/L
	Sodiumlactate	20 g/L
	NaOH	0.1 M
	рН	6

	Current (mA) —		Sodiumlac	etate		NaOH		
Time (min)	Current (mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0	18.53	2.78	A CONTRACTOR A	44.62	6.69		0.1000
20	47.6	20.86	3.13	0.35	42.33	6.35	0.34	0.1040
40	42.1	21.05	3.16	0.38	42.00	6.30	0.39	0.1060
60	36	21.25	3.19	0.41	41.53	6.23	0.46	0.1120
80	39.4	21.31	3.20	0.42	40.97	6.15	0.55	0.1200
100	42.7	21.64	3.25	0.47	40.76	6.11	0.58	0.1220
120	40.2	21.78	3.27	0.49	40.55	6.08	0.61	0.1240
140	44.1	21.92	3.29	0.51	40.27	6.04	0.65	0.1280
160	12.4	21.94	3.29	0.51	40.00	6.00	0.69	0.1300
180	32.6	22.09	3.31	0.53	39.70	5.95	0.74	0.1269

Total J_s Specific energy consumption (ε) % Recovery = 0.68 mol/m².h = 0.1632 KWh/Kg = 72 %

เพาลงการแมหาาวทยาละ

D10 :Operating condition 1	Voltage	8 V	
	Flow rate	6 ml/min	
	Lactic acid	40 g/L	
	Sodiumlactate	20 g/L	
	NaOH	0.1 M	
	рН	3	

Time		Sodiumlactate			Lactic acid			NaOH
(min)	Current (mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0.00	16.23	2.43		35.61	5.34		0.1102
20	10.90	16.54	2.48	0.05	35.31	5.30	0.05	0.1240
40	11.90	16.61	2.49	0.06	34.96	5.24	0.10	0.1240
60	11.60	16.87	2.53	0.10	34.43	5.16	0.18	0.1360
80	8.90	17.06	2.56	0.13	33.06	4.96	0.38	0.1380
100	9.10	17.38	2.61	0.17	32.08	4.81	0.53	0.1400
120	9.10	17.91	2.69	0.25	31.31	4.70	0.65	0.1460
140	9.80	18.12	2.72	0.28	30.80	4.62	0.72	0.1600
160	11.80	19.85	2.98	0.54	30.66	4.60	0.74	0.1620
180	11.60	20.43	3.06	0.63	30.28	4.54	0.80	0.1577

Total J_s Specific energy consumption (ϵ)

% Recovery

mol/m².h 0.80 = 0.3760 KWh/Kg = 79 %

84

D11 :Operating condition 11	Voltage	8 V
	Flow rate	6 ml/min
	Lactic acid	40 g/L
	Sodiumlactate	20 g/L
	NaOH	0.1 M
	рН	6

T· (•)	$C_{unrest}(mA) =$		Sodiumlactate		Lactic acid			NaOH
Time (min)	Current (mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0.00	29.27	4.39		44.54	6.68	6.68	0.1110
20	82.03	33.12	4.97	0.58	38.61	5.79	5.79	0.1340
40	73.05	37.23	5.58	1.19	34.53	5.18	5.18	0.1700
60	70.00	37.44	5.62	1.23	30.45	4.57	4.57	0.1820
80	56.12	37.45	5.62	1.23	30.07	4.51	4.51	0.2100
100	54.38	37.64	5.65	1.26	28.21	4.23	4.23	0.2240
120	50.04	37.92	5.69	1.30	28.62	4.29	4.29	0.2600
140	44.09	38.26	5.74	1.35	24.40	3.66	3.66	0.2840
160	40.08	42.39	6.36	1.97	28.84	4.33	4.33	0.3120
180	35.09	43.42	6.51	2.12	25.11	3.77	3.77	0.3365

Total J_s Specific energy consumption (ε)

% Recovery



D12 :Operating condition 12	Voltage	8 V
	Flow rate	6 ml/min
	Lactic acid	40 g/L
	Sodiumlactate	20 g/L
	NaOH	0.1 M
	pН	9

