Potential Use of Palmyra Sap and Oil Palm Sap for Lactic Acid Production by *Lactobacillus casei* TISTR 1500

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ชื่อวิทยานิพนธ์	ศักยภาพการใช้ของน้ำตาลโตนดและน้ำบีบแกนในลำต้นปาล์มน้ำมัน สำหรับผลิตกรดแลคติกโดย *Lactobacillus casei* TISTR 1500
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บทคัดย่อ

การศึกษาการผลิตกรดแลคติกจากน้ำตาลโตนดและน้ำบีบแกนในลำต้นปาล์มน้ำมันจากเชื้อ *Lactobacillus casei* TISTR 1500 โดยศึกษาปัจจัยของการไฮโดรไลซีสด้วยกรด การควบคุมพีเอช และการเสริมแหล่งอาหารของน้ำตาลโตนดและน้ำบีบแกนในลำต้นปาล์มน้ำมันต่อประสิทธิภาพการหมัก พบว่า การหมักกรดแลคติกด้วยน้ำตาลโตนดไม่ได้ส่งผลอย่างมีนัยสำคัญทั้งจากการไฮโดรไลซีสด้วยกรดหรือการควบคุมพีเอช การเติมแหล่งอาหาร MRS จะเพิ่มชีวมวลและผลได้ผลิตภัณฑ์ การเพิ่มความเข้มข้นของน้ำตาลทั้งหมดในน้ำตาลโตนดจนถึง 134.0 g L^{-1} ทำให้ค่าความเข้มข้นสุดท้ายกรดแลคติก น้ำหนักเซลล์แห้ง และผลผลิตเพิ่มมากขึ้น ค่าจลนพลศาสตร์ของน้ำตาลโตนดที่ความเข้มข้นน้ำตาลทั้งหมด 134.0 g L^{-1} พบว่า อัตราการเจริญจำเพาะ (μ) เท่ากับ 0.05 h^{-1} ผลผลิตสูงสุด (R_M) เท่ากับ 2.02 g lactic acid L^{-1} h^{-1} สัมประสิทธิ์ผลได้ของเซลล์ ($Y_{X/S}$) เท่ากับ 0.20 g cell g^{-1} sugar และผลได้ผลิตภัณฑ์ ($Y_{P/S}$) เท่ากับ 0.78 g g^{-1} การใช้น้ำบีบแกนในลำต้นปาล์มน้ำมันเป็นแหล่งคาร์บอนด้วย *Lactobacillus casei* TISTR 1500 พบว่า การควบคุมพีเอชไม่ได้ส่งผลอย่างมีนัยสำคัญต่อการผลิตกรดแลคติก การเติมแหล่งอาหาร MRS เป็นแหล่งคาร์บอนจะปรับปรุงชีวมวลและผลได้ผลิตภัณฑ์ ประสิทธิภาพการหมักของน้ำบีบแกนในลำต้นปาล์มน้ำมันด้วยแหล่งอาหาร MRS ในการผลิตกรดแลคติก สภาวะนิ่ง อุณหภูมิ 37°C พีเอช 5.5 น้ำตาลทั้งหมด 20 g L^{-1} สามารถปรับปรุงผลผลิตได้เท่ากับ 0.55 g L^{-1} h^{-1} น้ำบีบแกนในลำต้นปาล์มน้ำมันจึงเป็นแหล่งวัตถุดิบที่มีศักยภาพในการผลิตกรดแลคติกด้วย *Lactobacillus casei* TISTR 1500

การศึกษาเพื่อแยกกรดแลคติกจากน้ำหมักสังเคราะห์และน้ำหมักชีวภาพด้วยเรซินพอลิเมอร์ (Amberlite IRA402 และ Dowex™ 66) พบว่า สมดุลการดูดซับกรดแลคติกที่ความเข้มข้น 100 g L^{-1} ด้วยเรซินทั้งสองมีค่าเป็น 227.00 mg lactic acid g^{-1} dry resin และ 148.60 mg lactic acid g^{-1} dry resin ตามลำดับ ไอโซเทอร์มการดูดซับสามารถทำนายได้ดีด้วยแบบจำลองฟรุนดลิคช์ เปรียบเทียบกับแบบจำลองแลงเมียร์และแบบจำลองดูบินิน ราดัชเควิส การ

แยกคืนกรดแลคติกจากของผสมสังเคราะห์ที่ถูกดูดซับด้วยเรซิน Dowex™ 66 (46.75%) มีค่ามากกว่าเรซิน Amberlite IRA402 (34.35%) ด้วยน้ำเป็นตัวชะล้าง การศึกษาผลได้ของการคายซับ (desorption yield) ของกรดแลคติกในการดูดซับด้วยคอลัมน์ของเรซิน Dowex™ 66 จากน้ำหมักสังเคราะห์และน้ำหมักชีวภาพเท่ากับ 84.91% และ 75.16% ตามลำดับ

การศึกษาปฏิกิริยาเอสเตอริฟิเคชันของกรดแลคติกกับเอทานอลปนน้ำด้วยการกลั่นแบบเกิดปฏิกิริยาโดยมี Amberlyst 15 เป็นตัวเร่งปฏิกิริยาและการทำบริสุทธิ์เอทิลแลคเตทจากผลิตภัณฑ์เอสเตอร์ด้วยการกลั่นแบบสองขั้นตอน ในการศึกษาปฏิกิริยาเอสเตอริฟิเคชันซึ่งดำเนินการในปฏิกรณ์แบบกึ่งกะร่วมกับการกลั่น ภายใต้ความดันบรรยากาศ จากความเข้มข้นของเอทานอล 70.0-99.7 wt% พบว่า การเพิ่มความเข้มข้นของเอทานอลส่งผลอย่างมีนัยสำคัญต่อค่าการเปลี่ยนแปลงกรดแลคติก (conversion) และผลได้เอทิลแลคเตท (yield) เพิ่มขึ้น การแยกคืนเอทิลแลคเตทบริสุทธิ์ด้วยการกลั่นแบบสองขั้นตอน พบว่า การเพิ่มขึ้นของความเข้มข้นของเอทานอล 70.0-99.7 wt% ทำให้เกิดการเปลี่ยนแปลงขององค์ประกอบกรดแลคติกเพิ่มขึ้น องค์ประกอบโอลิโกเมอร์กรดแลคติกและโอลิโกเมอร์เอทิลแลคเตทเกิดการเปลี่ยนแปลงเพิ่มขึ้นที่ความเข้มข้นเอทานอลในช่วง 70.0-95.0 wt% ซึ่งสอดคล้องกับการเปลี่ยนแปลงเพิ่มขึ้นขององค์ประกอบของเอทิลแลคเตทในช่วงความเข้มข้นเอทานอลดังกล่าว ปริมาณน้ำเริ่มต้นจึงมีอิทธิพลอย่างมากต่อเอทิลแลคเตทในการทำปฏิกิริยาและการทำบริสุทธิ์ ความเข้มข้นของเอทานอล 95.0 wt% จึงมีศักยภาพสำหรับการผลิตเอทิลแลคเตทเพื่อใช้เป็นสารตั้งต้นในการไฮโดรไลซีสเป็นกรดแลคติก

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ABSTRACT

Production of lactic acid from sap of palmyra (*Borassus flabellifer* Linn.) and oil palm (*Elaeis guineensis*) as substrates by *Lactobacillus casei* TISTR 1500 were investigated in this study. The effects of acid hydrolysis, pH control and nutrient supplement of palmyra sap and oil palm sap on fermentation performance were investigated. It was found that lactic acid fermentation using palmyra sap was not significantly affected by either acid hydrolysis or pH control. The addition of MRS increased biomass and product yield. The final lactic acid concentration, dry cell weight and productivity were increased by increasing the total sugars of palmyra sap concentrations up to 134.0 g L^{-1}. The kinetic parameters for the palmyra sap at 134.0 g L^{-1} total sugars were calculated to be of: specific growth rate (μ) 0.05 h^{-1}, the maximum productivity (R_M) 2.02 g lactic acid L^{-1} h^{-1}, cellular yield coefficient ($Y_{X/S}$) 0.20 g cell g^{-1} sugar, and lactic acid yield ($Y_{P/S}$) 0.78 g g^{-1}. When oil palm sap was used as carbon source for *L. casei* TISTR 1500, pH control did not significantly affect the lactic acid production. The addition of MRS medium into oil palm sap improved the biomass and the product yield for which the lactic acid production in static flask at 37°C and pH 5.5 using 20 g L^{-1} of total sugars was improved to be of 0.55 g L^{-1} h^{-1}. Therefore, oil palm sap could be served as a good potential source of raw materials for efficient production of lactic acid by *L. casei* TISTR 1500.

Separation of lactic acid from a synthetic broth and fermentation broth using polymeric resins (Amberlite IRA402 and Dowex™ 66 resins) was studied. Experimental results of lactic acid adsorption equilibrium indicated that the adsorption capacity of lactic acid at 100 g L^{-1} onto Amberlite IRA402 and Dowex™ 66 resins was 227.00 mg lactic acid g^{-1} dry resin and 148.60 mg lactic acid g^{-1} dry

resin, respectively. The adsorption isotherms were closely predicted by the Freundlich model between the two isotherm models (Langmuir model and Dubinin-Radushkevich) tested. Moreover, the recovery of lactic acid from Dowex ^{TM} 66 resin (46.75%) was higher than Amberlite IRA402 resin (34.35%) when using water as an eluant from synthetic broth. The desorption yield of column adsorption by Dowex^{TM} 66 resin for synthetic broth and fermentation broth using water in washing step were of 84.91% and 75.16%, respectively.

Esterification of lactic acid with wet ethanol (70.0-99.7 wt%) by semi-batch catalytic distillation using a cation exchange resin (Amberlyst 15) as an acid catalyst and the purification of ethyl lactate from ester product by the two step distillation were studied. The effects of ethanol concentrations on the esterification and the purification were investigated. It was found that ethanol concentration significantly affected the on conversion and the reaction yield, which increased with the increment of ethanol concentration. The recovery of pure ethyl lactate through the two step distillation was studied. As the ethanol concentration (70.0-99.7 wt%) increased, the overall change of lactic acid component was negatively increased. Furthermore, lactic acid and ethyl lactate oligomer components increased with increasing ethanol concentration in the range from 70.0-95.0 wt%. Ethyl lactate component from mass balance increased with increasing ethanol concentration of 70.0 to 95.0 wt% in the two step distillation. Initial water content had a great influence since ethyl lactate was proportional to water concentration in a reactor and the distillation column. Ethanol concentration at 95.0 wt% was proven to be potential reactant for ethyl lactate production in order for hydrolysis to lactic acid.

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LIST OF ABBREVIATIONS AND SYMBOLS

Ac	=	Acetic Acid
q_e	=	Amount of Adsorption at Equilibrium
t	=	Breakthrough Time
$Y_{X/S}$	=	Cellular Yield Coefficient
$Y_{P/S}$	=	Conversion of Yield of Substrate to Product
MRS	=	de Man, Rogosa, Sharpe
R_L	=	Dimensionless Separation Factor
L_2E	=	Dimer Ethyl Lactate
L_2A	=	Dimer Lactic Acid
DCW	=	Dry Cell Weight
E	=	Energy of Adsorption
EtOH	=	Ethanol Component
L_1E	=	Ethyl Lactate
EtLa	=	Ethyl Lactate Component
C_e	=	Equilibrium Concentration of Lactic Acid in Solution
k_F	=	Freundlich Constant
R	=	Gas Constant
HPS	=	Hydrolyzed Palmyra Sap
IS	=	Internal Standard
C_0	=	Initial Concentration of Lactic Acid Solution
q_m	=	Isotherm Constant
L_1A	=	Lactic Acid
LA and LE Oligomers	=	Lactic Acid and Ethyl Lactate Oligomers
Lactic	=	Lactic Acid Component
k_L, a_L	=	Langmuir Constant
R_M	=	Maximum Productivity
N/A	=	Not Available
OPS	=	Oil Palm Sap
PS	=	Palmyra Sap
ε	=	Polanyipotential

LIST OF ABBREVIATIONS AND SYMBOLS (CONTINUED)

μ	=	Specific Growth Rate
T	=	Temperature
k_{Th}	=	Thomas Rate Constant
L_3E	=	Trimer Ethyl Lactate
L_3A	=	Trimer Lactic Acid
τ	=	Time Required for 50%Adsorbate Breakthrough
Q	=	Volume Flow Rate
H_2O	=	Water
k_{YN}	=	Yoon – Nelson Constant

CHAPTER 1

INTRODUCTION

1.1 Rational/Problem Statement

Lactic acid is a versatile industrial chemical used as an acidulant and preservative in food industry as well as other applications such as a controlled drug delivery agent in pharmaceutical industry (Sreenath *et al.*, 2001), a cleaning agent and green solvent in chemical, moisturizers and skin-lightening agents in cosmetic industry (Olmos-Dichara *et al.*, 1997). Moreover, it is employed as a precursor for a production of emulsifiers such as stearoyl-2-lactylates for baking industries (Schepers *et al.*, 2002). The current global demand for lactic acid is estimated at 70,000 (metric) tons per year. The huge expansion of lactic acid demand in a global market is driven greatly by development of more economically large-scale fermentation process. This can help reduce the cost of lactic acid and make it more attractive for various uses, for instance, a production of biodegradable plastics, namely poly lactic acid (PLA), which currently still have a higher price than petroleum-based plastics. Cargill Dow LLC, a primary US manufacturer of PLA, has reported that the global PLA market is likely to expand up to 500,000 (metric) tons per year by 2010 (Wee *et al.*, 2006). Such a great demand of lactic acid should be promised with cost-effective process of lactic acid production by microbial fermentation. Many efforts have been tried from many points of view to improve fermentation efficiency as well as to minimize the production cost. These include well-performing microbial bacteria selection, high productivity process development, implementation of economical process for lactic acid recovery and application of low-cost feedstock and chemicals in fermentation process.

Lactic acid has been produced by either fermentation or a synthetic method. Approximately, 90% of the total lactic acid produced worldwide has been obtained by bacterial fermentation, and which the rest has synthesized by the hydrolysis of lactonitrile. The chemical synthesis of lactic acid always results in racemic mixture of lactic acid, which is a major disadvantage. Fermentative production of lactic acid offers the advantages in both utilization of renewable carbohydrates and production of

optically pure L- or D-lactic acid depending on the strain selected (Patil *et al.*, 2006). The efficiency and economics of the ultimate lactic acid fermentation is however still a problem from many points of view and media compositions play a vital role in the improvement of such the process. In recent years, research effort is focused on looking for new and effective nutritional sources and new progressive fermentation techniques enabling the achievement of both high substrate conversion and high production yields (Bulut *et al.*, 2004). A number of substrates have been used for biotechnological production of lactic acid, including glucose, sucrose, lactose, maltose, mannose, xylose, and galactose. The most pure product has been obtained when the pure sugar was fermented, resulting in the lower purification cost. However, there is economically unfavorable, because pure sugars are expensive and lactic acid is a relatively cheap product. To replace these refined and costly raw materials, the application of agricultural resource is promising to be used as resource an attractive because of their low prices (John *et al.*, 2006; Tanaka *et al.*, 2006). Using cheap raw materials as a fermentation substrate for lactic acid is an alternative way in reducing the cost of lactic acid production.

Palmyra palms (*Borassus flabellifer* Linn.) are abundant in the southern part of Thailand from Phetchaburi to Songkhla provinces. Most palmyra palms populate in Songkhla province, approximately 3 millions plants. The most important product of palmyra palm is the sap. The total and reducing sugars in palmyra sap, which is rich in sucrose as dominant sugar, vary in the range of 10.36%-16.94% and 0.88%-3.56%, respectively (Naknean *et al.*, 2010).

Oil palm (*Elaeis guineensis*) is widely planted for its edible oil in tropical countries such as Malaysia, Indonesia and Thailand. Palm oil is the most produced plant oil, with a worldwide production of 4.3 million tons in 2008. Oil palm sap has been reported to contain large quantities of high glucose content sap. Glucose has been found to be the dominant sugar in all parts, accounting for approximately 86.9%, 86.3% and 65.2% of the total free sugars obtained for the inner, middle and outer parts, respectively (Kosugi *et al.*, 2010). By contrast, Eze and Organ (1988) reported that oil palm sap collected in Nigeria, by tapping at the base of the inflorescence, contained sucrose as the dominant sugar (10% w/v). Similar results have been

reported on sap of Raphia palm (*Raphia hookeri*) in Nigeria, with sucrose as the dominant sugar (Obahiagbon and Osagie, 2007). The discrepancy may be due to the difference in varieties, species and/or cultivation conditions. Another possibility is that the sugar composition of sap collected from felled palm trunk differs from that of sap collected by tapping the base of the inflorescence. Oil palm sap has been found to be rich in various kinds of amino acids, organic acids, minerals and vitamins. Based on these findings, the sap can be fermented to produce ethanol using the sake brewing yeast strain, *Saccharomyces cerrevisiae* Kyokai no.7, and lactic acid using the homolactic acid bacterium, *Lactobacillus lactis* ATCC19435 (Kosugi *et al.*, 2010).

During the fermentation process, sugars are converted into lactic acid and organic acids. Then, fermentation broth of lactic acid contains impurities such as residual sugars, nutrients and other organic acid, apart from cell mass. These impurities are needed to be removed for obtaining pure lactic acid. The recovery of lactic acid is rather difficult due to its chemical behavior, as it shows strong affinity to water and low volatility. Therefore, its purification is the most cost-incentive processing step. Separation process takes a large part of the total cost in lactic acid production. Commercially, lactic acid is recovered as calcium lactate by precipitation. The disadvantages of this method are the high product loss during crystallization and be environmental problems caused by the formation of a large quantity of calcium sulphate (gysum). Many separation methods such as reactive extraction, adsorption, electrodialysis and esterification hydrolysis with distillation have been studied for lactic acid recovery (Boniardi *et al.*, 1997; Siebold *et al.*, 1995; Chen and Lee, 1997; Evangelista and Nikolov, 1996; Kwon *et al.*, 1996; Planas *et al.*, 1999; Jeantet *et al.*, 1996; Russo *et al.*, 1996). Out of these impurities, other organic acids are effectively removed only by esterification-distillation step. Esterification is efficiently carried out by employing reactive distillation using cation exchange resin columns acting as a packing material as well as a catalyst. However, lactic acid, fed to these columns must be free of residual sugars and colour. This purification can be achieved by using adsorption. The adsorption is a process suitable for recovering substance produced in dilute concentrations and in complex solutions, such as fermentation broth. Synthetic

polymeric sorbents such as PVP (Zheng *et al.*, 1996), IRA-420 (Antonio *et al.*, 2000), IRA-400 (Cao *et al.*, 2002), DOWEX-50W (Choi and Hong, 1999) with varying degrees of basicity have been used in the recovery of carboxylic acid from fermentation broth (Evangelista and Nikolov, 1996).

In this study, first, we determined physical and chemical composition of agricultural sources, palmyra sap and oil palm sap to evaluate their suitability as substrates for the production of lactic acid by *Lactobacillus casei* TISTR 1500, and to investigate the effects of acid hydrolysis of palmyra sap to obtain the sap that contains high fermentable reduced sugar for use as a substrate for bioconversion. The effect of pH control and nutrient supplementation of the palmyra sap, hydrolyzed palmyra sap and oil palm sap were also investigated for maximizing biomass and lactic acid production. The influence of pH on lactic acid production was studied by comparing fermentations between two conditions: with initial pH of 5.5 that was allowed to vary, and with a constant pH at 5.5 in stirred tank bioreactor.

Secondly, the study to focus on the use of anionic polymeric resin for the separation of lactate from synthetic mixtures and fermentation broth. The ion-exchange characteristics were determined in term of ion exchange isotherms. Also, the influence of components in broth and the ability of lactic acid desorption from resins were investigated.

Finally, the research was focused at developing a technology of producing ethyl lactate from lactic acid with wet ethanol and using heterogeneous catalyst and concentrated lactic acid in order for hydrolysis to pure lactic acid.

1.2 Objectives of the study

1. To produce lactic acid using *Lactobacillus casei* TISTR 1500 cultured in palmyra sap and oil palm sap as substrates.
2. To study adsorption and desorption of lactic acid from synthetic mixtures and fermentation broth using Amberlite IRA 402 and Dowex^{TM} 66 resin.
3. To produce ethyl lactate from lactic acid and wet ethanol as catalyzed by Amberlyst 15 using catalytic distillation combined with two step distillation in order for hydrolysis to pure lactic acid.

CHAPTER 2

LITERATURE REVIEWS

2.1 Lactic acid-producing microorganism

Lactic acid has a long history of uses for fermentation and preservation of human foodstuff (Davison *et al.*, 1995). It was first discovered in sour milk by Scheele in 1780, who initially considered it as a milk component. In 1789, Lavoisier named this milk component as acide lactique, which became the possible origin of the current terminology for lactic acid production. In 1857, however, Pasteur discovered that it was not a milk component, but a fermentation metabolite generated by certain microorganism (Benninga, 1990).

Lactic acid is now considered to be one of the most useful chemicals, used in the food industry as a preservative, acidant, and a flavouring agent, in the textile and pharmaceutical industries, and in chemical industries as a raw material for the production of lactate ester, propylene glycol, 2, 3-pentanedione, propionic acid, acrylic acid, acetaldehyde, and dilactide (Varadarajanand and Miller, 1999; Akerberg and Zacchi, 2000). Lactic acid consumption has increased considerably because of its role as a monomer in the production of biodegradable PLA, which is well-known as a sustainable bioplastic material (Datta *et al.*, 1995; Litchfield, 1996). The worldwide demand for lactic acid is estimated roughly to be 130,000 to 150,000 (metric) tones per year (Mirasol, 1999). However, the global consumption of lactic acid is expected to increase rapidly in the near future. Nature Works LLC, a major PLA manufacturer established in the US, expects that the global PLA market may increase to 500,000 (metric) tones per year by the year 2010 (http://www.chemsystems.com).

Lactic acid can be produced by either microbial fermentation or chemical synthesis (Figure 2.1) (Wee *et al.*, 2006). In the early 1960s, a method to synthesize lactic acid chemically was developed due to the need for heat-stable lactic acid in the baking industry (VickRoy, 1985). There are two optical isomers of lactic acid: L-(+)-lactic acid and D-(-)-lactic acid. Lactic acid is classified as GRAS (generally

recognized as safe) for use as a food additive by the US FDA (Food and Drug Administration), but D(-)-lactic acid is at times harmful to human metabolism and can result acidosis and decalcification (Datta *et al.*, 1995). Although racemic DL-lactic acid is always produced by chemical synthesis from petrochemical resources, an optically pure L-(+)-or D(-)-lactic acid can be obtained by microbial fermentation of renewable resources when the appropriate microorganism that can produce only one of the isomers is selected (Hofvendahl and Hahn-Hagerdal, 2000). The optical purity of lactic acid is crucial to the physical properties of polylactic acid (PLA), and an optically pure L-(+)- or D(-)-lactic acid, rather than racemic DL-lactic acid, can be polymerized with high crystallinity. PLA is suitable for commercial uses (Lunt, 1998; Sodergard and Stolt, 2002). Therefore, the biotechnological production of lactic acid has received a significant amount of interest recently, since it offers an alternative to environmental pollution caused by the petrochemical industry and the limited supply of petrochemical resources.

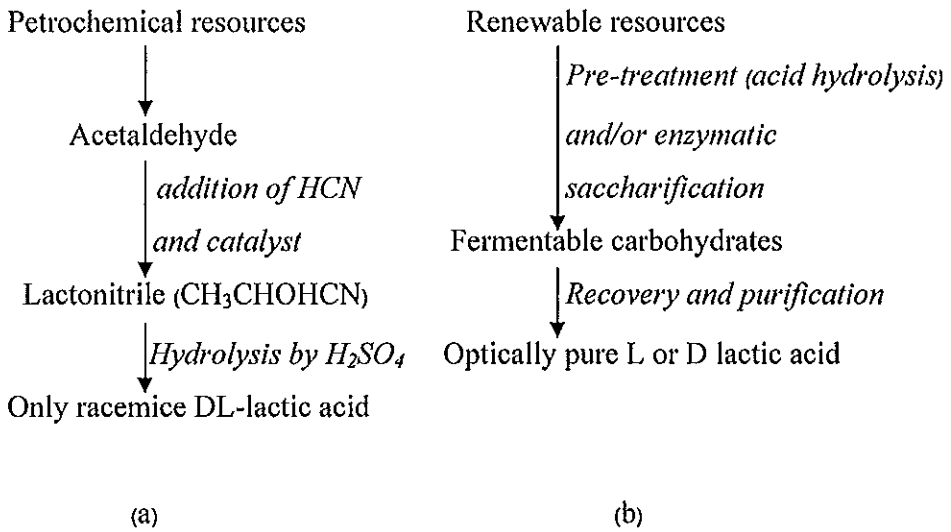


Figure 2.1 Overview of two industrially manufacturing processes of lactic acid (a) chemical synthesis and (b) microbial fermentation (Wee *et al.*, 2006)

Lactic acid bacteria (LAB) are a group of related bacteria that produce lactic acid as a major metabolic product. LAB has the property in producing lactic acid from carbohydrates through fermentation. Moreover, LAB can grow at temperature 5-45°C and not surprisingly are tolerant to acidic conditions, with most strains able to grow at pH 4.4. The growth is optimum at pH 5.5-6.5 and the organisms have complex nutritional requirements for amino acids, peptides, nucleotide bases, vitamins, minerals, fatty acids and carbohydrates. The genus is divided into three groups based on fermentation patterns:

- Homofermentative: this group of LAB produces more than 85% lactic acid from glucose. The LAB ferment one mole of glucose to two moles of lactic acid, generating a net yield of 2 moles of ATP per mole of glucose.

- Heterofermentative: produce 50% lactic acid. The LAB in this group ferment 1 mole of glucose to 1 mole of lactic acid, 1 mole of ethanol, and 1 mole of carbon dioxide. One mole of ATP is generated per mole of glucose, resulting in less growth per mole of glucose.

- Less well known heterofermentative species which produce DL-lactic acid, acetic acid and carbon dioxide.

In Table 2.1, species of the genera *Lactobacillus, Soprolactobacillus, Streptococcus, Lueconostoc, Pediococcus*, and *Bifidobacterium* were listed.

Table 2.1 List of homo- and hetero-fermentative lactic acid bacteria and configuration of lactic acid produced (Wee *et al.*, 2006)

Bacteria	Homo-	Hetero-	Configuration
Lactobacillus			
L. delbrueckii	+	-	D(-)
L. lactis	+	-	D(-)
L. bulgaricus	+	-	D(-)
L. casei	+	-	L(+)
L. plantarum	+	-	DL
L. curvatus	+	-	DL
L. brevis	-	+	DL
L. fermentum	-	+	DL
Sporolactobacillus			
S. inulinus	+	-	D(-)
Streptococcus			
S. faecalis	+	-	L(+)
S. cremoris	+	-	L(+)
S. lactis	+	-	L(+)
Leuconostoc			
L. mesenteroides	-	+	D(-)
L. dextranicum	-	+	D(-)
Pediococcus			
P. damnosus	+	-	DL
Bifidobacterium			
B. bifidum	-	+	L(+)

2.2 Factors affecting lactic acid production

2.2.1 pH

The fermentation pH is either set at the beginning or then left to decrease due to acid production or controlled by base titration, extraction, adsorption, and electro dialysis of LA. The effect of pH has been studied by fermenting at various pH values.

pH has been known to be the most important parameter related to the stability of the enzyme (Wasewar *et al.*, 2004). When glucose was converted into lactic acid, the pH fell. As a result, the rate of enzymatic reaction decreased. If the pH drops further, deactivation of enzyme was accelerated. It was essential to control the pH so that the rate of reaction was maintained at its maximum level. The enzymatic reaction suffered not only from the substrate inhibition but also the competitive inhibition by lactic acid. Moreover, the pH effects on bacterial cell growth were in part due to the concentrations of dissociated and undissociated forms of lactic acid in the broth. Zhang *et al.* (2007), indicating that pH was one of the most crucial operational factors affecting lactic acid production. The effect of pH has been demonstrated by conducting fermentation at various pH values. In all cases, titration to a constant pH resulted in higher or equal lactic acid concentration, yield and productivity in comparison without pH control. When pH was not controlled, it dropped from 5.85 to 3.2 in 24 h. After that, no more lactate was produced. Only 3.3 g lactate L^{-1} was produced compare with 96 g L^{-1} when pH was held constant at 6.0. It was recognized that a favorable pH range was 5.0-6.0 (Hofvendahl and Hahn-Hagerdal, 2000). Huang *et al.* (2003) and Tay and Yang (2002) found that production of acid, ethanol and fumaric acid decreased as pH decreased from 6.0 to 4.0. The result of Miura *et al.* (2003) showed that the highest lactic acid yield (93 g L^{-1}) was achieved at pH 6.0-6.5. A pH below 5.7 was only optimal for *Lactobacillus* strains, which were known to tolerate to lower pH more than lactococci (Kashket, 1987). Fu and Mathews (1999) reported lactic acid production from lactose without pH control and indicated that lactose slightly inhibited cell growth in the exponential growth phase, while there was no effect at the stationary and the death phases. Mussatto *et al.* (2008) reported the effects of medium supplementation and pH control on lactic acid production from brewer's spent grain. Addition of 5 g L^{-1} yeast extract enhanced the lactic acid volumetric productivity, attaining 0.53 g L^{-1} h^{-1}, 18% higher than that obtained from non-supplemented hydrolysate. Addition of MRS broth (except the carbon source) was still better, providing a productivity of 0.79 g L^{-1} h^{-1}. In all cases, the lactic acid yield factor was of 0.7 g g^{-1} glucose consumed, but the fermentations stopped after 24 h due to the pH drop from 6.0 to 4.2, resulting in large amounts of

residual glucose (38-41 g L^{-1}). Fermentation running pH-controlled at 6.0 gave better results than those where the initial pH was not controlled. The best result, 35.54 g L^{-1} lactic acid (0.99 g g^{-1} glucose consumed) was obtained during the pH-controlled fermentation of hydrolysate medium supplemented with MRS. The volumetric productivity at the end of this fermentation was 0.59 g L^{-1} h^{-1}, with a maximum yield of 0.82 g L^{-1} h^{-1} during the first 12 h.

2.2.2 Carbon sources

In order for the biotechnological production of lactic acid to be feasible, cheap raw materials are necessary, because polymer producers and other industrial users usually require large quantities of lactic acid at a relatively low cost. Raw materials for lactic acid production should have the following characteristics: cheap, low levels of contaminants, rapid production rate, high yield, little or no by-product formation, and ability to be fermented with little or no pre-treatment and year-round availability (VickRoy, 1985). When refined materials are used for production, the costs for product purification should be significantly reduced. However, this is still economically unfavorable because the refined carbohydrates are so expensive that eventually result in higher production costs of lactic acid. Cheap raw materials, such as whey, molasses, starch waste, whole wheat flour and beet- and cane-sugar have been used for the fermentative production of lactic acid.

Oh *et al.* (2005) studied lactic acid production from agricultural resources as cheap raw materials. The agricultural resources such as barley, wheat, and corn were hydrolyzed by commercial amylolytic enzymes and fermented into lactic acid by *Enterococcus faecalis* RKY1. Although no additional nutrients were supplemented to those resources, lactic acid productivities were obtained at >0.8 g L^{-1} h^{-1} from barley and wheat. When 200 g L^{-1} h^{-1} of whole wheat flour was hydrolyzed by amylolytic enzymes after the pre-treatment with 0.3% (vv^{-1}) sulfuric acid and sterilized by filtration, *E. faecalis* RKY1 efficiently produced lactic acid with 2.6 g L^{-1} h^{-1} of lactic acid productivity and 5.90 g L^{-1} of maximal dry cell weight without additional nutrients. Lactic acid productivity and cell growth could be enhanced from 31% to 12%, higher than those of non-adapted RKY1 by adaption of *E. faecalis*

RKY1 to CSL-based medium. When the medium was contained 200 g L^{-1} of whole wheat flour hydrolyzate, 15 g L^{-1} of corn steep liquor and 1.5 g L^{-1} of yeast extract, lactic acid productivity and maximal dry cell weight were obtained at 5.36 g L^{-1} h^{-1} and 14.08 g L^{-1}, respectively.

Bulut *et al.* (2004) reported on the effect of different sources on L-(+)-lactic acid production by *Rhizopus oryzae* using glucose, sucrose, beet molasses, carob pod and wheat bran as substrates. The highest lactic acid concentration was obtained when 150 g L^{-1} glucose was present in the medium as the sole carbon source. In this case, the lactic acid yield was approximately 60% by weight based on the amount of glucose consumed. Wheat bran was found to be an unsuitable substrate for this particular fermentation. Pasteurization of molasses increased lactic acid production rate compared to that of untreated molasses. Likewise, 58 g L^{-1} lactic acid was obtained by using the supernatant containing sugars extracted from carob pod. This medium could therefore be considered as an alternative carbon source for lactic acid production.

Dumbrepatil *et al.* (2008) reported utilization of molasses sugar for lactic acid production by *Lactobacillus delbrueckii* subsp. *delbrueckii* mutant Uc-3 in batch fermentation. Efficient lactic acid production from cane sugar molasses by *L. delbrueckii* mutant Uc-3 using molasses was not significantly affected by yeast extract concentrations. The final lactic acid concentration increased with the increment of molasses sugar concentration up to 190 g L^{-1}. The maximum lactic acid concentration of 166 g L^{-1} was obtained at a molasses sugar concentration of 190 g L^{-1} with the productivity of 4.15 g L^{-1} h^{-1}. Such a high concentration of lactic acid with high productivity from molasses has not been reported previously, and hence mutant Uc-3 could be a potential candidate for economic production of lactic acid from molasses at a commercial scale.

Panesar *et al.* (2010) studied the production of L-(+)-lactic acid using *Lactobacillus casei* from whey. Fermentations were performed without any pH control. The optimization of the fermentation conditions resulted in significant decrease in fermentation time, besides increase in lactose conversion to lactic acid.

The optimized process conditions resulted in high lactose conversion (95.62%) to L-(+)-lactic acid production (33.73 g L^{-1}) after an incubation period of 36 h.

Laopaiboon *et al.* (2010) reported acid hydrolysis of sugarcane bagasse for lactic acid production. After lignin extraction, the conditions were varied in terms of hydrochloric (HCl) or sulfuric (H_2SO_4) concentration (0.5-5% vv^{-1}), reaction time (1-5 h) and incubation temperature (90-120 °C). The maximum catalytic efficiency (E) was 10.85 under the conditions of 0.5% of HCl at 100°C for 5 h, which the main components in the hydrolysate were glucose, 1.50 g L^{-1}; xylose, 22.59 g L^{-1}; arabinose, 1.29 g L^{-1}; acetic acid, 0.15 g L^{-1} and furfural, 1.19 g L^{-1}. To increase yield of lactic acid production from the hydrolysate by *Lactobacillus lactis* IO-1, the hydrolysate was detoxified through amberlite and supplemented with 7 g L^{-1} of yeast extract. The main products of the fermentation were lactic acid, 10.85 g L^{-1}; acetic acid, 7.87 g L^{-1}; formic acid, 6.04 g L^{-1} and ethanol, 5.24 g L^{-1}.

Ohkouchi and Inoue (2006) studied factors for direct and effective lactic acid production from food wastes by *Lactobacillus manihotivorans* LMG 18011, and optimum conditions for simultaneous saccharification and fermentation using soluble starch and food waste as substrates. The productivity was found to be affected by three factors: (1) initial pH, which influenced amylase production for saccharification of starch, (2) culture pH control which influenced selective production of L-(+)-lactic acid, and (3) manganese concentration in medium which improved in production rate and yield of lactic acid. The optimum initial pH was 5.0-5.5, and the fermentation pH for the direct and effective fermentation from starchy substrate was 5.0 based on the yield of L-(+)-lactic acid. Under these conditions, 19.5 g L-(+)-lactic acid was produced from 200 g food wastes by *L. manihotivorans* LMG 18011.

Using cheap raw materials as a fermentation substrate for lactic acid is an alternative to reduce the cost of lactic acid production.

Palmyra palms (*Borassus flabellifer* Linn.) can be found in tropical countries. In Thailand, palmyra palms are crowed in southern part of Thailand from Phetchaburi to Songkhla provinces. Most populations of palmyra palms are in Songkhla province, approximately 3 millions plants (Department of agricultural

extension Thailand, 2001; Taybui, 1984). The most important product of palmyra palm is the sap or juice. The tapping process of palm sap involves the bruising of the interior of the developing inflorescences by means of a wooden maller or tong, thereby stimulating sap flow. Sap is collected by cutting the outer end at the head of the inflorescences normally in the morning and the evening. Three to six inflorescence are tided together and inserted into a suitable container for sap collection, usually using an earthware pot (in Sri Lanka) or a bamboo tube (in Thailand) (Davis and Johnson, 1987). Fresh sap is sweet, oyster white colour and transient, with nearly neutral pH (Gupta *et al.*, 1980).

Oil palm (*Elaeis guineensis*) is widely planted for its edible oil in tropical countries such as Malaysia, Indonesia and Thailand. The production of palm oil is 39 Mt per year in 2007, which is the most produced plant oil in the world. In general, the palm starts bearing oil-contained fruits in 2.5 years after planted and its productivity become lower after 20-25 years. Therefore it is necessary to cut the old palms and to replant new seeding at plantation sites. When replanting, oil palms are cut and most of them are discarded or burnt at the plantation site. Therefore, efficient ways for utilizing oil palm trunks is desired for ideal oil palm plantation and sustainable palm oil industry. The palm trunk structure is not strong enough for use as lumber, and thus, only the outer part of the trunk, which is relatively strong, is partially utilized for plywood manufacturing. In the plywood production process, the inner part is discarded in large amounts due to its extremely weak physical properties. Meanwhile, it is known that palm sugar and palm wine are produced from sap obtained by tapping the inflorescence of varieties of palm species. Sap squeezed from the inner part of oil palm or oil palm sap contains approximately 90% of total sugars (Kosugi *et al.*, 2010).

2.3 Downstream purification processes for recovery of lactic acid from the fermentation broth

2.3.1 Conventional lactic acid recovery processes

The most frequently used calcium carbonate precipitation technology includes the following steps (Figure 2.2)

1. Removal of the biomass materials by a rotary vacuum filter

2. Addition of calcium carbonate to clarify fermentation liquor to precipitate calcium lactate ($Ca_2C_3H_6O_3$)

3. Separation of calcium lactate from the fermentation liquor by a second rotary vacuum filter

4. Regeneration of lactic acid by addition of sulfuric acid (H_2SO_4) to the calcium lactate cake the precipitate of calcium sulfate (gypsum, $CaSO_4$) is formed in the step.

5. Precipitation and isolation of the gypsum, This process step is usually repeated several times in order to remove the readily carbonizable substances (RCS), the main impurities existing in the lactic acid fermentation broth. The quality of lactic acid produced is determined by the RCS presence, lower quantity means higher product quality.

6. Use of anion and cation exchangers to remove the metal ions and other ionic species, resulting in the high purity lactic acid solution.

7. Decoloration of the lactic acid solution by use of activated carbon

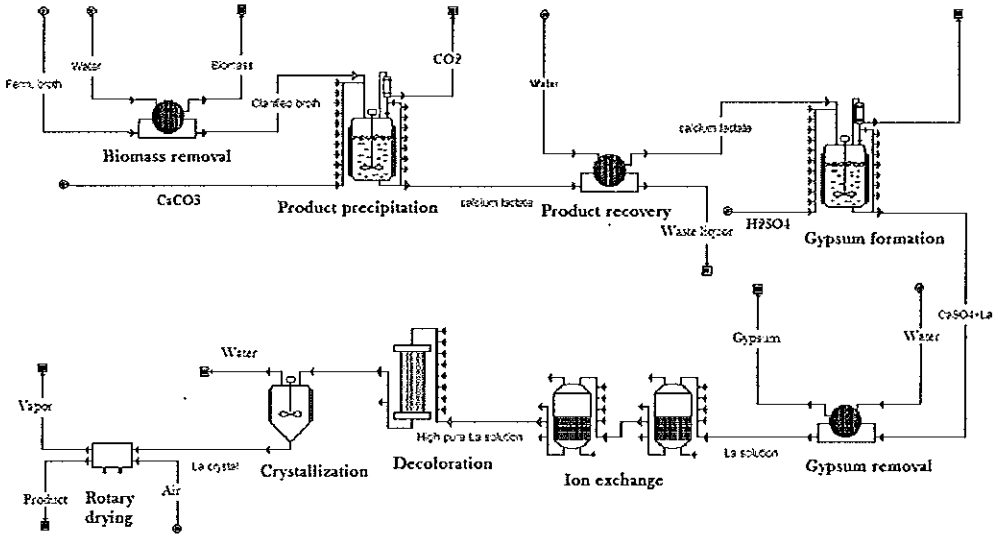


Figure 2.2 Flow sheet of the conventional process lactic acid recovery from its fermentation broth based on precipitation technology (Wu, 2009)

2.3.2 Novel lactic acid purification process

The filtered fermentation broth mainly contains impurities such as residual sugar compounds, colour and other organic acids. These impurities can be removed by reactive extraction, adsorption, electrodialysis and esterification hydrolysis with distillation.

Recovery of carboxylic acids from fermentation broths presents a challenging separation problem, because of the dilute, complex nature of fermentation broths. Methods of recovery that utilize separating agents, such as solid sorbents, that are selective for carboxylic acids are attractive and reported by many researchers (King, 1987; Tung and King, 1994). Important characteristics of extractants and solid sorbents are of high capacity for the acid as opposed to water and substrate (e.g., glucose), regenerability depending upon the process configuration, and biocompatibility with microorganisms. Many fermentation, such as that for lactic acid production, are subjected to end-product inhibition (Yabannavar and Wang, 1991; Davison and Thomson, 1992). If a solid sorbent can be used in situ or in an external recycle loop, higher overall yields can be achieved.

Many fermentation to produce carboxylic acids operate most effectively at pH above pKa of the acid product. For example, lactic acid (pKa=3.76) is typically produced at pH 5-6. One approach for recovering carboxylic acids from such solutions is to use agents that are sufficiently basic to retain substantial capacity several pH units above the pKa of the carboxylic acid. Tung and King (1994) investigated extraction and sorption of lactic and succinic acids using different basic extractants and polymeric sorbents. The results showed that the uptake in the pH range of 5-6 varied substantially from one agent to another and was strongly dependent upon the basicity and capacity of the agent. Agents to be used in fermentation processes should provide high selectivity between the product carboxylic acid and substrate sugars. In addition for conservation of substrate, one reason for high selectivity sugars in a product such as lactic acid to cause discoloration. Reported measurements of uptake capacities for sugars and for selectivity achieved between carboxylic acids and sugars with extractants and/or solid sorbents are few and fragmentary.

Kaufman *et al.* (1994) screened a series of solid sorbents preliminarily for possible utilization in biparticle fluidized-bed fermentation to produce lactic acid from immobilized *Lactobacillus delbreuckii* by measuring the selectivity between acids and sugars as well as other properties. Kaufman *et al.* (1995) presented selectivities between lactic acid and glucose obtained from a fixed-bed of the sorbent Amberlite IRA-35 under a particular set of operating conditions. Evangelista *et al.* (1994) reported breakthrough curves for lactic acid and glucose during the fixed-bed adsorption with in actual fermentation broths.

For typical lactic acid fermentations, the sorbent should demonstrate substantial uptake within the pH range of 5-6 (Tung and King, 1994; Dai and King, 1995).

Ion exchange technique is widely used in bio-separation (Houwing *et al.*, 2002; Tong *et al.*, 2001) and several different ion exchangers such as poly(4-vinyl pyridine) resin (PVP) (Zheng *et al.*, 1996), IRA-420 (Antonio *et al.*, 2000), IRA-400 (Srivastava *et al.*, 1992; Cao *et al.*, 2002) have been studied on the recovery of lactic

acid in the past few years. However, little systematic research has been done on the effect of different operating conditions on the separation efficiency of lactic acid and especially on the purification performances of lactic acid.

2.4 Ion exchange chromatography

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

Ion exchangers are cross-linking polymeric resins, consisting It consists of insoluble matrix to which charged groups have been covalently bound. In Figure 2.3, 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. Both positively and negatively charged exchangers have been developed. 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 exchangers. The resin is flushed with another newly exchanged ion in solution in order to remove and substitutes the previously 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 simultaneously obtained from the back wash effluent.

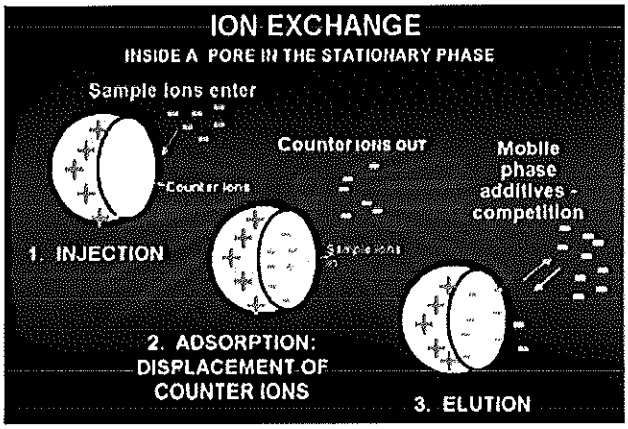


Figure 2.3 Mechanism of ion exchange (Boonkong, 2006)

2.4.1 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 (concentration in the solid phase versus concentration in the fluid phase (gas or liquid)) are plotted as adsorption isotherm as shown in Figure 2.4. The concentration in the solid phase is expressed as q, kg adsorbate (solute) kg^{-1} adsorbent (solid), and in the fluid phase (gas or liquid) as c, kg adsorbate m^{-3} fluid.

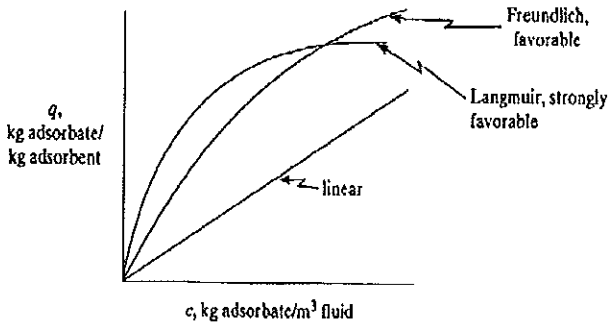


Figure 2.4 Three common types of adsorption isotherms (Henry, Langmuir and Freundlich) (Geankoplis, 2004)

2.4.2 Adsorption isotherm

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^{-1} adsorbent. This linear isotherm is not common, but in the dilute region it can be used to estimate data for many systems.

2. Langmuir isotherm

The Langmuir isotherm has a theoretical basis and is given in the following, where K_L and a_L are empirical constants:

$$q_e = \frac{K_L a_L C_e}{1 + a_L C_e}$$

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 $1/q_e$ versus $1/C_e$, the slope is $1/K_L a_L$ and intercept is $1/K_L$ (Langmuir, 1918)

3. Freundlich isotherm

The Freundlich isotherm equation, which is empirical, often approximates data for many physical adsorption systems and is particularly useful for liquid (Freundlich, 1906);

$$q_e = K_F C_e^{1/n}$$

Where K_F 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.

4. Dubinin-Radushkevich isotherm (Dubinin and Radushkevich, 1947)

The experimental data was fitted to Dubinin-Radushkevich isotherm model in order to determine the adsorption type. The non linear model is given by the following equation

$$q_e = q_m \exp\left(-k\varepsilon^2\right)$$

Where q_m is the maximum adsorption capacity (mg g^{-1}), k is a constant relating to the adsorption energy of (mol^2 kJ^{-2}), ε is the Polanyi potential, C_e is the equilibrium concentration of solute in aqueous solution (mg L^{-1}), R is the gas constant and T is the temperature (K)

$$\varepsilon = RT \ln\left(1 + \frac{1}{C_e}\right)$$

2.4.3 Adsorption process

1. Batch adsorption

Batch adsorption is often used to adsorb solutes from liquid solutions when the treated amount is small such as 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

$$q_F M + c_F S = qM + cS$$

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

Where 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 (Geankoplis, 2004).

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 is 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 reaches 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 2.5(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 take place at a slightly far from the inlet. At a later time t_2, the profile or mass-transfer zone where most of the concentration changes take place moves farther down the bed. The concentration profiles shown are for the fluid phase. Concentration profiles for the concentration of adsorbates on the solid are similar. The solid at the entrance is nearly saturated and this concentration remains almost constant down to the mass-transfer zone, where it is dropped off rapidly to almost zero. The dashed line for time t_3 shows the concentration in the fluid phase in equilibrium with the solid. The difference in concentrations is the driving force for mass-transfer.

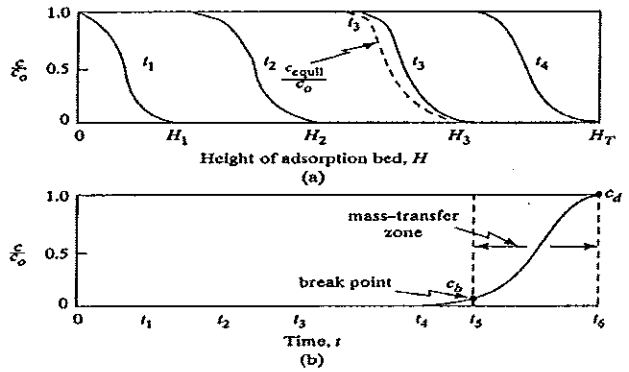


Figure 2.5 Concentration profiles for adsorption in fixed bed (a) profiles at various positions and times in the bed, (b) breakthrough concentration profile in the fluid at the outlet of bed (Geankoplis, 2004)

2.4.4 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.

2.4.5 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 (Geankoplis, 2004).

Keil and Greiner (1985) disclosed the separation of lactic acid from a fermentation medium with an adsorbent comprising a polymer with tertiary amino groups as described in US Patent 4,552,905 (Keil *et al.*, 1985).

Kawabata *et al.* (1982) separated carboxylic acid by using a polymer adsorbent of pyridine skeletal structure and a cross link structure. The polymer adsorbent showed good selectivity and high adsorption capacity for carboxylic acids even in the presence of salts. The selected substances were aliphatic alcohol, aliphatic ketone, and carboxylic ester.

Evangelista and Nikolov (1996) recovered lactic acid from fermentation broth by weak base polymer adsorbents MWA-1, IRA-35, and VI-15. The pH for the adsorption of lactic acid was below its pKa and fermentation broth was acidified by using cation exchange resin instead of using inorganic acid to eliminate possible competition between inorganic acid and lactate in the subsequent adsorption steps. Methanol and 5% NH_4OH were used as eluants. Although 1.5 times of bed volume of 5%NH_4OH could recover all the adsorbed lactic acid from MWA-1

column, product purity was not high. However, 6.8 times of bed volume of methanol could completely desorbed lactic acid from VI to 15 anion exchange resin with higher purity.

Simulated moving bed (SMB) chromatography is a continuous separation process that has many important industrial applications (Ruthven and Ching, 1989). The SMB consists of a circle of chromatographic columns. The circle is typically divided into four zones by two inlet ports and two outlet ports. The four ports move periodically by one column length along the mobile phase flow direction to follow the migrating solute bands. The port movement maintains product purity and yield, while achieving simulated countercurrent movement between the adsorbent and the fluid (Broughton and Gerhold, 1961). In comparison to batch chromatography, the processes of SMB have higher yield and product purity as well as lower solvent consumption. They have been used, for the separation of hydrocarbon and high fructose corn syrup (Ruthven and Ching, 1989). Recently, the method has been developed for the separation of racemic mixtures or isomers (Juza *et al.*, 2000; Xie *et al.*, 2003).

Lee *et al.* (2004) explored the feasibility of developing the SMB processes for separating of lactic acid from other organic acids present in a fermentation broth. Poly (4-vinylpyridine) resin (PVP), which was found to have a high capacity and selectivity for organic acids in previous study (Hritzko *et al.*, 1999), was chosen as the adsorbent. A systematic design method, which is based on the standing wave analysis for Langmuir isotherm systems, was tested for this separation (Xie *et al.*, 2003). Adsorption isotherms and mass transfer parameters for the two organic acids were determined from frontal chromatograms. These parameters were first validated by comparing the single column frontal chromatograms with simulations based on a rate model (Whitley, 1990). The validated parameters were applied in the standing wave design to find the four zone flow rates and step time for a laboratory SMB unit. High product purity (99.9%) and relatively high yield (>93%) were achieved in the SMB experiments. The SMB column profiles and effluent histories can be better explained if the adsorption isotherm of lactic acid is represented by a modified Langmuir equation. The standing waved sign method was

extended to nonlinear systems with modified Langmuir isotherms. Rate model simulations show that this method can achieve higher purity and higher yield for this separation than the design based on the Langmuir isotherms.

Chen and Ju (1998) examined polyvinyl pyridine (PVP) and activated carbon for the adsorption characteristics pertinent to their application in lactic acid fermentation. For PVP, the linear adsorption constant (K_{ad}) was between 0.7 and 1.0 for an equilibrium pH range of 3-9. The pH was adjusted by acid or base addition, similar to pH control in fermentation. The values of K_{ad} in the pH adjusted systems were much lower than that reported for pure lactic acid solutions, i.e., about 9.7. Furthermore, no clear effect of pH was observed. These are attributed mainly to the competition of anions (Cl and lactate) for the adsorption sites of protonated pyridinal. Its adsorption capacity was also found to decrease with the base regeneration (by about 14% each time) after being contacted with the culture broth. These factors limit its potential application in lactic acid fermentation. Activated carbon was much more effective in lactic acid/lactate adsorption than PVP. The adsorption further favored lower pH under acid (HCl) addition. Activated carbon has been reported to adsorb glucose. However, the presence of glucose in 0-70 g L^{-1} was found to have an insignificant effect on lactate adsorption. Cells of *L. delbrueckii* also adsorbed rapidly on activated carbon. This cell adsorption had a negative effect on lactate adsorption.

Kulprathipanja and Oroshar (1991) studied separation of lactic acid from a fermentation broth by using an adsorbent comprising a water-insoluble macro-reticular gel or weakly basic anionic exchange resin or pyridine functional groups or a strongly basic anionic exchange resin. The resins are in sulphate form and have across-linked acrylic or styrene resin matrix. The adsorption mechanism is shown in Figure.2.6. For tertiary amine and pyridine function containing ion exchange resins, the lone electron pair of the nitrogen atom enables nitrogen atom to form hydrogen bond with sulfate ion. IRA-400, a strongly basic quaternary ammonium ion exchange resin, has positive charge and can form ionic bond with sulfate ion. The sulfate form of quaternary ammonium of anion exchange resin has a weakly basic property and can adsorb lactic acid through acid-base interaction. Consequently, the adsorption of lactic acid is not affected by inorganic salt in fermentation broth. Lactic acid is

desorbed with water or diluted inorganic acid. The pH of the feed is maintained below the ionization constant (pKa) of the lactic acid to obtain high selectivity.

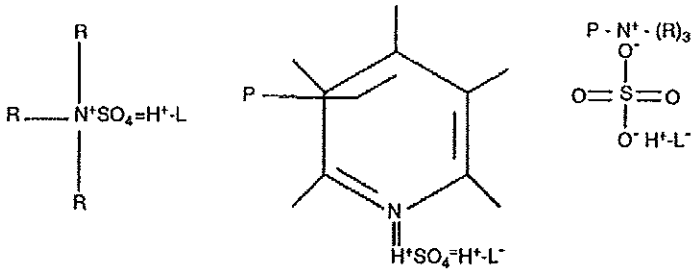


Figure 2.6 The adsorption mechanism (Joglekar *et al.*, 2006)

Several different ion exchangers such as IRA-400, PVP, and IRA-92, Amberlite were tested. IRA-92, a weakly basic exchanger, is selected as the ion exchanger for the purification of lactic acid because of its fair purity and recovery yield of lactic acid comparable with other resins' results.

Tong *et al.* (2004) examined the capacity of IRA-92 to lactic acid in the fermentation broth, and then, optimized the operating conditions, and finally gained a fair product of lactic acid with a higher purity, recovery yield and productivity with only one-step chromatographic procedure from the supernatant of the fermentation broth. The experimental results demonstrated that when the pH of the fermentation broth is 6.0, the recovery yield, purity and productivity in the lactic acid purification were the highest. With the decrease of the sample volume loaded and of flow rate, the recovery yield and the purity were improved but the productivity apparently reduced. In the experimental scale, the scale-up of purification process exhibited little influence on the recovery yield and the purity. Under the preliminary optimal conditions, the yield, purity and specific productivity were of 82.6%, 96.2% and 1.16 g LA (g-resin day)$^{-1}$, respectively.

Raya-Tonetti *et al.* (1999) studied the use of strong anionic exchange resin (IRA-400) to recover lactic acid directly from fermentation in an up flow fluidized bed column, resulting in 0.18 g lactic acid g^{-1} resin bound with a subsequent

elution of 94%. When the culture broth was heated and adjusted pH to 8.0, 0.4 g lactic acid was bound per gram of resin, with a subsequent elution of 90%. L-(+) and D-(-) lactic acid isomers distribution was analyzed in the elution product resulting in an increase of L-(+) isomer concentration. The resin did not alter its binding capacity even after 23 cycles. The lactic acid recovery attained was not dependent on the increase of the resin poured to the columns but was due to the increase in the hydraulic residence time and back mixing which resulted in a high contact time between lactate ions and resin particles.

Srivastava *et al.* (2000) separated lactic acid by using IRA-400 column coupled with fermentor. This study was focused on improving fermentation yield, whereas the separation performance of IRA-400 was not studied. The Amberlite IRA-400 resin has proper pore size and high adsorption capacity for recovery of lactic acid and it can adsorb lactic acid in wide pH range.

Cao *et al.* (2002) studied the application of Amberlite IRA-400 anion exchange resin for the recovery of L-(+)-lactic acid from fermentation broth. Adsorption isotherm and breakthrough curves for the separation of (L+)-lactic acid were obtained at pH 5.0 and 2.0, respectively. Recovery experiments coupled with fermentation were carried out successfully by using a column without autoclaving. Different types of adsorption isotherms were found at pH above and below the pKa(3.86) of lactic acid. 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, and dissociation constant, were of 222.46 mg g^{-1} wet resin and 60.7 mg mL^{-1}, respectively. The breakthrough curve for the separation of lactic acid from fermentation broth was also obtained. The maximum adsorption capacity (197.09 mg g^{-1} wet resin) at pH 5.0 was much higher than that at pH 2.0 (106 mg g^{-1} wet resin). Proper elution and washing conditions were sought by using H_2SO_4, methanol, ammonia or their mixtures as eluants. When column separation was performed at pH 5.0 by using 50% (vv^{-1}) methanol as washing solvent and 1.0 M H_2SO_4 as eluant, the total yield was 86.21%. However, the total yield was 92.11% when the column separation was performed at pH 2.0 and water was used as eluant.

2.5 Ethyl lactate production

A great number of lactate esters are known. Particularly interesting ones are the methyl, ethyl and n-butyl esters. There are used in pharmaceutical and cosmetic industries and as solvents for varnishes, nitrocellulose and polyvinyl compounds. Methyl, ethyl and propyl lactates are water-soluble while butyl lactate is only slightly soluble. The lower esters are prepared by direct esterification while the lactic esters of higher alcohols are prepared from methyl or ethyl lactate by transesterification with the appropriate alcohol. Several methods have been used to prepare esters of lactic acid; these methods can be classified into the following main groups:

A: Direct esterification of lactic acid and alcohol.

B: Transesterification of one ester into another by reaction with alcohol.

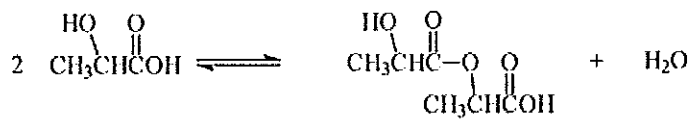
C: Conversion of a metal lactate or ammonium lactate into an ester by treatment with alcohol.

D: Reaction of a metal lactate with an alkyl halide (Ozen, 2004).

2.5.1 Intermolecular Esters

It has been observed experimentally that dilute (<20 wt%) lactic acid solutions contain only lactic acid monomer (LA_1). However, many process involving lactic acid, including polymerization and esterification, require concentrated lactic acid solutions, and lactic acid in these solutions undergoes intermolecular self-esterification to form higher oligomers. This oligomerization occurs to an increasing degree at high acid concentration, low water concentration, and high temperature.

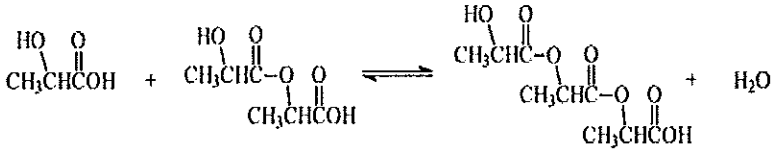
In oligomerization, two molecules of lactic acid first react to form a linear dimer, commonly called lactoyllactic acid (LA_2), along with a mole of water.



(2.1)

Lactic Acid (LA_1) Lactoyllactic acid (LA_2)

Lactic acid also forms a cyclic dimmer noted as lactide, but this compound is known to be unstable in water. Lactoyllactic acid (LA_2) can further esterify with LA_1 to form the trimerlactoyl lactoyllactic acid (LA_3); this process can further continue to give higher chain intermolecular polyesters LA_4, LA_5 and so on.



(2.2)

Lactoyl-lactoyllactic acid (LA_3)

2.5.2 Reaction pathways

Based on the reactant and product composition profiles during esterification of the 88 wt% lactic acid solution, a set of reaction pathways have been defined for the kinetic model. The set of reactions, which are given in reactions (1)-(5) below, describe lactic acid monomer esterification as well as oligomer formation and esterification. All oligomers that are larger than dimmers (L_2 and L_2E) are lumped together as L_3 and L_3E; the quantities of larger oligomers are small enough that they do not significantly influence the reaction, either kinetically or with regarding to overall acid concentration and mass balances. Esterification of the 50 wt% and 20 wt% lactic acid solutions is completely described by reactions (2.3), (2.4), and (2.6) respectively.

$$L_1 + EtOH \underset{k_1/K_1}{\overset{k_1}{\rightleftharpoons}} L_1E + H_2O \quad (2.3)$$

$$L_2 + EtOH \underset{k_2/K_2}{\overset{k_2}{\rightleftharpoons}} L_2E + H_2O \quad (2.4)$$

$$L_3 + EtOH \underset{k_3/K_3}{\overset{k_3}{\rightleftharpoons}} L_3E + H_2O \quad (2.5)$$

$$L_2 + H_2O \underset{k_4/K_4}{\overset{k_4}{\rightleftharpoons}} 2L_1 \quad (2.6)$$

$$L_3 + H_2O \underset{k_5/K_5}{\overset{k_5}{\rightleftharpoons}} L_1 + L_2 \quad (2.7)$$

2.5.3 Catalysis

The properties possessed by ion-exchange resins have resulted in the development of many procedures and processes for use in both research and industry. Many industrially important reactions involving acid or bases as catalysts can also be carried out using cation-exchange or anion-exchange resins since standard ion-exchange resins are insoluble acids or bases.

Catalysis with solid ion-exchange resins has the following advantages over the use of homogeneous catalysts like sulfuric acid (Roy and Bhatia, 1987)

1. The catalyst can be readily removed from the reaction product by decantation or simple filtration.

2. Continuous operations in columns are possible.

3. The purity of the products is higher since side reactions can be completely eliminated or are less significant.

4. It is possible to isolate the reaction intermediates.

5. Ion exchange resins can differentiate between small and large molecules.

6. Environmentally safe.

7. No corrosion.

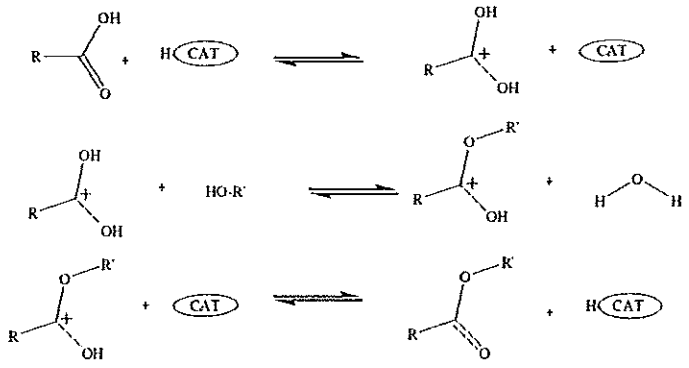
8. A higher local concentration of H^+/OH^-

For liquid phase esterification reactions, the use of ion-exchange resin as solid catalysts increases with regarding to their advantageous properties. In comparison with the conventional homogeneous catalysts, esterification of lactic acid with methanol (Choi *et al.*, 1996), benzyl alcohol with acetic acid (Roy and Bhatia, 1987), synthesis of butyl lactate (Dassay *et al.*, 1994), synthesis of isopropyl lactate (Yadav and Kulkarni, 2000) and esterification of ethanol with acetic acid etc., were carried out and all have been proved to be active catalysts.

Esters can be formed by the reaction of a carboxylic acid with an alcohol forming the ester and water molecules. This (reversible) reaction, also called the intermolecular dehydration reaction, is very important and is a common type of reaction in chemical industry. The general esterication reaction is shown below:

Acid + alcohol $\leftrightarrow$ ester + water

The reaction is initiated by the transfer of a proton from the catalyst to the carboxylic acid. The proton becomes attached to one of the lone pairs on the oxygen which is double-bonded to the carbon. The transfer of the proton to the oxygen gives it a positive charge. This results in affair amount of positive charge on the carbon atom. Then, the positive charge on the carbon atom is attacked by the hydroxyl group of the alcohol molecule. After that, a molecule of water is lost from the ion. Finally, the catalyst is recovered by the transfer of proton from the ion to the catalyst surface. This mechanism is represented by the following scheme:



(2.8)

(2.9)

(2.10)

The donation of a proton is commonly assumed to be a fast step, while the nucleophilic substitution is usually assumed to be slow followed by fast steps resulting in the formation of ester and water and the recovery of the catalyst.

Ion-exchange resins are also attracting attention as promising catalyst carriers, which show higher activity than the unsupported form of the resin for the synthesis of Methyl Tert-Butyl Ether (MTBE), esterification of acetic acid with 1-pentanol, and hydration of 2-methylpropene. Resulting activities were attributed by the synergy created from the protons originating both from the ion exchanger and heteropoly acid catalysts.

2.5.4 Catalytic distillation

There is considerable academic and industrial interest in the area of reactive (catalytic) distillation (Highler *et al.*, 1999). Because of the potential benefits of this technology, the number of publications on the theoretical and experimental performance of special reactive distillation process are rapidly increasing (Chen *et al.*, 2000; Chen *et al.*, 2002). Catalytic distillation seems to be an energy saving process with lower investment and operating costs in comparison to the traditional processes (Hanika *et al.*, 2001). Reactive distillation is an emerging technology that has considerable potential as an alternative process for carrying out equilibrium limited liquid phase chemical reactions, exothermic reactions, poor raw materials usage due to selectivity losses, or excessive flow sheet complexity.

The synthesis of ethyl lactate from ethanol and lactic acid has been studied in a semi-batch reactor combined with a pervaporation unit (Delgado *et al.*, 2007). They found that the yield lactate exceeds the corresponding thermodynamic equilibrium via selective removal of water from the reaction mixture through the membrane. Therefore, water is generated in this reaction and it always preferable to eliminate the water from the initial reaction mixture to drive the equilibrium in the forward direction. In order to isolate ethyl lactate, it is necessary to firstly remove large amounts of water by distillation, which requires consumption of large amounts of energy. In addition, if the concentration of lactic acid decreases from 100 to 50%, the size of the reactor doubles to produce the same amount of product.

CHAPTER 3

MATERIALS AND METHODS

Materials

3.1 Microorganism and inoculum

Lactobacillus casei TISTR 1500, obtained from Department of Biotechnology, Faculty of Agro-Industry, Rajamangala University of Technology Srivijaya, was the microorganism used in the experiments. It was maintained at 4°C in plate culture on MRS (de Man, Rogosa, Sharpe) agar media with the following composition (in g L^{-1}): proteose peptone, 10; beef extract, 10; yeast extract, 5; glucose, 20; polysorbate 80, 1; ammonium citrate, 2; sodium acetate, 5; magnesium sulphate, 0.1; maganese sulphate, 0.05; dipotassium phosphate, 2; and agar, 15. The inoculum was prepared by transferring a loopful of cells to 250 mL conical flask containing 50 mL sterile MRS broth (the same composition of MRS agar, but without agar). The flasks were incubated at 37°C for 24 h for seed culture. Ten milliliters of this culture was then transferred to a 250 mL Erlnemeyer flask containing 90 mL MRS broth, and incubated at the same conditions. Finally, the cells were harvested by centrifugation (8,000 rpm, 15 min) and directly resuspended in the fermentation medium to obtain a cell concentration of 1.0 g L^{-1} at the beginning of the fermentation.

3.2 Raw material and characterization

Palmyra sap was collected from planters in Songkhla province, Thailand. The Palmyra sap is listed as: pH 4.30, moisture content 86.20%, total sugars (as sucrose) 134.00 g L^{-1}, total soluble solid 14.80°Brix and total nitrogen 0.03 g L^{-1}. The palmyra sap was hydrolyzed by adding 1 mL of 20% sulfuric acid in 100 mL of palmyra sap solution (Kadam *et al.*, 2006). The acidified palmyra sap solution was heated in a boiling water bath for 20 min. The physical and chemical properties of hydrolyzed palmyra sap were listed as: pH 1.60, moisture content 87.88%, total sugars 181.53 g

L^{-1} (glucose 91.90 g L^{-1} and fructose 89.63 g L^{-1}), total soluble solid 17.60°Brix, and total nitrogen 0.03 g L^{-1}.

Oil palm sap was collected by using a laboratory-scale hydraulic press. The sap was centrifuged at 6,000 rpm for 15 min and the supernatant was stored at -20 °C before use. The physical and chemical properties of oil palm sap were listed as: pH 7.49, moisture content 97.06%, total sugars 19.17 g L^{-1} (glucose 16.58 g L^{-1} and fructose 2.59 g L^{-1}), total soluble solid 3.4°Brix, and total nitrogen 0.06 g L^{-1}.

We characterized three sap samples collected from same source in Songkhla province, in the southern of Thailand. Collected sap was kept in an icebox (4°C) to inhibit the activity of microorganisms during transportation (30 min) to the department of chemical engineering, Prince of Songkla University, Hat Yai Campus. The physical and chemical property of each sample was determined within a day. Average compositions of the samples were given in Table 3.1.

Table 3.1 Physical and chemical properties of palmyra sap, hydrolyzed palmyra sap and oil palm sap

Parameters	Carbon source			Unit
	Palmyra sap	Hydrolyzed palmyra sap	Oil palm sap	
pH	4.30	1.60	7.49	-
Moisture	86.20	87.88	97.06	%
Total sugars	134.00	181.53	19.17	g L^{-1}
glucose	-	91.90	16.58	g L^{-1}
fructose	-	89.63	2.59	g L^{-1}
sucrose	134.00	-	-	g L^{-1}
Total soluble solid	14.80	17.60	3.40	°Brix
Total nitrogen	0.03	0.03	0.06	g L^{-1}

3.3 Chemicals

Chemicals used in the analysis were analytical grade: lactic acid (88 wt%), acetic acid (99 wt%), ethyl lactate (>98 wt%) and glucose were purchased from Sigma-Aldrich. Ethanol (>98 wt%) was achieved from Specialized R&D Center for Alternative Energy from Palm Oil Crops at Prince of Songkla University.

A strongly acidic cation exchange resin, Amberlyst 15 in the H^+ form, was obtained from Sigma-Aldrich and was used without modification. This ion exchange resin was consisted of a sulfonated styrene crosslinked with 8% divinylbenzene. Its total exchange capacity was of 4.7 meq g^{-1}.

The resin used in experiments was Amberlite IRA 402 and Dowex™ 66 (see Table 3.2).

Table 3.2 Characteristics of resins used in the study

Resin	Manufacturer	Active group	Form	Basicity	pH-range
Amberlite IRA402	Rohm and Haas	Quaternary ammonium	OH	Strong	0-14
Dowex™ 66	Rohm and Haas	Tertiary amine	Free base	Weak	0-7

Methods

An overview of ethyl lactate production in order for hydrolysis to pure lactic acid was shown in Figure 3.1.

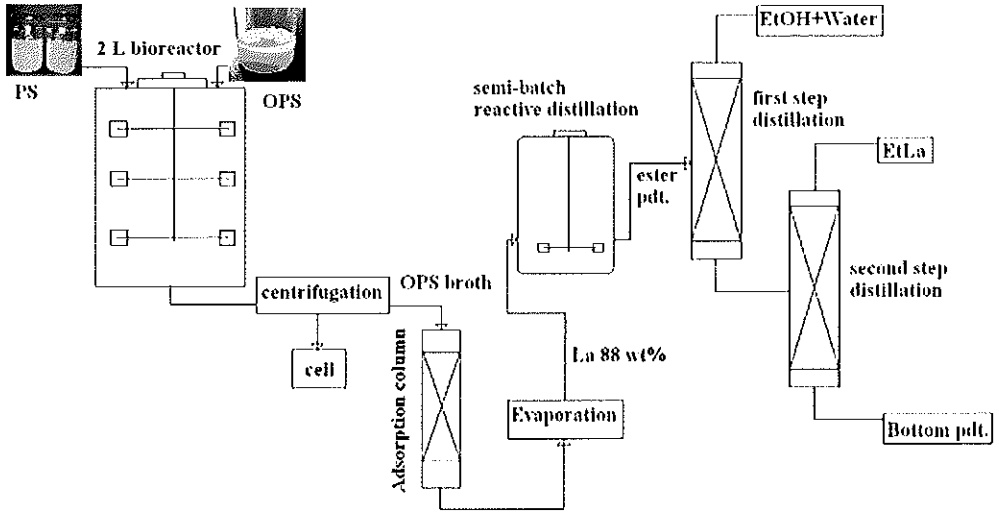


Figure 3.1 A schematic diagram of ethyl lactate production in order for hydrolysis to pure lactic acid

3.4 Fermentation

3.4.1 Lactic acid fermentation

L. casei TISTR 1500 was used for this study because it produced L(+)-lactic acid and was acidotolerant with an optimum pH of 5.5. The bacterium was pre-cultured on MRS medium. These saps were diluted to a final total sugars concentration at 20.0 g L^{-1} by basis on initial total sugars of palmyra sap. Oil palm sap was used directly without dilution. After formulation, 100 mL of media were transferred to 250 mL Erlenmeyer flasks and sterilized at 121°C for 15 min.

1. Time course of lactic acid production in static flask cultivation

The flasks were statically incubated on a static flask at 37°C for 24 h. Samples were taken every 2 h to measure lactic acid concentration, total sugars and dry cell weight.

2. Type of carbon source

The effect of different carbon sources; monosaccharide (glucose and fructose), and disaccharide (sucrose) at 20 g L^{-1} concentration, was studied. The flasks were statically incubated on a static flask at 37°C for 14 h. Samples were taken every 2 h to measure lactic acid concentration, total sugars and dry cell weight.

3. Effect of total sugars using palmyra sap as carbon source

Cultivation was performed in MRS medium (without glucose) containing palmyra sap with total sugars concentration at 10.0, 20.0, 40.0, 60.0 and 134.0 g L^{-1}. The flasks were statically incubated on a static flask at 37°C for 14 h. Samples were taken every 2 h to measure lactic acid concentration, total sugars and dry cell weight.

4. Effect of medium supplementation

Cultivation was performed in with and without MRS medium containing palmyra sap, hydrolyzed palmyra sap and oil palm sap as carbon source. The flasks were statically incubated on a static flask at 37°C for 48 h. Samples were taken every 6 h to measure lactic acid concentration, total sugars, dry cell weight and pH.

5. Effect of pH control

Cultivation under uncontrolled and controlled pH were compared in 2 L stirred tank bioreactor with a working volume of 1 L (Figure 3.2). The culture temperature was 37°C. The pH was maintained at 5.5 with 2.0 N NaOH during fermentation. Samples were taken every 12 h to measure lactic acid concentration, total sugars, dry cell weight and pH.

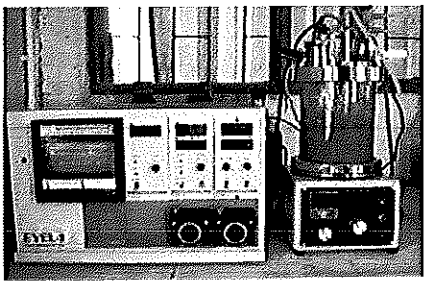


Figure 3.2 Bioreactor

3.4.2 Analytical methods

Cell growth was measured by diluting the culture broth with distilled water to obtained optimum dilution. After mixing, the absorbance was measured by UV-spectrophotometer (1601, Shimadzu) at 660 nm (Kurane *et al.*, 1994).

Dry cell weight was determined by centrifugation of culture broth (2 mL) at 8,000 rpm for 15 min. The cell sediments were dried for 24 h at 105°C and then weighed to constant weight after cooling in a desiccator (Dermlim *et al.*, 1999).

Lactic acid and acetic acid concentrations in supernatant were conducted by means of Gas chromatography (GC) analysis. GC (GC-14A, Shimadzu, Japan) was equipped with a BP-20 GC column (30m x 0.53mm) using flame ionization detector (Sura-apinan *et al.*, 2010).

Residual sugar (sucrose, glucose and fructose) in the supernatant was determined by HPLC apparatus (System controller: SCL-10A *VP*, Liquid chromatograph: LC-10AD *VP*, Degasser: DGU-12A, RI detector: RID-10A, Auto injector: SIL-10AD *VP*, Column oven: CTO-10AS *VP*, Shimadzu, Japan), equipped with NH_2 column and a refractory index detector, adapted from Liu and Steinbuchel (1997).

Total sugars concentrations were analyzed by the Dubois method using phenol and sulphuric acid (Dubois *et al.*, 1956).

Moisture content of each sample was determined by drying at 105°C for 48 h (A.O.A.C., 2000).

Total soluble solid was measured using Atago hand-held refractometer and reported in degree brix (°Brix) (A.O.A.C., 2000).

3.4.3 Fermentative parameters

The fermentation parameters were determined: the specific growth rate (μ, h^{-1}), defined as the ratio of logarithm of biomass concentration produced to elapsed time (h); cellular yield coefficient ($Y_{X/S}$, g g^{-1}), defined as the ratio of the total cell mass presented in the medium to sugar consumed; conversion yield of substrate to product ($Y_{P/S}$, g g^{-1}), defined as the ratio of lactic acid produced to sugar consumed; and maximum productivity (R_M, g L^{-1} h^{-1}), calculated as the ratio of lactic acid concentration to the fermentation time (Prit, 1975).

3.5 Adsorption

3.5.1 Equilibrium adsorption

Adsorption isotherms were determined by using a 1:10 (w:v) ratio of resin and starting solution. First, the binary component equilibrium adsorption was determined using the initial concentration (C_0) of lactic acid solution from 20 to 100 g L^{-1}. Second, multiple components equilibrium adsorption was investigated using the initial concentration of lactic acid, acetic acid glucose and magnesium sulphate at 20, 5, 5, and 0.005 g L^{-1}, respectively. The bottles were placed in water bath with shaking for 24 h, which was sufficient for system equilibration. Each of equilibrium concentrations of the solutions were measured using GC/FID.

After 24 h of shaking, equilibrium concentrations of lactic acid (C_e) in solutions were determined and the amount of adsorption (q_e, g lactic acid per g dry resin) was calculated from Equation (3.1).

$$q_e = \frac{V(C_0 - C_e)}{W} \qquad (3.1)$$

3.5.2 Desorption

To determine the amount of lactic acid eluted from the adsorption of lactic acid onto the Amberlite IRA 402 and Dowex^{TM} 66 resins, 20 mL of 20 g L^{-1} lactic acid solution were introduced into a series of 150 mL conical flasks and then shaken with 1 g resin for 24 h. The amount of lactic acid adsorbed was calculated using equation (3.1). Afterward, 1 g of the resin that adsorbed lactic acid was placed in 20 mL ethanol/water mixture of 90, 50 and 0 wt%, sealed and then shaken at 30°C. Samples were taken for 24 h. The amount of lactic acid desorbed was determined by GC/FID. The desorption yield (%) was calculated by the following equation (3.2)

$$\textit{Desorption yield (\%)} = \frac{\textit{Amount lactic acid into the elution medium } x\ 100}{\textit{Amount of lactic acid adsorbed onto resin}} \qquad (3.2)$$

3.5.3 Column adsorption

Column adsorption experiments were performed in a glass column (Figure 3.3). The free space at the top of the column allowed the expansion of the resin. A peristaltic pump transported either broth or lactic acid solution to column at average flow rate of 1.0 mL min^{-1}. Glass wool was put on the top and bottom of the bed to obtain a good liquid distribution. In addition, the adsorption step was performed at room temperature and pH 5.5 was adjusted by adding 50%NaOH. Adsorbed lactic acid on the resin was eluted by 1.0 M H_2SO_4 at a flow rate of 1 mL min^{-1}. The effluent was collected and analyzed for lactic acid concentration.

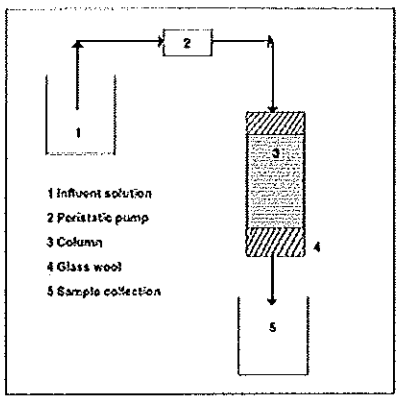


Figure 3.3 Flow chart of down flow packed bed

3.6 Esterification

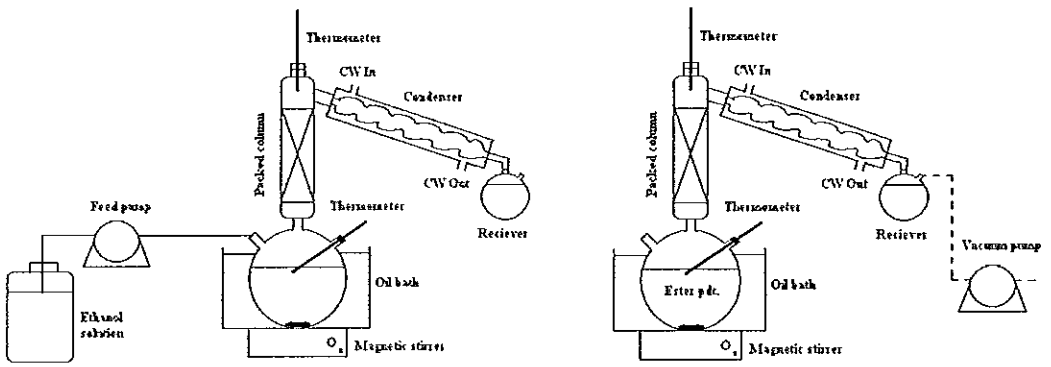
3.6.1 Semi-Batch Reactive Distillation

The apparatus for esterification on semi-batch reactive distillation was schematically shown in Figure 3.4A. The experiments were carried out in a three-necked Pyrex flask reactor of capacity 0.5 L. A packed column was placed in one of the opening (one other opening was connected to an ethanol feed and another to a temperature measuring device). A condenser was attached to the end of packed column through which chilled water circulated. The reactor was heated by immersing it in a constant temperature of which was maintained within (±0.5°C) of the desired temperature. Agitation in the reaction was conducted with a magnetic stirrer to suspend the resins, and the stirring rate was controlled at 600 rpm. In all experimentals, cation-exchange resins were first charged into the reactor; the second reactant, the aqueous lactic acid and ethanol solution were charged into the reactor (the initial molar ratio of ethanol to lactic acid, 3:1) and heated to the desired reaction temperature (95°C). When water was removed and condensed in the receiver, the ethanol solution was fed into the reactor using a peristaltic pump at flow rate 0.036 moL min^{-1}. This time was taken as the starting time for the experiments. When 0.5 hr reaction time was reached, the catalyst was separated with the filter.

3.6.2 Two Step Distillation

Whole liquid was carried out into two step distillation unit (Figure 3.4B) after finishing the esterification reaction to purify ethyl lactate in this unit. Then, ester products were distilled under packed bed distillation column which has an inner diameter of 35 mm and total height 340 mm. The 250 mm packed column is a packing section containing raschig rings (8 mmx5 mm dia.). The volatilities of products play an important role in the operation of distillation process. The order of volatilities of products involved in this experiment is as follows: ethanol>water>ethyl lactate>lactic acid. The Antoine equation was used in this work to calculate the vapor pressure of each component (Riddick *et al.*, 1996). Therefore, distillation process was

operated under a vacuum condition of 700 mmHg below atmosphere pressure. In first step, the temperature was set at 55°C for recovering unreacted ethanol and water, and increased to 110°C in second step for purification of ethyl lactate.



(A) (B)

Figure 3.4 The schematic diagram of (A) semi-batch catalytic distillation and (B) two-step distillation unit

Material balances calculation of lactic acid, ethanol, ethyl lactate and water were conducted by mean of GC analysis. Conversion of lactic acid, yield of ethyl lactate and overall change were defined by the following equation (3.3), (3.4) and (3.5), respectively.

$$\%\,Conversion = \frac{Number\ of\ moles\ of\ lactic\ acid\ consumed\ in\ the\ reaction}{The\ total\ moles\ of\ initial\ lactic\ acid} x100 \qquad (3.3)$$

$$\%\,Yield = \frac{Number\ of\ moles\ of\ the\ ethyl\ lactate\ formation}{Number\ of\ moles\ of\ lactic\ acid\ in\ feed} x100 \qquad (3.4)$$

$$\%Overall\ change = \frac{(Total\ weight\ of\ i\ component\ with\ distillation - Total\ weight\ of\ i\ component\ without\ distillation)}{Total\ weight\ of\ i\ component\ without\ distillation} x100 \qquad (3.5)$$

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Potential Use of *Lactobacillus casei* TISTR 1500 for the Bioconversion from Palmyra Sap and Oil Palm Sap to Lactic Acid

In this study, we determined the physical and chemical compositions of agricultural resources, palmyra sap (PS), hydrolyzed palmyra sap (HPS) and oil palm sap (OPS) to evaluate their suitability as substrate for the production of lactic acid by *Lactobacillus casei* TISTR 1500, and to investigate the effect of acid hydrolysis of palmyra sap to obtain the sap that contains high fermentable reduced sugar for use as a substrate for bioconversion. The effects of pH control and nutrient supplementation of the palmyra sap, hydrolyzed palmyra sap and oil palm sap were also investigated for maximizing biomass and lactic acid production. The compounds used on the formation of MRS broth media (free the glucose) were evaluated as nutrient sources. The influence of pH on lactic acid production was studied by comparing fermentations between two conditions: with initial pH 5.5 that was allowed to vary, and with constant pH at 5.5 in stirred tank bioreactor.

4.1.1 Lactic acid fermentation with a single carbon source (glucose, fructose, and sucrose)

Time course studied were conducted on growth and lactic acid production by *L. casei* TISTR 1500 in MRS medium (pH 5.5) using 20 g L^{-1} glucose as carbon source for 24 h at 37°C. The profile of growth (DCW), lactic acid production and total sugars utilization were shown in Figure 4.1.1. The bacterium grew rapidly within the first 14 h, correlating with the rapid decline of total sugars, which was due to the sugar being metabolized by cells and the cell forming lactic acid. The maximum amount of lactic acid (22.06 g L^{-1}) was produced from 20 g L^{-1} glucose within 14 h of fermentation, with an increase in dry cell weight from 1.52 to 5.05 g L^{-1}.

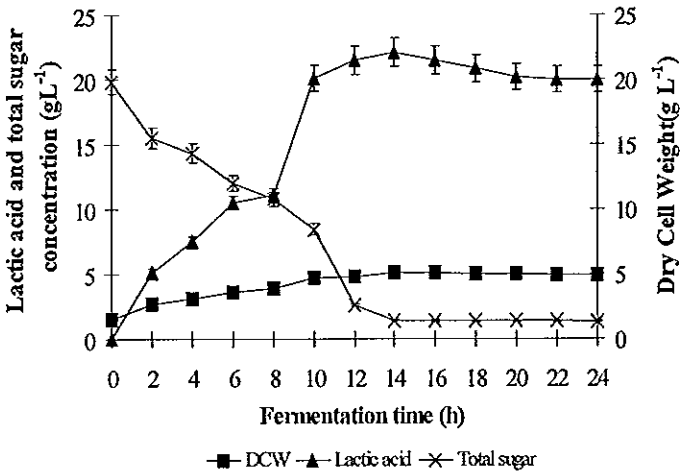


Figure 4.1.1 Time course on lactic acid production in static flask cultivation with MRS medium

The kinetic parameters were as follows: specific growth rate (μ), 0.06 h^{-1}; the product yield ($Y_{P/S}$), 1.20 g lactic acid g $sugar^{-1}$; cellular yield coefficient ($Y_{X/S}$), 0.20 g cell g $sugar^{-1}$; and the maximum productivity (R_M), 1.58 g lactic acid L^{-1} h^{-1}.

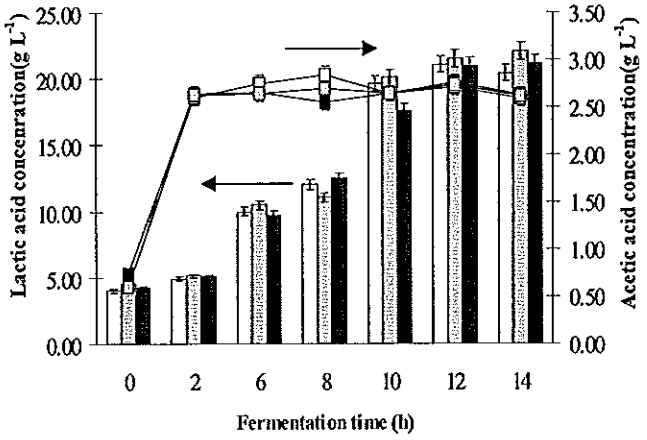


Figure 4.1.2 Profiles of lactic acid (piece symbols) and acetic acid (line symbols) formation with glucose (▤), fructose (■) and sucrose (□) as carbon sources during batch cultivation of *L. casei* TISTR 1500

Typical kinetics of lactic acid formation by *Lactobacillus casei* TISTR 1500 during the fermentor cultivation with media containing glucose, fructose and sucrose as the carbon source were shown in Figure 4.1.2. When *L. casei* TISTR 1500 was cultivated on these three carbon sources, lactic acid formation patterns were similar.

Table 4.1.1 Fermentation of *L. casei* TISTR 1500 using glucose, fructose and sucrose as the sole carbon source

Carbon source	Dry Cell Weight ($g\ L^{-1}$)	Final lactic acid ($g\ L^{-1}$)	Residual sugar ($g\ L^{-1}$)
Glucose	5.22±0.02	22.06±0.18	0.11±0.04
Fructose	5.05±0.01	21.15±0.35	0.50±0.08
Sucrose	4.78±0.04	20.41±0.21	0.10±0.05

Note: Batch fermentations were performed on 250 mL static flask with working volume of 100 mL at pH 5.5, 37°C for 14 h. Results are the average of data from triplicate experiments.

As shown in Table 4.1.1, the lactic acid production and the dry cell weight reached 20.41-22.06 g L^{-1} and 4.78-5.22 g L^{-1}, respectively. The results showed that there was slightly difference in lactic acid concentration when using sucrose, fructose and glucose. Hence, these sugars consumption by *L. casi* TISTR 1500 reflected its ability to efficiency use carbon sources available in palmyra sap.

4.1.2 Lactic acid production from original palmyra sap

The influence of concentrations of original palmyra sap on lactic acid fermentation was determined through the culturing of *L. casei* TISTR 1500 on a static flask at 37°C, pH 5.5 and 14 h using 10.0-134.0 g L^{-1} total sugars of palmyra sap. The obtained results (Table 4.1.2) showed that final lactic acid, dry cell weight and productivity increased with increasing total sugars of palmyra sap from 10.0 up to 134.0 g L^{-1}. The increase in product yields was 9.86% at 134.0 g L^{-1} total sugars of palmyra sap. However, the total sugars of palmyra sap consumption decrease from 94.96% to 27.19% with increasing total sugars of palmyra sap concentration up to 134 g L^{-1}, which resulted in the high total sugars concentration, would inhibit biomass and product formation. In addition, the most abundant sugar was sucrose, the high concentration of which maked the high viscosity of the liquid (Bulut *et al.*, 2004). Lactic acid yields, based on total sugars consumed were ranged between 0.71 g g^{-1} and 0.78 g g^{-1}. It is well known that sucrose is poorly metabolized by microorganisms compared to glucose (Bulut *et al.*, 2004). Therefore, in order to hold the high ability of *L. casei* TISTR 1500, the hydrolysis of sucrose in palmyra sap to glucose was also conducted prior to fermentation. The maximum dry cell weight was obtained up to 8.51 g L^{-1} and the highest productivity of lactic acid was found to be 2.02 g L^{-1} h^{-1}. All the maxima were obtained at 134.0 g L^{-1} total sugars of palmyra sap.

The optimum of total sugars of palmyra sap for lactic acid fermentation by batch culture of *L. casei* TISTR 1500 seemed to be 134.0 g L^{-1} total sugars of palmyra sap based on economic considerations of final lactic acid and productivity. However, total sugars of palmyra sap was not fully consumed in the presence of the high total sugars of palmyra sap and approximately 73% of total sugars of the palmyra sap remained unused in the fermentation medium. Time cultivation had to be more than 14 h to consume sugar by *L. casei* TISTR 1500. The kinetic parameters of the palmyra sap of 134.0 g L^{-1} total sugars revealed that the maximum productivity (R_M) was 2.02 g lactic acid L^{-1} h^{-1} and lactic acid yield ($Y_{P/S}$) was 0.78 g g^{-1}.

Table 4.1.2 Effect of total sugars of palmyra sap on lactic acid production, dry cell weight and productivity

Total sugars of palmyra sap ($g L^{-1}$)	Residual sugar ($g L^{-1}$)	Final lactic acid ($g L^{-1}$)	Dry cell weight ($g L^{-1}$)	Lactic acid yield ($g g^{-1}$)	Productivity ($g L^{-1} h^{-1}$)
10.0	0.58±0.04	7.78±0.97	1.30±0.2	0.71±0.06	0.56±0.10
20.0	2.76±0.50	12.00±0.42	1.53±0.1	0.72±0.02	0.86±0.04
40.0	16.46±0.76	17.00±0.28	2.75±0.3	0.74±0.02	1.21±0.03
60.0	34.92±0.12	18.95±0.20	4.60±0.2	0.76±0.06	1.35±0.02
134.0	97.00±1.41	28.35±0.22	8.51±0.3	0.78±0.02	2.02±0.02

Note: Batch fermentations were performed on 250 mL static flask with working volume 100 mL with MRS medium at pH 5.5, 37°C for 14 h. Results were the average of data from triplicate experiments.

The profile of growth (dry cell weight), pH, lactic acid production and total sugar utilization were shown in Figure 4.1.3. The total sugars of palmyra sap concentrations of 10.0, 20.0, 40.0, 60.0, and 134.0 g L^{-1} provided rapid growth rate (maximum dry cell weight of 1.30, 1.53, 2.75. 4.60 and 8.51 g L^{-1}, respectively). Lactic acid from *L. casei* TISTR 1500 increased as total sugars concentrations increased up to 134.0 g L^{-1}. Therefore, the carbon source concentration affected the efficiency of substrate conversion to lactic acid. The high carbon source concentration resulted in high lactic acid concentration. In this study, both lactic acid and acetic acid were the by-products in the fermentation broth (0-1.43 g L^{-1}) (Figure 4.1.3(D)). Ethanol and formic acid were not detected. Acetic acid could be occurred by both post-pyruvate and pre-pyruvate (Hofvendahl and Hahn-Hagerdal, 2000). Therefore, not only heterofermentation occurred but also mixed acid fermentation occurred. The hetero- or mixed acid fermentation routes give not only lactic acid, but formic acid and acetic acid as byproducts (VickRoy, 1985). Although total sugars concentration at 134.0 g L^{-1} gave the highest biomass and lactic acid production, the total sugars of palmyra sap at 20 g L^{-1} was selected as the total sugars concentration to improve the economic carbon source from palmyra sap containing the same sugar concentration as in MRS media contains 20 g L^{-1} glucose in lactic acid production.

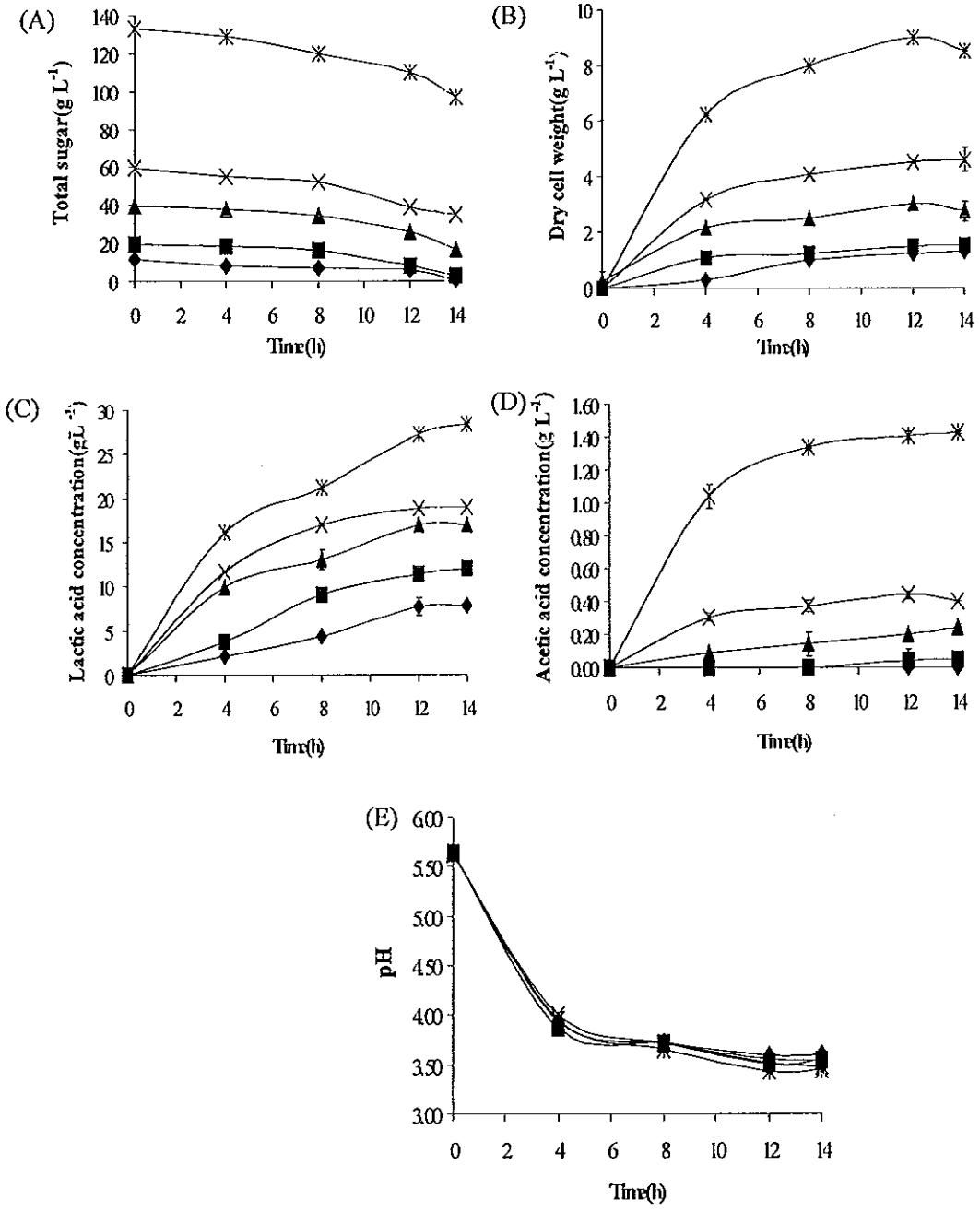


Figure 4.1.3 Kinetic profiles of total sugars consumption (A), dry cell weight (B), lactic acid concentration (C), acetic acid concentration (D) and pH (E) over time of fermentation for lactic acid production by *L. casei* TISTR 1500 with (♦): 10 g L^{-1}, (■): 20 g L^{-1}, (▲): 40 g L^{-1}, (x): 60 g L^{-1}, (*): 134 g L^{-1} total sugars of palmyra sap.

4.1.3 Effect of medium supplementation on lactic acid production

Experiments were initially carried out to investigate the influence of fermentation performance with and without MRS medium addition to the palmyra sap, hydrolyzed palmyra sap and oil palm sap for lactic acid production in static flask at 37°C and pH 5.5 using 20 g L^{-1} of total sugars. The results, shown in Figure 4.1.4, clearly demonstrated that *L. casei* TISTR 1500 slightly grew in the without-addition MRS medium of the palmyra sap, hydrolyzed palmyra sap and oil palm sap. This might be due to the effects of some nutrient source presenting in these saps but there were insufficient nutrients for growth of bacteria. Products from fermentation were increased against time and were relatively constant at 42 h. The two highest main product concentrations detected were lactic acid and acetic acid. Palmyra sap, hydrolyzed palmyra sap and oil palm sap contained sufficient sugar content to be used as carbon source for lactic acid production. However, MRS media, which contains yeast extract, peptone and meat extract, was supplemented to these saps to support growth as the saps did not contain significant amount of nitrogen and minerals.

In Figure 4.1.4 a strong *L. casei* TISTR 1500 growth was observed. Results clearly showed that the palmyra sap, hydrolyzed palmyra sap and oil palm sap with addition MRS medium improved the fermentation by *L. casei* TISTR 1500 compared to the without-addition MRS medium. Lactic acid bacteria are considered fastidious microorganisms and have complex nutrient requirement due to their limited ability to biosynthesize B-vitamins and amino acids (Mussato *et al.*, 2008). Therefore, to achieve optimal cultivation conditions, the fermentation medium should contain minerals, B-vitamins, amino acids, fatty acids, purines and pyrimidines for bacteria growth and biological activity. Hofvendahl and Hahn-Hagerdal (2000) compared several studies concerning lactic acid production in fermentation media supplemented with different kinds of nutrients and reported on the positive aspect that addition of MRS broth components promotes a better fermentation performance when compared with addition of yeast extract. This could be explained by considering that yeast extract is also present in the MRS medium composition together with other nutrients such as meat extract, peptone and some salts.

Figure 4.1.4(A) showed sugar consumption in with and without addition MRS medium to the palmyra sap, hydrolyzed palmyra sap and oil palm sap. The consumption of total sugars (91.78-99.38%) in the with-addition MRS medium of the palmyra sap, hydrolyzed palmyra sap and oil palm sap was higher than that in the medium without-addition MRS (46.93-62.31%). The total sugars uptake in the with-addition MRS medium to these saps continued until the end of the fermentation (42 h).

Cell growth was favored in the palmyra sap, hydrolyzed palmyra sap and oil palm sap with the addition MRS medium (Figure 4.1.4(B)). The bacterial grew rapidly within 36 h and ceased after that. This was correlated to the rapid decline of pH due to that the sugar was metabolized by cells to form acidic metabolites. This caused growth inhibition and lactic acid production.

It is worth emphasizing that all these assays were performed without pH control which clearly affected the fermentation performance. As shown in Figure 4.1.4(D), at the beginning of the process (12 h), pH decreased from 5.53 to 3.48 in all fermentation media as a consequence of lactic acid production by the microorganism. This affected the microorganism metabolism that acted better in a pH range between 5.0 and 7.0 (Lasekan *et al.*, 2007). Moreover, pH 5.5 has been used for lactic acid production using *L. helveticus* (Ghaly *et al.*, 2004). Hydrogen ion concentration of a medium had the maximum influence on microbial growth. The pH has affected at least two aspects of microbial cells, i.e. functioning of its enzymes and the transport of nutrients into the cell. It has limited the synthesis of metabolic enzymes responsible for the synthesis of new protoplasm. In addition, the pH values still affect the RNA and protein synthesis. When microorganisms were grown on either side of their optimum pH range, there may be an increasing lag phase (Panesar *et al.*, 2010).

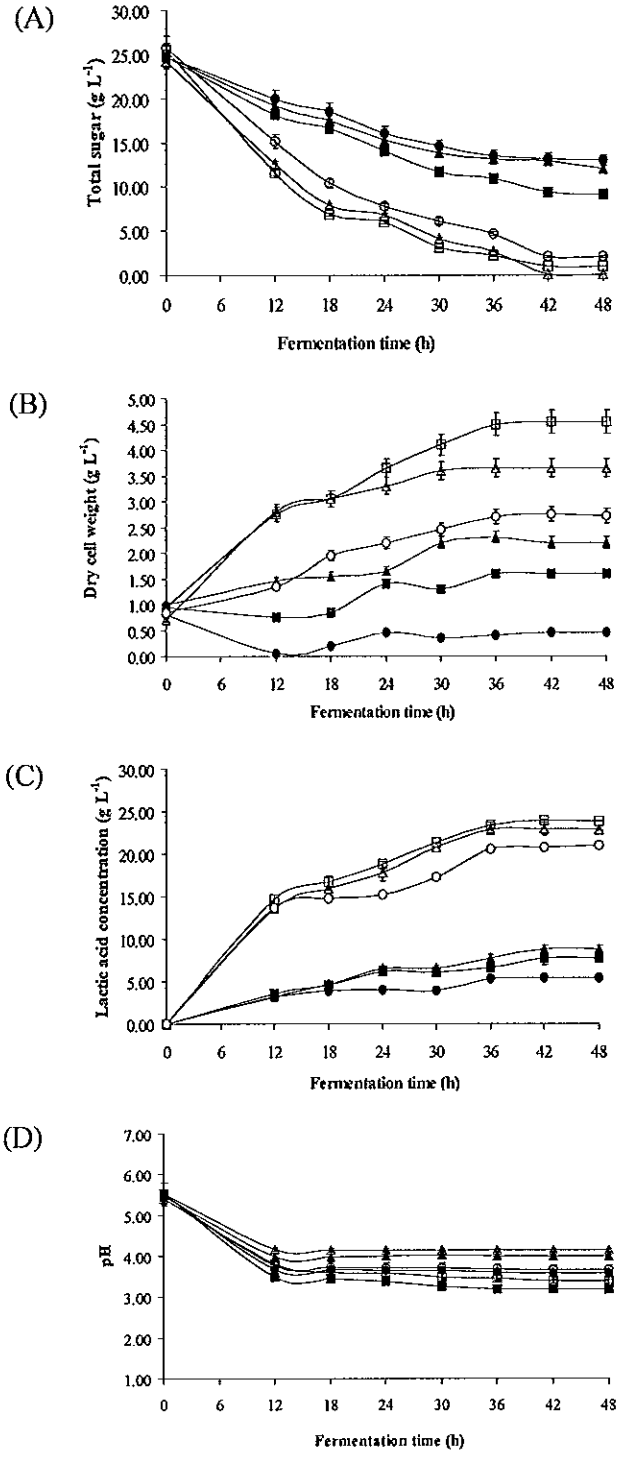


Figure 4.1.4 Kinetic profiles of total sugars consumption (A), dry cell weight (B), lactic acid production (C) and pH (D) over time of fermentation for lactic acid production by *L. casei* TISTR 1500 with (•): PS, (■): HPS, (▲): OPS, (o): PS+MRS, (□): HPS+MRS, (Δ): OPS+MRS

Table 4.1.3 Kinetic parameters of lactic acid production by *L. casei* TISTR 1500 from different carbon sources

Carbon source	Residual sugar ($g\ L^{-1}$)	Lactic acid ($g\ L^{-1}$)	Yield ($g\ g^{-1}$)	Maximum DCW ($g\ L^{-1}$)	Productivity (42h) ($g\ L^{-1}h^{-1}$)
PS	13.00±0.42	5.41±0.20	0.46±0.05	0.45±0.03	0.13±0.007
HPS	9.10±0.28	7.71±0.18	0.49±0.02	1.60±0.02	0.18±0.006
OPS	12.10±0.28	8.85±0.12	0.70±0.02	2.20±0.05	0.21±0.004
PS+MRS	2.08±0.11	20.81±0.21	0.88±0.01	2.72±0.04	0.50±0.007
HPS+MRS	0.98±0.03	23.87±0.23	0.97±0.06	4.55±0.03	0.57±0.008
OPS+MRS	0.13±0.03	22.90±0.15	0.95±0.01	3.65±0.04	0.55±0.005

Note: Batch fermentations were performed on 250 mL static flask with working volume of 100 mL at pH 5.5, 37°C for 42 h. Results were the average of data from triplicate experiments.

PS: palmyra sap; HPS: hydrolyzed palm sap; OPS: oil palm sap; MRS: deMan Rogosa and Sharpe.

The kinetic parameters of batch cultivation in static flask were given in Table 4.1.3. Kinetics values obtained from cultivation with MRS medium in the palmyra sap, hydrolyzed palmyra sap and oil palm sap were higher than those from the palmyra sap, hydrolyzed palmyra sap and oil palm sap without the MRS medium. Lactic acid yields, based on total sugars consumed, were obtained in the range of 46.0-97.0%, Moreover, the maximum lactic acid productivity (0.57 g L^{-1} h^{-1}) and the dry cell weight (4.55 g L^{-1}) were found in the MRS-contained hydrolyzed palmyra sap. Results were shown in Table 4.1.4. The addition of MRS medium enhanced the product yield of palmyra palm (91.30%), hydrolyzed palmyra sap (97.96%) and oil palm sap (35.71%). The fermentation performance in oil palm sap supplemented with MRS slightly increases. It could be explained that the sap presents a high content in nitrogen source (Table 3.1). In addition, it was found to be rich in various kinds of

amino acids, minerals and vitamins (Kosugi *et al.*, 2010). Thus, addition of MRS in oil palm sap slighty improves enhancement of product yield. Moreover, there was a slightly significant increases in the amount of product yield (6.52%) when palmyra sap with MRS medium was hydrolyzed. The reason was that acid hydrolysis using sulfuric acid caused in releasing of some toxic compounds or inhibitors (Laopaiboon *et al.*, 2010). Thus, palmyra sap with MRS medium could well serve as a carbon source for *L. casei* TISTR 1500 without the acid hydrolysis.

Table 4.1.4 Lactic acid production for various sources of carbon, acid hydrolysis and MRS medium during 42 h of flask cultivation (initial total sugars concentration: 20 g L^{-1})

Carbon source	Enhancement of product yield (%) of lactic acid production	
	by acid hydrolysis	by MRS medium
Palmyra sap	6.52	91.30
Hydrolyzed palm sap	-	97.96
Oil palm sap	-	35.71

4.1.4 Effect of pH control on fermentation

Level of pH is one of the most important environment parameters affecting cell growth and product formation. In general, effects of pH on cell growth and product accumulation vary with different microorganisms, medium composition, and operational conditions. Some literatures (Hofvendahl and Hahn-Hagerdal, 2000; Idris and Suzana, 2006; Chang *et al.*, 2001) dealing with conditions of *Lactobacillus* reported the optimal pH for cell growth and lactic acid production. To date, no reports have been found about the effects of pH control on cell growth and lactic acid production in a lab-scale fermentor with palmyra sap, hydrolyzed palmyra sap and oil palm sap as substrates.

Lactic acid-producing bacteria (LAB) are constantly confronted with acidified environments, making acid stress part of the life cycle of LAB due to their ability to ferment sugars into lactate. Knowledge on metabolic process stress response caused by low pH in certain strains of development of many biotechnology products. The purpose of this experiment was to evaluate the inhibitory effect on growth and lactic acid production by *L. casei* TISTR 1500 exposed to conditions of stress caused by acidification of the medium. In this work, all fermentation cases started in 2 L fermentor containing 1.0 L optimal medium, incubation temperature 37°C, with and without control of pH (5.5). The results were displayed in Figure 4.1.5.

First, it is important to note that microorganisms were able to grow and produced lactic acid in both culture media tested (with and without pH control). There was a similar growth pattern, reaching a stationary phase after 48 h of fermentation. During the initial 12 h, a similar performance was observed in fermentations with and without pH control. However, the consumption of total sugars, lactic acid production and cell growth were influenced by the fermentation pH (Figure 4.1.5).

According to some authors (Hofvendahl and Hahn-Hagerdal, 2000; Kashket, 1987), weak acid, e.g., lactic acid, inhibit bacterial growth because as the external pH declines, the acid is protonized as soon as it is exported out of the bacteria. Uncharged, it diffuses back into the cell and dissociates due to the higher

intracellular pH. The cell then has to use ATP to pump out protons and energy eventually is depleted, causing growth stop and the bacteria die.

Lactic acid production in pH-controlled palmyra sap, hydrolyzed palmyra sap and oil palm sap supplemented with MRS components were of 27.12 g L^{-1}, 28.34 g L^{-1} and 26.49 g L^{-1}, respectively. While in the medium without pH control only 23.50 g L^{-1}, 25.08 g L^{-1} and 22.90 g L^{-1} were obtained (Figure 4.1.5(B)). These values represented approximately increasing in lactic acid concentration of 15%, 13% and 16%, respectively, when the pH of the MRS-supplemented palmyra sap, hydrolyzed palmyra sap and oil palm sap was controlled. The result was obtained similarly by Wee *et al.* (2006) on the subject of *Enterococcus faecalis* RKY1 grown on molasses preferred neutral or alkali conditions for lactic acid fermentation. When acidic condition (pH 5.0) was used in lactic acid production, cell growth was ceased after 10 h.

L. casei TISTR 1500 was able to grow and produce lactic acid with highest efficiency in the MRS-supplemented hydrolyzed palmyra sap, which could have favored the bioconversion process since the higher the cell concentration the larger the amount of substrate that can be consumed and converted into products (lactic acid and acetic acid) (Figure 4.1.5(C)).

According to Idris and Suzana (2006), lactic acid production depends on microbial growth, thus an increase in microbial growth promotes an increase in the lactic acid production. Mussato *et al.* (2008) found that after 60 h fermentation lactic acid production by *L. delbrueckii* UFV H2B20 in brewer's grain cellulosic hydrolysate supplemented with MRS components, and pH controlled value at 6.0, $Y_{P/S}$ and R_M values of 0.99 g g^{-1} and 0.59 g L^{-1} h^{-1}, respectively, were obtained.

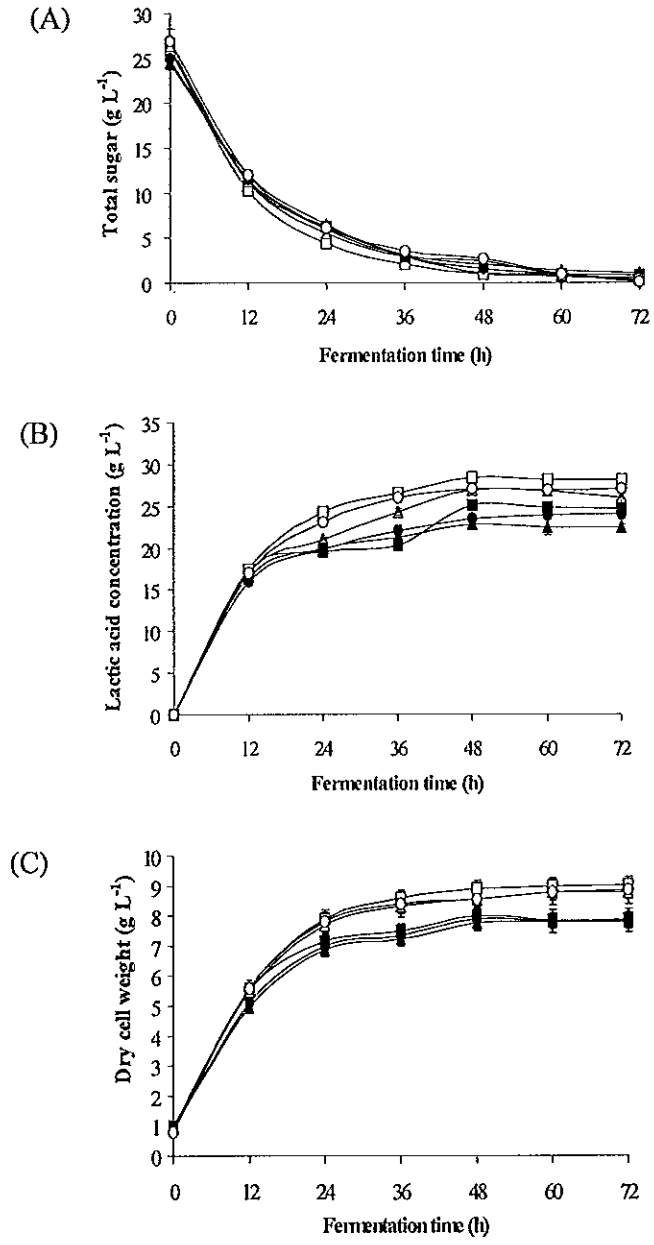


Figure 4.1.5 Effect of the pH control on total sugars consumption (A), lactic acid production (B) and dry cell weight of *L. casei* TISTR 1500 (C) in media: palmyra sap with MRS components (●) without pH control; (o) with pH control; hydrolyzed palmyra sap with MRS components (■) without pH control; (□) with pH control; oil palm sap with MRS components (▲) without pH control; (Δ) with pH control.

Table 4.1.5 summarized the fermentative parameters obtained in the fermentation runs with and without pH control. It was found that lactic acid yield and productivity were higher than those in the fermentation runs with pH control. The reason could be from the pH inhibition and the low cell growth provoked the inability of the strain in using the remaining sugars. As the fermentation continued, the rate slowed down because of the accumulation of lactic acid so pH values fell causing metabolic inhibition. This indicated the importance of pH control. The result was obtained similarly by Tango and Ghaly (1999). They reported that productions of 10 g L^{-1} of lactic acid from fermentation of cheese whey without pH control using *Lactobacillus helveticus*. The pH inhibition and the final lactic acid concentration were suggested to have a strong inhibitory effect.

It was found that the kinetic parameters of pH controlled of the MRS-supplemented hydrolyzed palmyra sap were highest with the following results: conversion yield of substrate to product ($Y_{P/S}$) 1.11 g lactic acid g^{-1} total sugars, cellular yield coefficient ($Y_{X/S}$) 0.32 cells g^{-1} total sugars, and the maximum productivity (R_M) 0.59 g lactic acid L^{-1} h^{-1}. The results of lactic acid yield were more than one. It might be explained by the utilization of nutrient sources other than the considered sugars or by hydrolysis of the oligosaccharides, which altered the mass of sugars by the incorporation of water molecules (Karp *et al.*, 2010). In the culture, both for the uncontrolled and controlled pH, not only lactic acid but also acetic acid (0.20-0.26 g L^{-1}) were the main products in the fermentation broth. These results indicated that glucose, the main sugar in the hydrolyzed palmyra sap and oil palm sap, was metabolized to lactic acid via heterolactic fermentation pathway or mixed acid fermentation.

Table 4.1.5 Fermentation parameters of lactic acid production by *L. casei* TISTR 1500 in different fermentation media

	PS+MRS		HPS+MRS		OPS+MRS	
	Without pH control	With pH control	Without pH control	With pH control	Without pH control	With pH control
Lactic acid ($g\ L^{-1}$)	23.50±0.250	27.12±0.360	25.08±0.120	28.34±0.350	22.90±0.240	26.89±0.400
Acetic acid ($g\ L^{-1}$)	0.20±0.030	0.22±0.050	0.22±0.040	0.23±0.110	0.25±0.120	0.26±0.080
$Y_{P/S}$ ($g\ g^{-1}$)	0.95±0.061	1.01±0.022	0.99±0.004	1.11±0.012	0.98±0.049	1.04±0.001
$Y_{X/S}$ ($g\ g^{-1}$)	0.28±0.015	0.30±0.008	0.27±0.011	0.32±0.003	0.30±0.005	0.31±0.020
R_M ($g\ L^{-1}\ h^{-1}$)	0.49±0.007	0.56±0.011	0.52±0.004	0.59±0.010	0.48±0.007	0.56±0.012

Note: Batch fermentations were performed on a 2.0 L stirred tank bioreactor with 1 L working volume at pH 5.5, 37°C for 48 h. Results were the average of data from triplicate experiments.

PS: palmyra sap; HPS: hydrolyzed palm sap; OPS: oil palm sap; MRS: deMan Rogosa and Sharpe.

As tabulated in Table 4.1.6, with pH control of palmyra sap, hydrolyzed palmyra palm and oil palm sap, product yield were of 6.32%, 12.12% and 6.12%, respectively. This indicated that the effect of pH control of palmyra sap, hydrolyzed palmyra palm and oil palm sap rendered only a slightly significant increase in lactic acid production. Therefore, pH control from palmyra sap, hydrolyzed palmyra palm and oil palm sap might not be required. Cost-effectiveness of controlled versus uncontrolled pH for lactic acid production should be further studied.

Table 4.1.6 Lactic acid production for various sources of carbon and pH control during 42 h of flask cultivation (initial total sugars concentration: 20 g L^{-1})

Carbon source	Enhancement of product yield by pH control (%)
Palmyra sap	6.32
Hydrolyzed palm sap	12.12
Oil palm sap	6.12

With increasing interest in producing biotechnological products from low-cost and renewable biomass, production of lactic acid from various raw agricultural materials has gained considerable attentions recently. Many microorganisms, including lactic acid bacteria (LAB), have been investigated for production of lactic acid. Some examples of microbial lactic acid production from agricultural resources by LAB were shown in Table 4.1.7. Relatively low lactic acid concentrations were obtained when oil palm sap (Kosugi *et al.*, 2010), sugarcane bagasse (Laopaiboon, 2010), and whey (Ghasemi *et al.*, 2009) were used for lactic acid production. However, higher concentration of lactic acid were reported using hydrolyzed cane sugar (Kadam *et al.*, 2006), brewer's spent grain (Mussato *et al.*, 2008), wheat bran (Li *et al.*, 2010), molasses (Kotzamanidis *et al.*, 2002 ; Dumbrepatil *et al.*, 2008), and cashew apple juice (Silveira *et al.*, 2010). In the present study, palmyra sap yielded productivity less than many of those reported since it contained high sucrose content raw material, which can only be slowly metabolized by lactic acid bacteria. In contrast, high productivity of lactic acid could be obtained by using oil palm sap compared to palmyra sap. Hence, oil palm sap is potentially more feasible and more efficient in lactic acid production by *L. casei* TISTR 1500.

Table 4.1.7 Data reported on batch fermentations for lactic acid from agricultural resources

Microorganism	Raw material	Initial sugar ($g\ L^{-1}$)	Lactic acid ($g\ L^{-1}$)	Productivity ($g\ L^{-1}\ h^{-1}$)	Reference
Lactobacillus delbrueckii	Hydrolyzed cane sugar	150.00(sucrose)	128.50	3.20	Kadam *et al.* (2006)
Lactobacillus lactis ATCC19435	Oil palm sap	18.95(total sugars)	17.04	0.24	Kosugi *et al.* (2010)
Lactobacillus delbrueckii	Brewer's spent grain	50.00 (glucose)	35.54	0.59	Mussato *et al.* (2008)
Lactobacillus lactis IO-1	Sugarcane bagasse	30.00(xylose)	10.85	0.17	Laopaiboon (2010)
Lactobacillus rhamnosus	Wheat bran	25.00 (wheat bran hydrolysate)	75.00	3.75	Li *et al.* (2010)
Lactobacillus delbrueckii NCIMB 8130	Molasses	100.00 (molasses sugar)	90.00	3.80	Kotzamanidis *et al.* (2002)
Lactobacillus delbrueckii Uc-3	Molasses	148.00 (molasses sugar)	129.00	4.30	Dumbrepatil *et al.* (2008)
Lactobacillus delbrueckii NCIMB 8130	Molasses	190.00 (molasses sugar)	166.00	4.15	Dumbrepatil *et al.* (2008)
Lactobacillus bulgaricus	Whey	50.00(lactose)	20.80	0.30	Ghasemi *et al.* (2009)
Lactobacillus casei B-442	Cashew apple juice	50(reducing sugar)	47.37	2.36	Silveira *et al.* (2010)
Lactobacillus casei TISTR 1500	Palmyra sap	134.00 (total sugars)	28.35	2.02	This study
Lactobacillus casei TISTR 1500	Oil palm sap	20.00(total sugars)	22.90	0.55	This study

4.2 Adsorption of Lactic acid using Anion Exchange Resin as Adsorbents

Ion exchange was studied in two processes, batch adsorption and column adsorption. These processes contained adsorption and desorption steps.

4.2.1 Adsorption isotherms

Adsorption isotherm of Amberlite IRA402 and Dowex^{TM} 66 resin were determined. The amount of adsorbed lactic acid (q_e, mg g^{-1}) was plotted versus initial lactic acid concentration (20-100 g L^{-1}) that shown in Figure 4.2.1. The amount of lactic acid adsorbed (q_e) was calculated using Equation (3.1). Result indicated that the isotherm existed as those of Type II isotherm. Furthermore, the maximum experimental adsorption capacities of lactic acid at 100 g L^{-1} onto Amberlite IRA402 (strong base anion resin) and Dowex^{TM} 66 (weak base anion resin) resins were of 227.00 lactic acid g^{-1} dry resin and 148.60 mg lactic acid g^{-1} dry resin, respectively. The results indicated that Amberlite IRA402 has more adsorption capacity than Dowex^{TM} 66, suggesting that strong-base resins showed the highest capacities, which was in agreement with Moldes *et al.* (2003). Two simultaneous phenomena happened when strong-base resins were used in OH^- form: ion exchange (hydroxyl ions were exchanged with lactate ions because of their different affinity), and neutralization reactions with the hydronium ions present in the medium. Both effects tend to increase the pH of media, leading to increase lactate/molecule lactic acid ratios owing to the displacement of the equilibrium. In contrast, weak-base resin in free-base mechanism being the adsorption of the molecular form of lactic acid. The difference in behavior between strong- and weak-base resins could explain their different capacities. Moreover, the strong base resin in OH^- form presented higher capacities than the weak base (in free base form) because the strong base resins present usually low affinities for the OH^- ion; this fact allowed them to exchange easily (Evangelista and Nikolov, 1996). Additionally, as mentioned previously, strong base resins in OH^- form can interact with both dissociated and undissociated forms of lactic acid, but the weak base resins in free base interact only with the undissociated form (which is in the minority at pH of 4.85).

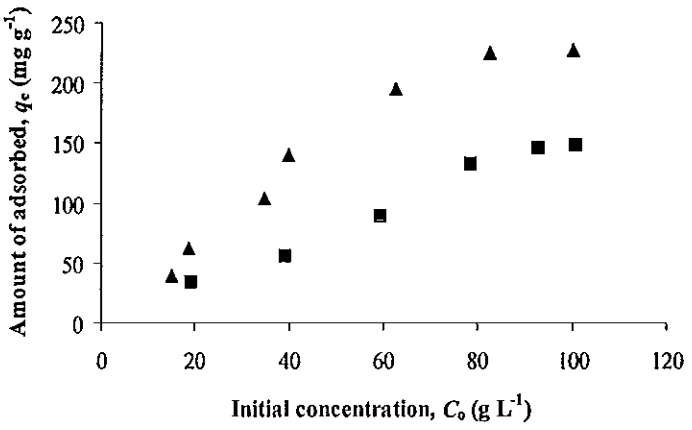


Figure 4.2.1 Equilibrium adsorption isotherm for lactic acid onto (▲) Amberlite IRA402 and (■) Dowex ™ 66 resins in binary component system at 30°C at pH 5.5.

4.2.2 Modeling of the adsorption isotherms

The relationship between adsorbed lactic acid concentration and concentration of the solution at equilibrium is described by isotherm models, of which Langmuir and Freundlich are the most widely referred equations. Langmuir isotherm model, given in Equation (4.2.1), is representative of monolayer adsorption occurring on an energetically uniform surface on which the adsorbed molecules are not interactive. Accordingly, equilibrium is attained once the monolayer is completely saturated (Aksoy and Aydin, 2009).The non-linear model is transformed into Equation (4.2.2) so that the corresponding constants can be computed.

$$q_e = \frac{K_L a_L C_e}{1 + a_L C_e} \tag{4.2.1}$$

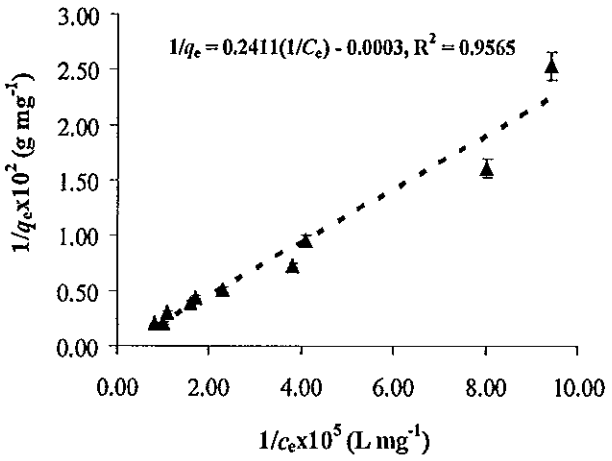
$$\frac{1}{q_e} = \frac{1}{K_L a_L C_e} + \frac{1}{K_L} \tag{4.2.2}$$

The values of Langmuir constants K_L and a_L (see Table 4.2.1) were obtained from the intercept and slope of the plot between ($1/q_e$) vs. ($1/C_e$) presented in Figure 4.2.2.

Table 4.2.1 Fitting parameters of models

Langmuir model			
adsorbents	k_L (mg g^{-1})	a_L (L mg^{-1})	R^2
Amberlite IRA402	-3333.33	-1.24E-03	0.9565
Dowex™ 66	588.24	4.00E-03	0.9955
Freundlich model			
adsorbents	k_F (mg g^{-1})	$1/n$ (mg L^{-1})	R^2
Amberlite IRA402	4.87	0.9644	0.9782
Dowex™ 66	3.03	0.8838	0.9893
Dubinin-Radushkevich			
adsorbents	k (mol^2 kJ^{-2})	q_m (mg g^{-1})	R^2
Amberlite IRA402	0.9436	0.6240	0.9710
Dowex™ 66	2.2141	0.9520	0.9885

(A)



(B)

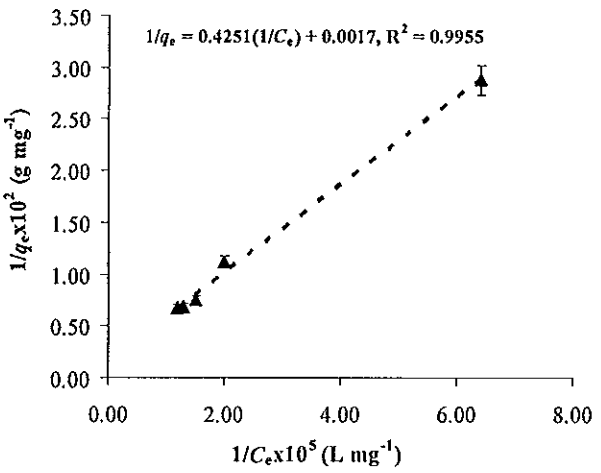


Figure 4.2.2 Langmuir adsorption isotherm of lactic acid adsorbed on resins (A) Amberlite IRA 402 and (B) Dowex™ 66 at temperature 30°C and pH 5.5

From linear equation; Figure (A) $y = 0.2411x - 0.0003$

Y – intercept was $1/k_L$; $1/k_L = -0.0003$

$k_L = -3{,}333.33$

Figure (B) $y = 0.4251x + 0.0017$

Y – intercept was $1/k_L$; $1/k_L = 0.0017$

$k_L = 588.24$

Slope was $1/ k_L a_L$; Figure (A) $1/ k_L a_L = 0.241$; $a_L = -1.24E-03$

Figure (B) $1/ k_L a_L = 0.425$; $a_L = 4.00E-03$

At pH 5.5, the Langmuir constant, k_L and a_L of Amberlite IRA 402 and Dowex™ 66 resin were -3,333.33 and -1.24E-03; 588.24 and 4.00E-03, respectively.

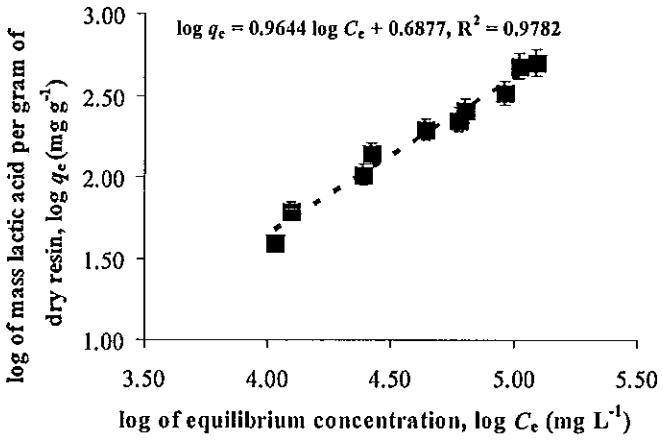
Contradictory to Langmuir, Freundlich model, shown in Equation (4.2.3), describes the adsorption on an energetically heterogeneous surface on which the adsorbed molecules have been interactive (Aksoy and Aydin, 2009).The model has been linearized to give Equation (4.2.4)

$$q_e = K_F C_e^{1/n} \quad (4.2.3)$$

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (4.2.4)$$

The values of K_F and n (see in Table 4.2.1) were calculated from the slope and intercept of the plot between ln q_e and ln C_e (Figure 4.2.3).

(A)



(B)

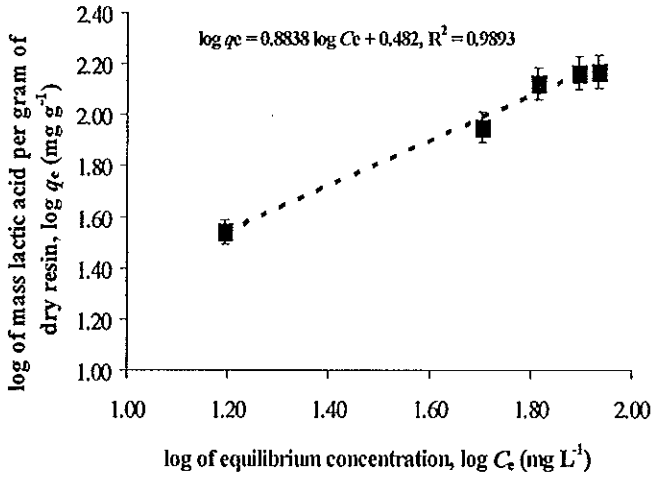


Figure 4.2.3 Freundlich adsorption isotherm of lactic acid adsorbed on resins (A) Amberlite IRA 402 and (B) Dowex™ 66 at temperature 30°C and pH 5.5

From linear equation; Figure (A) $y = 0.9644x + 0.6877$

Y – intercept was k_F; $k_F = 4.87$

Figure (B) $k_F = 3.03$

Slope was $1/n$; Figure (A) $1/n = 0.9644$

Figure (B) $1/n = 0.8838$

The experimental data was fitted to Dubinin-Radushkevich isotherm model in order to determine the adsorption type. The non-linear model given with Equation (4.2.5) is reduced to linear form as in Equation (4.2.6)

$$q_e = q_m \exp(-k\varepsilon^2) \quad (4.2.5)$$

$$\ln q_e = \ln qm \left(-k\varepsilon^2\right) \quad (4.2.6)$$

Where ε is the Polanyipotential, which is calculated from Equation (4.2.7)

$$\varepsilon = RT \ln\left(1 + \frac{1}{C_e}\right) \quad (4.2.7)$$

The isotherm constants q_m and k (see in Table 4.2.1) were calculated from the slope and intercept of the plot of ln q_m vs. ε^2. The mean free energy of adsorption (E) was calculated using the value of k according to Equation (4.2.8)

$$E = 2k^{-0.5} \quad (4.2.8)$$

Accordingly, The E values of lactic acid adsorbed on Amberlite IRA 402 and Dowex ™ 66 resins were of 0.7279 kJ mol^{-1} and 0.4752 kJ mol^{-1}, respectively. The common regard about E was that it depicted adsorption by ion-exchange when its value was between 8 kJ mol^{-1} and 16 kJ mol^{-1}. The value of E calculated in this study was substantially lower than 8 kJ mol^{-1}, indicating that the adsorption of lactic acid on both resins occured via physical adsorption due to weak vander Waals forces (Aksoy and Aydin, 2009).

A dimensionless separation factor R_L was calculated by Equation (4.2.9) for confirmation of the efficiency of adsorption.

$$R_L = \frac{1}{(1 + bC_0)} \tag{4.2.9}$$

While $0<R_L<1$ denotes favorable adsorption, $R_L>1$ is an indication of unfavorable adsorption. The value for $R_L>1$ is an indication of unfavorable adsorption (Aksoy and Aydin, 2009). The values for R_L of lactic acid adsorbed on Amberlite IRA 402 and Dowex™ 66 resins were of 0.0704 and 0.0157, respectively. The values of R were all in the range 0-1 which indicated the favorability of lactic acid adsorption both resins. The amount of lactic acid adsorbed (q_e) on both resins plotted against its equilibrium concentration at 30°C was shown in Figure 4.2.4. Three isotherm models, the Freundlich, Langmuir and Dubinin-Radushkevich, were tested for best-fit and their non-linear. Three models can be linerized and then the fitting parameters were determined graphically by linear regression. Based on the fitted curves, the Freundlich model was found to provide a best-fit between 2-model tested (Figure 4.2.4).

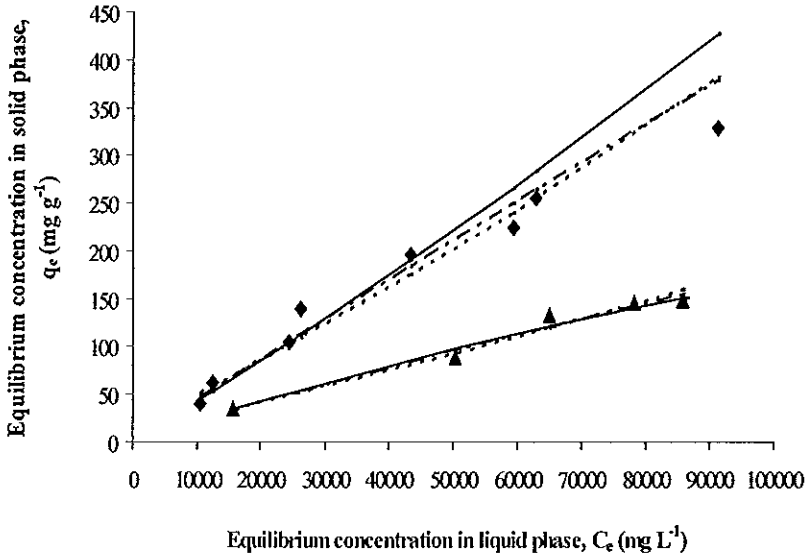


Figure 4.2.4 Lactic acid equilibrium adsorption isotherms of Amberlite IRA402 (♦) and Dowex™ 66 (▲) resins at 30°C. Fitting to Langmuir (——), Freundlich (- - -) and Dubinin-Radushkevich (·····) models

4.2.3 Effect of other component in aqueous mixture

The amount of lactic acid adsorbed onto Amberlite IRA 402 and Dowex ™ 66 resin with multicomponent system was compared at 20 g L^{-1} lactic acid for investigating the influence of other components in aqueous solution. An adsorption capacity for lactic acid in mixture with inorganic salts and sugar was shown in Figure 4.2.5. From this figure, the results showed that lactic acid, acetic acid and inorganic salts were adsorbed on two resins whereas glucose was not significant on adsorption. According to Houwing *et al.* (2002) the strong base resin in OH^- form had higher adsorption capacities than the weak base in free base form. Lactic acid was adsorbed higher than acetic acid in two anion resin due to the stronger acidity of lactic acid (pKa=3.76) than acetic acid (pKa=4.76). The stronger the acidity, the more preferentially is the acid adsorbed, because the adsorption of carboxylic acids onto basic polymeric adsorbents can be regarded as an acid/base neutralization reaction (Yoshida and Kishimoto, 1995).

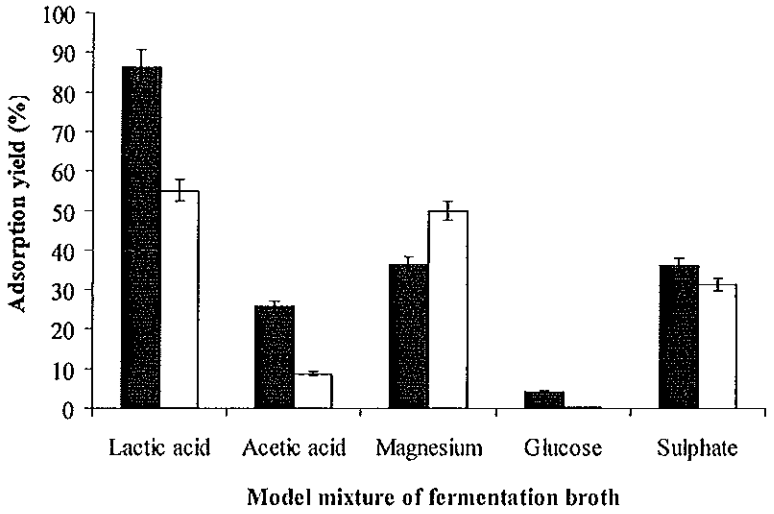


Figure 4.2.5 The amount of lactic acid adsorbed onto (■) Amberlite IRA402 and (□) Dowex™ 66 resin with multicomponent at 20 g L^{-1} lactic acid concentration

Glucose, the carbon and energy source for cells is the major individual component in the fermentation broth other than lactic acid. Figure 4.2.5 showed the amount of the capacity of glucose adsorbed onto two resins. The amount of glucose adsorbed was so small, indicating that negligible adsorptive capacity of ion exchange resin for glucose. Similar behavior has been reported by Aljundi *et al.* (2005), and showed that glucose has not significantly affected lactic acid adsorption on activated carbon.

4.2.4 Desorption

In this part, lactic acid adsorbed resins were investigated in order to determine the ability of lactic acid desorption in synthetic fermentation broth. Figure 4.2.6 showed the desorption yield of lactic acid from the Amberlite IRA402 and Dowex™ 66 resins using different ethanol concentrations. The desorption yield of lactic acid from Dowex™ 66 was higher than Amberlite IRA402 resin. This might be explained that the Amberlite IRA402 resin might be of high affinity for the lactic acid adsorption. Therefore, lactic acid eluted from the resin seemed to be very difficult,

which affected the possibility of resin reuse. Moreover, the desorption yield of lactic acid both resin increased with decreasing ethanol concentration in eluants for both two resins. According to Antonio *et al.* (2000), the mixture of methanol and H_2SO_4 was not effective for elution L-(+)-lactic acid. Methanol is an organic solvent with low dielectric constant, which probably reduced the elution by H_2SO_4. However, methanol was a good eluant for lactic acid adsorbed on VI-15, a weak basic anion exchange resin (Evangelista *et al.*, 1994). Different characteristics in the elution of lactic acid are expected when different types of resins were used. The elution recovery of lactic acid from IRA-67 using methanol was only 9.6% and water was 22.3% (Cao *et al.*, 2002). Moreover, the highest elution recovery of 85.07% was obtained with water. From the industrial point of view, water is better than methanol as eluant due to its economy and facility (Cao *et al.*, 2002).

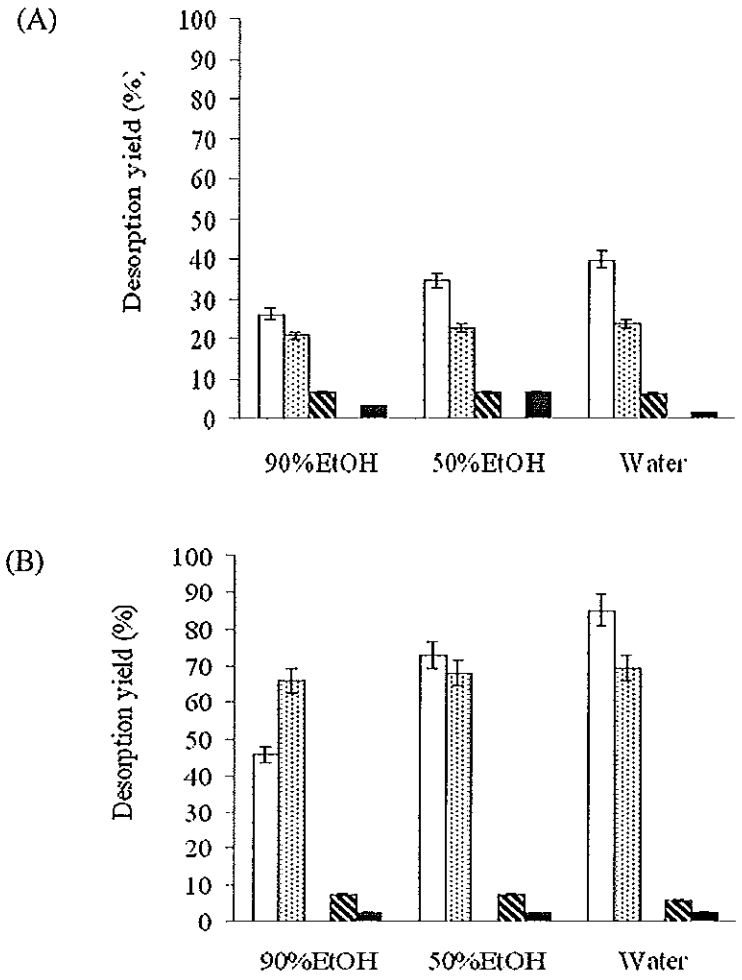


Figure 4.2.6 Desorption yield (%) of synthetic broth (□: lactic acid; ▨: acetic acid; ▨: magnesium; ▧: sulphate; ■: glucose) from (A) Amberlite IRA402 and (B) Dowex™ 66 resins using different ethanol concentrations

In Figure 4.2.7, the recovery yield (%) of lactic acid adsorbed on Amberlite IRA402 and Dowex™ 66 with water as eluant was 34.35% and 46.75%, respectively. Therefore, the recovery of lactic acid from Dowex ™ 66 was higher than Amberlite IRA402 resin from synthetic broth.

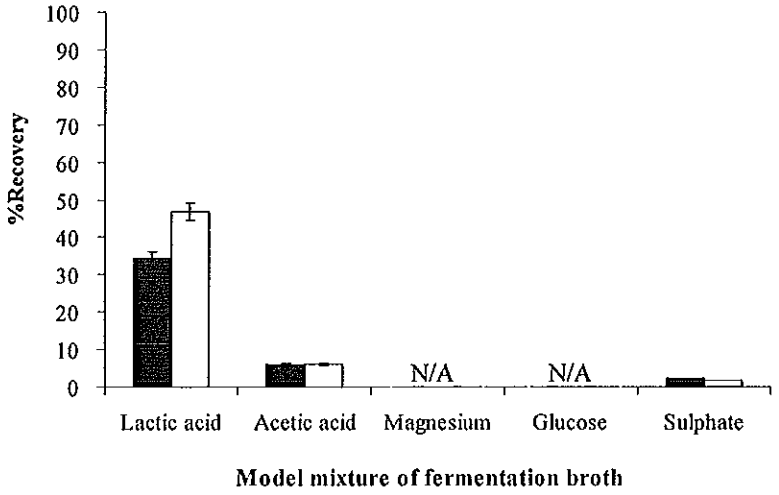


Figure 4.2.7 Recovery (%) of lactic acid from (■) Amberlite IRA402 and (□) Dowex ™ 66 resin

4.2.5 Column adsorption

Column adsorption was studied in the optimal elution for lactic acid purification in column separation.

1. Breakthrough curve

The breakthrough curves of two components (lactic acid and acetic acid) were shown in Figure 4.2.8. These components have not a t_B (breakthrough time) but have a t_E (exhaustion time) at 60 min and 70 min of lactic acid and acetic acid, the concentration of both acids at the outlet of a column were measured as a function of time. The adsorption capacity of lactic acid and acetic acid onto Dowex ™ 66 resin were of 0.37 mg g^{-1} and 0.26 mg g^{-1}, respectively.

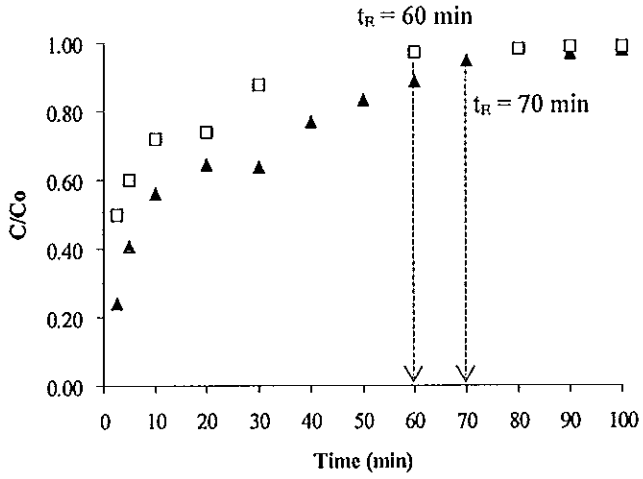


Figure 4.2.8 Breakthrough curve of (□) lactic acid and (▲) acetic acid on Dowex ™ 66 resin

Good separation performance required steeper and narrower breakthrough curve. Therefore, according to the results shown in Figure 4.2.8, lactic acid provided good adsorption performance comparison to acetic acid.

2. Modeling of column adsorption

Full-scale column operation can be designed on the basis of data collected at laboratory level. Many mathematical models have been proposed in the past for the evaluation of efficiency and applicability of the column models for large - scale operations. To design a column adsorption process it is necessary to predict the breakthrough curve or concentration-time profile and adsorption capacity of the adsorbent for the selected adsorbate under the given set of operating conditions. Many models have been developed to predict the adsorption breakthrough behavior with high degree of accuracy. The Thomas model and Yoon-Nelson model were used in this study to analyze the behavior of the selected adsorbent-adsorbate system.

2.1 Application of the Thomas Model

Successful design of a column adsorption process requires prediction of the concentration-time profile or breakthrough curve for the effluent. Designing the maximum adsorption capacity of an adsorbent is also needed.

Traditionally, The Thomas model is used to fulfill the purpose. The model has the following form

$$\frac{C_T}{C_0} = \frac{1}{1+\left(\frac{k_{Th} q_e x}{Q} - k_{Th} C_0 t\right)} \qquad (4.2.10)$$

Where K_{Th} is the Thomas rate constant (L min^{-1} mg^{-1}) and Q is the volumetric flow rate (L min^{-1}). The linearized form of the Thomas model is as follows:

$$\ln\left[\frac{C_0}{C_T} - 1\right] = \frac{k_{Th} q_e x}{Q} - k_{Th} C_0 t \qquad (4.2.11)$$

The kinetic coefficient K_{Th} and the adsorption capacity of the bed q can be determined from a plot of ln ($C_0/C_T - 1$) against t at given flow rate. In Figure 4.2.9, the K_{Th} and q_e values (R^2=0.9782) were 2.9x10^{-6} L min^{-1} mg^{-1} and 6.71x10^{-3} mg g^{-1}, respectively.

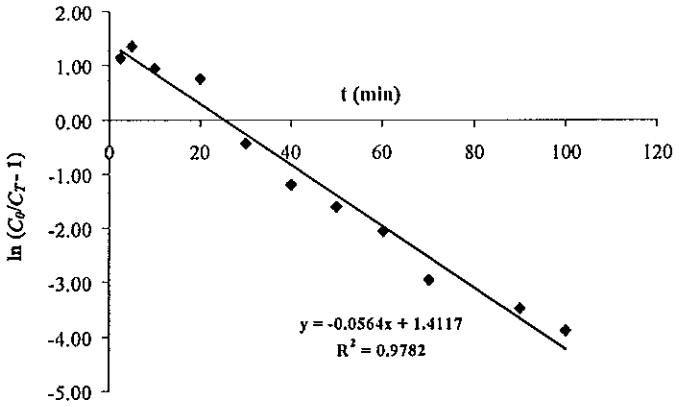


Figure 4.2.9 Thomas adsorption model of lactic acid adsorbed on Dowex™ 66 resin at temperature 30°C

2.2 Application of the Yoon and Nelson Model

This model is based on the assumption that the rate of decrease in the probability of adsorption for each adsorbate molecule is proportional to the

probability of adsorbate adsorption and the probability of adsorbate breakthrough on the adsorbent. The Yoon and Nelson model is not only less complicated than other models, but also requires no detailed data concerning the characteristics of adsorbate, the type of adsorbent, and the physical properties of adsorption bed. The Yoon and Nelson equation regarding to a single component system is expressed as

$$\frac{C_t}{C_0 - C_t} = \exp(k_{YN}t - \tau k_{YN})$$

Where k is the rate constant (L min^{-1}); τ, the time required for 50% adsorbate breakthrough (min) and t is the breakthrough (sampling) time (min). The linearized form of the Yoon and Nelson Model is as follows:

$$\ln\left(\frac{C_t}{C_0 - C_t}\right) = k_{YN}t - \tau k_{YN}$$

A plot of ln (C_t/C_0-C_t) versus t gives a straight line with a slope of k_{YN} and intercept of -τk_{YN} (Figure 4.2.10). It was found that the k_{YN} and τ values (R^2 = 0.9818) were of 0.0399 L min^{-1} and 2.36 min, respectively.

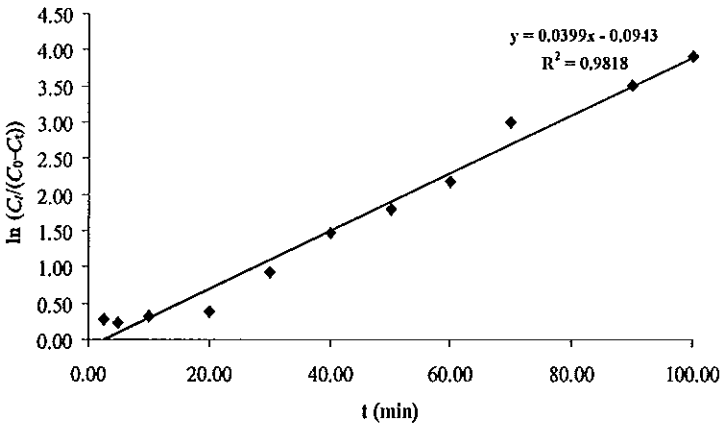


Figure 4.2.10 Yoon and Nelson adsorption model of lactic acid adsorbed on Dowex™ 66 resin at a temperature of 30°C

The breakthrough curves of lactic acid, acetic acid and glucose in aqueous solution were shown in Figure 4.2.11. The concentration of these organic acids in

effluent increased rapidly with elution time. The Thomas and Yoon-Nelson models were used to describe breakthrough curves of lactic acid, acetic acid and glucose in aqueous solution on the Dowex™ 66 resin, respectively. The calculated and experimental were in good agreement. The correlation coefficients of Yoon -Nelson models of breakthrough curves of lactic acid, acetic acid and glucose were of 0.9818, 0.9765, and 0.9558, respectively.

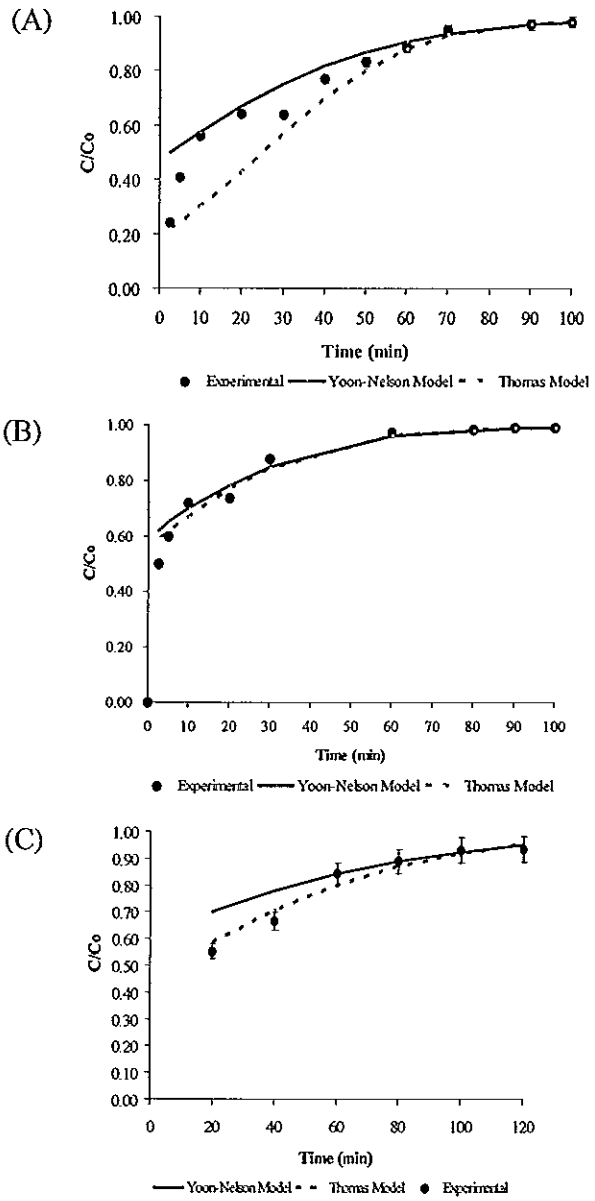


Figure 4.2.11 Comparison of the experimental and the predicted breakthrough curves for (A) lactic acid, (B) acetic acid and (C) glucose according to Thomas and Yoon-Nelson model (at 30°C, pH 5.5 and C_0 20 g L^{-1})

3. Desorption process in column

3.1 The effect of eluants to lactic acid recovery

Different ethanol concentrations were used for washing step to study the efficiency. It could reduce the product loss in washing step as results shown in the Figure 4.2.12. It was found that increasing ethanol concentration from 0% to 99.9% decreased lactic acid yield from 94.02% to 52.81% in washing step. When washing water changed adsorption environment, lactic acid loaded on resin was desorbed. Moreover, adsorption equilibrium was shifted to the direction of dissociation in the presence of washing water. Cao *et al.* (2002) studied washing column with water resulted in high product loss (40.82%). However, washing column with 50% methanol showed much lower product loss (only 10.57%) and total yield of 86.21%. Higher concentration of methanol would further reduce the product loss. The effect of increasing ethanol concentrations were not affected the acetic acid yield (20.48-22.69%wt) in this study.

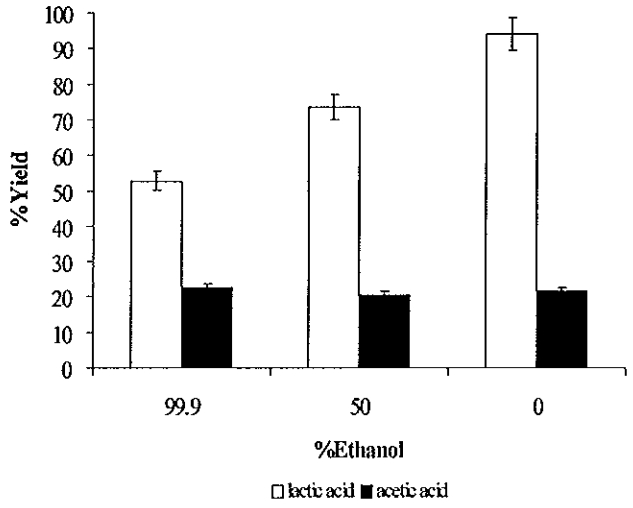


Figure 4.2.12 Effect of ethanol on washing step during recovery of lactic acid and acetic acid in shake flask using Dowex^{TM} 66 resin (at 30°C, pH 5.5 and C_0 20 g L^{-1})

3.2 Lactic acid recovery in column adsorption

The results of column separation were shown in Table 4.2.2. Washing the column with water also resulted in high product yield (84.91%). However, washing column with 99.9% ethanol showed much lower product yield (68.731%). It demonstrated that elution of lactic acid was easily performed. The twice column volume of water or 50% methanol could elute almost lactic acid from the loaded column. From the industrial point of view, water was better than methanol as eluant due to its economy and facility (Cao *et al.*, 2002).

Table 4.2.2 Column separation of lactic acid from lactic acid solution adsorbed on Dowex™ 66 resin

Solution	Concentration ($mg\ mL^{-1}$)	Volume (mL)	Total amount (mg)	Parameters		
				Adsorption (%)	Desorption Yield (%)	Lactic acid (%)
Washing step with water						
Loading	17.37	130	2258.10			
Effluent	16.46	100	1646.00	27.11		72.89
Washing	2.26	230	519.80		84.91	23.02
Eluate	0.55	170	93.50			4.14
Washing step with 50% ethanol						
Loading	16.39	130	2130.70			
Effluent	14.60	100	1460.00	31.48		68.52
Washing	2.47	200	494.00		73.65	23.18
Eluate	0.89	200	178.00			8.35
Washing step with 99.9% ethanol						
Loading	17.19	120	2062.80			
Effluent	16.00	90	1440.00	30.19		69.81
Washing	2.14	200	428.00		68.73	20.75
Eluate	0.99	200	198.00			9.60

From the results in Table 4.2.2, fermentation broth of oil palm sap contains: glucose 0.41 g L^{-1}; lactic acid 22.90 g L^{-1} and acetic acid 0.25 g L^{-1} (Table 4.2.4) was applied on Dowex^{TM} 66 ion exchange resin column at a flow rate of 1.0 mL min^{-1} and washed step with water until the resins were saturated. The saturated resins were eluted with water at a flow rate of 1.0 mL min^{-1}. The concentration of lactic acid in eluate was determined by GC/FID. The results were shown in Table 4.2.3. The experimental results demonstrated that when pH of the fermentation broth was 5.5 with water elution, the recovery yield in the lactic acid purification was 75.16%.

Table 4.2.3 Column separation of lactic acid from fermentation broth of oil palm sap adsorbed on Dowex^{TM} 66 resin

				Parameters		
Solution	Concentration (mg mL^{-1})	Volume (mL)	Total amount (mg)	Adsorption (%)[a]	Desorption Yield (%)[b]	Recovery (%)[c]
Loading	22.90	130	2977.00			
Effluent	20.39	100	2039.00	31.51		
Eluate	3.52	200	705.00		75.16	23.68

Note: [a]Adsorption (%) = amount of lactic acid adsorbed onto resin/ amount of loaded lactic acid x 100

[b]Desorption yield (%) = amount of lactic acid desorbed into the elution medium/ amount of lactic acid adsorbed onto resin x 100

[c]Recovery (%) = collected lactic acid/ amount of loaded lactic acid x 100

Table 4.2.4 Concentration of *L. casei* TISTR 1500 fermentation broth before adsorption and after desorption in separation column at pH 5.5 by Dowex^{TM} 66 resin

	Concentration (g L^{-1})	
Broth composition	Before adsorption	After desorption
Glucose	0.41	0.01
Lactic acid	22.90	3.52
Acetic acid	0.25	0.05

4.3 Esterification of Lactic Acid with Wet Ethanol by Semi-Batch Catalytic Distillation Combined with Two Step Distillation

This study was to focus at developing a technology of producing ethyl lactate from lactic acid with wet ethanol by using heterogeneous catalyst and concentrated lactic acid with simultaneously in order for hydrolysis to lactic acid.

4.3.1 Esterification in semi-batch catalytic distillation

Ethanol and lactic acid are reacted reversibly to form water and ethyl lactate, as shown below, with normal boiling points annotated:

Lactic acid + Ethanol ↔ Ethyl lactate + Water

(216°C) (78.5°C) (156°C) (100°C)

As an excess of water was present in the reaction mixture, conversion was greatly restricted by the equilibrium limitation. If one of the products could be effectively removed from the reaction mixture, then it would be possible to achieve very high conversions of limiting reactant. The reaction was very efficiently carried out in catalytic distillation wherein simultaneous reaction and distillation caused to shift the equilibrium in forward direction, which led to very high conversion of the limiting reactant at relatively low mole ratios (Seo *et al.*, 1999). Therefore, the semi-batch catalytic distillation was designed to remove water from reaction mixture at reaction temperature of 95°C. Although esterification is reversible reaction, removal of ethanol from reaction mixture was not shifted the equilibrium to the backward direction because excess alcohol was added before beginning the reaction. In addition, ethanol solution was continuously fed to maintain molar ratio of ethanol with lactic acid. Hence, the reaction was shifted to forward direction (Kumar and Mahajani, 2007).

The study case of aqueous lactic acid with wet ethanol was run using concentrated lactic acid (88 wt%) because it has a lower alcohol requirement and contains less water to be evaporated in the column, and wet ethanol concentration in the range of 70.0-99.7 wt%. The effect of ethanol concentration on esterification conversion of lactic acid and yield of ethyl lactate in the reaction medium were shown

in Figure 4.3.1. Results showed that esterification conversion and yield increased with each increment of ethanol concentration. When the amount of catalysts was fixed, an increase in ethanol concentration resulted in an accumulation of ethanol concentration in the reaction mixture and increasing molar ratio ethanol : lactic acid, hence increased the catalyst loading to ethanol ratio during the reaction. This increase in the catalyst loading to ethanol ratio resulted in higher ethyl lactate yield and high conversion of lactic acid. The increase in reactant concentration decreased the water of reaction mixture. Lower water level enabled more amount of ethyl lactate produced in the esterification reaction.

Since production rate of lactic acid which was esterified into ethyl lactate in the reactor increased, the final selectivity of ethyl lactate increased. Delgado *et al.* (2007) reported the sorption experiments in the esterification of lactic acid with ethanol, that water adsorbed most strongly, followed by ethanol, lactic acid and ethyl lactate. This was similar to the results obtained from Zhang *et al.* (2004) through FTIR experiments which proved that water adsorbed much stronger on the resin surface than lactic acid and ethyl lactate for the macro porous resin. They also found that the adsorption coefficient of water was greater than that of ethanol over catalysts, which reached to the conclusion that water could be adsorbed more strongly than ethanol on the active sites of the catalysts. Thus, increasing water content in ethanol decreased selectivity of ethyl lactate in esterification.

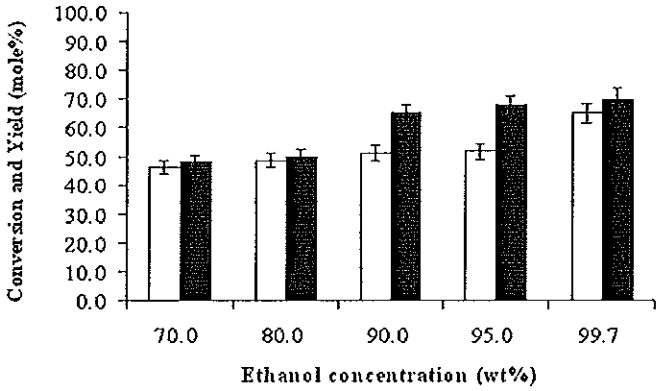


Figure 4.3.1 Conversion (□) and yield (■) vs. ethanol concentration of 70, 80, 90, 95 and 99.7wt% in semi-batch reactive distillation (initial lactic acid concentration, 88 wt%; catalytic concentration, 3.0 wt%; reaction temperature, 95°C; initial molar ratio of ethanol to lactic acid of 3:1; and reaction time, 0.5 hr)

However, this study found that the esterification yield of ethyl lactate was in the range of 47.75-69.83% (Figure 4.3.1). It could be explained that when ethanol was heated at reaction temperature of 95°C, which was higher than a boiling point of ethanol (78.5°C) in removing water from reaction mixture, the ethanol was vaporized from liquid phase reaction, which resulted in decreasing reaction rate and yield. Moreover, this reaction mixture not only esterified but also oligomerized which occured from the use of concentrated lactic acid concentration to produce ethyl lactate (Vu *et al.*, 2005). Based on these results, the effect of side reaction reduced the yield of ethyl lactate. We did not expect to achieve complete conversion of lactic acid to ethyl lactate and high yield of ethyl lactate in single stage catalytic distillation, but these ester products must contain low impurities due to pure ethyl lactate can easily be recovered by simple distillation.

4.3.2 Material balances of two step distillation

Ester products in esterification with ethanol concentrations of 70.0-99.7 wt% were calculated using mass balance before distillation. In this product, not only the ethyl lactate, but also the lactic acid and ethyl lactate oligomers appear in the reaction medium.

The liquid remained in the reactor were composed of unreacted ethanol (EtOH), water (H_2O), unreacted lactic acid, ethyl lactate (EtLa), and lactic acid and ethyl lactate oligomer (LA and LE Oligomers) components (Figure 4.3.2). Results showed that increasing ethanol concentration increased the weight percent of ethyl lactate and ethanol, but decreased the weight percent of water. Lactic acid, and LA and LE oligomer components were obtained in the range of 14.67 to 17.22 wt% and 7.49 to 8.27 wt%, respectively. The LA and LE oligomer components were slightly decreased with increasing ethanol concentration due to the characteristics of the system which occured multiple reactions (esterification, oligomerization, transesterification and hydrolysis) in the reaction mixture. When ethanol concentration decreased in reaction mixture, the transesterification (alcoholysis) of the oligomer esters to ethyl lactate were less reactive for this reaction (Asthana *et al.*, 2005).

The LA and LE oligomer components remained in the reactor due to concentrated lactic acid solutions undergoing intermolecular self-esterification to form higher oligomers. In oligomerization, two molecules of lactic acid first reacted to form a linear dimmer, commonly called lactoyllactic acid (L_2A), along with a mole of water. Lactoyllactic acid was further esterified with lactic acid to form the trimer lactoyllactic acid (L_3A); this process was continued by giving higher chain intermolecular polyesters L_4A, L_5A and so on. These oligomers were formed in esterification and hydrolysis reactions as given in Equation (1-5)

$$L_1A + EtOH \leftrightarrow L_1E + H_2O \quad (1)$$

$$L_2A + EtOH \leftrightarrow L_2E + H_2O \quad (2)$$

$$L_3A + EtOH \leftrightarrow L_3E + H_2O \quad (3)$$

$$L_2A + H_2O \leftrightarrow 2L_1A \quad (4)$$

$$L_3A + H_2O \leftrightarrow L_1A + L_2A \quad (5)$$

Where L_1A; L_2A and L_3A are lactic acid; and lactic acid and its oligomers, respectively; L_1E; L_2E, and L_3E are ethyl lactate; and ethyl lactate and its oliogomers, respectively (Vu *et al.*, 2005).

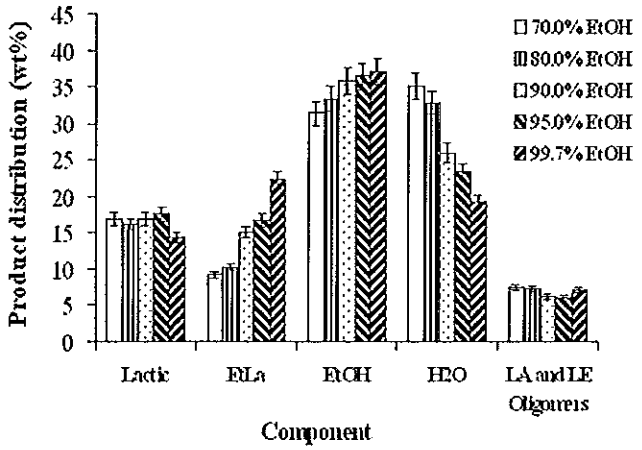


Figure 4.3.2 Product components obtained from esterification reaction in the semi-batch catalytic distillation

Ester product from the ester reactor was distilled in the first step distillation column to remove unreacted ethanol and water components into distillate stream (top stream) under the conditions of 55°C temperature and 700 mmHg vacuum pressure. The bottom stream consisting of unreacted lactic acid, ethyl lactate, and lactic acid and ethyl lactate oligomer components were purified to the second step distillation column. Moreover, ethyl lactate component was separated in the overhead product (Figure 4.3.3). Result demonstrated that ethanol, water and ethyl lactate

components were distilled into distillate stream as shown in Figure 4.3.3(A). Ethyl lactate component was obtained in the distillate stream because of two predominant reasons: the rectifying section in the lab-scale column was too short to facilitate separation, and ethyl lactate formed a minimum-boiling azeotrope with water (Asthana, 2005). Furthermore, it was also found that increased ethanol concentration increased the weight percent of ethanol, but decreased the weight percent of water in Figure 4.3.3(A). Ethanol and water from the first step distillation unit were removed because ethyl lactate was easily recovered from the bottom distillation unit and water in the bottom stream. The separation of ester product from water by distillation led to undesirable ester hydrolysis (Asthana *et al.*, 2005). However, lactic acid, and LA and LE oligomer components were not contained in distillate stream due to these components have a low vapor in first step distillation unit.

The bottom stream (Figure 4.3.3(B)) contains lactic acid, ethyl lactate, water, and lactic acid and ethyl lactate oligomer components. It could be seen that ethanol component was not obtained in this stream because it was removed by distillation into distillate stream. Furthermore, increasing ethanol concentration increased the weight percent of ethyl lactate, and lactic acid and ethyl lactate oligomer components, but decreased the weight percent of lactic acid and water. As water content in ethanol reactant decreased, the weight percent of lactic acid and ethyl lactate oligomer components increased from 8.60 wt% to 35.79 wt%.

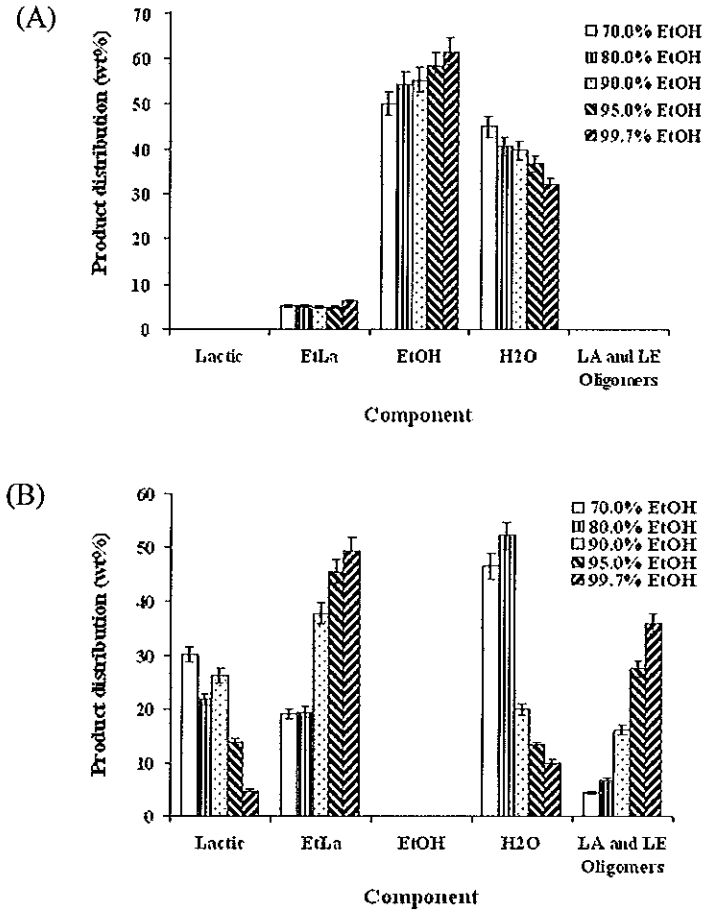


Figure 4.3.3 Product components obtained from (A) distillate stream and (B) bottom stream of the first distillation unit

Residual products in the bottom stream from the first step distillation column were distilled with the second step distillation column at a temperature of 110°C and under pressure of 700 mmHg to purify ethyl lactate component.

The effect of ethanol concentration (70.0 wt% to 99.7 wt%) on product distribution of different component was evaluated. In this unit, the ethyl lactate component was separated into the distillate stream. It contained ethyl lactate and water (Figure 4.3.4). It was found that increasing ethanol concentration increased the weight percent of ethyl lactate from 18.33 wt% up to 81.76 wt%. Ethyl lactate could be easily removed by distillation, since ethanol and ethyl lactate did not form

azeotrope (Delgado *et al.*, 2007). However, it decreased the weight percent of water in distillate stream (Figure 4.3.4(A)).

The weight percent of lactic acid and water decreased with increasing ethanol concentration in bottom stream (Figure 4.3.4(B)). On the other hand, a trend was observed in the case of the weight percent of lactic acid and ethyl lactate oligomers components that it increased because lactic acid and ethyl lactate oligomer components were formed from result of oligomerization reaction. Water and ethyl lactate were distilled to purify ethyl lactate from the bottom stream in the second step distillation unit. Lactic acid can be formed the oligomer components because water was removed from the solution. It could be explained that the hydroxyl on one molecule formed an ester with the carboxylic acid on another molecule. Asthana *et al.* (2005) found that eliminate water and ethanol from the bottom stream were desirable because ethyl lactate could easily be recovered from the bottom stream in a single column, and the oligomer components increased.

The physical properties of the oligomeric byproduct mixtures by mass balance contained 20.00, 30.00, 40.50, 61.37 and 79.00 wt% (Figure 4.3.4(B)), and had high viscous and brown colour at room temperature. The result was in good agreement with Vu *et al.* (2005) as they reported that concentrated solutions of lactic acid were fluidized at 120°C and highly viscous at room temperature.

Two steps of distillation were used to purify ethyl lactate from ester products. This process was evaluated with overall mass balance in their components as shown in Figure 4.3.5. It