Time		Sodiumlactate			Lactic acid			NaOH
(min)	Current (mA)	C (g/L)	Amount(g)	Increasing amount (g)	C(g/L)	Amount(g)	Reducing amount (g)	C (M)
0	0.00	22.52	3.38		49.72	5.95		0.1000
20	58.20	24.79	3.72	0.34	48.93	5.42	0.12	0.1120
40	52.10	24.98	3.75	0.37	46.52	5.35	0.48	0.1200
60	73.70	25.91	3.89	0.51	44.35	5.17	0.80	0.1400
80	74.90	27.19	4.08	0.70	43.38	4.87	0.95	0.1600
100	73.90	28.37	4.26	0.88	41.85	4.82	1.18	0.2080
120	70.09	29.61	4.44	1.06	41.77	4.61	1.19	0.2400
140	85.20	31.43	4.71	1.34	39.76	4.67	1.49	0.2540
160	89.00	35.57	5.34	1.96	37.44	4.67	1.84	0.2600
180	96.00	38.12	5.72	2.34	35.67	4.40	2.11	0.2692

Total J_s Specific energy consumption (ε) % Recovery = 2.97 mol/m².h = 0.7890 KWh/Kg = 100 %

จุฬาลงกรณ์มหาวิทยาลัย



Figure D1 The HPLC chromatogram of lactic acid from electrdialysis purification

APPENDIX E

Calculation of ion-exchange recovery, flux (J_s), specific

energy consumption (ϵ) , and elctrodialysis recovery

สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

E1: Ion-exchange recovery

Table E1 Amount of adsorption and desorption of fixed-bed adsorptionwith $1.0MH_3PO_4 + 1.0MH_2SO_4$:70:30

	Amount of adsorption(g)	Amount of desorption(g)
Glucose	0.0232	0.0165
Lactic acid	0.2277	0.1740
Fumaric acid	0.0000	0.0000
Ethanol	0.1113	0.0044

%Lactic acid recovery = $\frac{\text{Amount of desorption (g)}}{\text{Amount of adsorption (g)}} \times 100$

$$= \frac{0.1740 \text{ g}}{0.2277 \text{ g}} \times 100$$

% Purity

 $\frac{\text{Amount of lactic desorption (g)}}{\text{Total amount of desorption (g)}} \times 100$

 $= \frac{0.1740 \text{ g}}{0.0165 + 0.1740 + 0.0044 \text{ g}} \ge 100$

= 90 %

	$\mathbf{J}_{s} = -$	$\frac{\Delta m_s}{A_m \Delta t}$
$\mathbf{J}_{\mathbf{s}}$	is	Flux of lactate
Δm_s	is	mass of lactate through the membrane
A _m	is	area of membrane
Δt	is	time

Example; Amount of lactate increasing from 4.39 g to 6.51 g through the effective area of membrane 29.25 cm^2 for 3 hours.

$$J_{s} = \frac{\Delta m_{s}}{A_{m}\Delta t}$$
$$J_{s} = \frac{(6.52 - 4.39)/90}{(29.25/100000)x3}$$

= 2.69 mol/m²h

E4: Specific energy consumption (ε)

$$\varepsilon = \frac{\Phi \int I dt}{\Delta m_s}$$

$$\varepsilon = Specific energy consumption$$

$$\Phi = voltage (V)$$

$$I = current$$

$$t = time$$

$$\Delta m_s = mass of lactate through the membrane$$

Example; According to table D1 in appendix D : voltage was 5 V and amount of lactate increasing from 2.25 g to 2.84 g

$$\varepsilon = \frac{\Phi \int Idt}{\Delta m_s}$$
$$\varepsilon = \frac{\phi \times \text{peak area of I vs t plot}}{\Delta m_s}$$

$$\varepsilon = \frac{5 \times 0.1069}{2.84 - 2.25}$$
$$\varepsilon = 0.9096 \text{ KWh/Kg}$$

E5: Elctrodialysis recovery

Example; According to table D1 in appendix D

% Recovery
$$= \frac{\text{Total increasing amount of lactate (g)}}{\text{Total reducing amount of lactic acid (g)}} \times 100$$

$$=\frac{0.59 \text{ g}}{1.55 \text{ g}} \times 100$$
VITA

Ms. Wasinee Boonkong was born on Wednesday 19th August, 1981, in Chachoengsao, Thailand. In 2004, she graduated with a Bachelor's degree of Science in Chemistry, from Chulalongkorn University. After that, she has been studied for a Master's degree of Science in Petrochemical and polymer science, Faculty of Science, Chulalongkorn University, and completed the program in 2006.



สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย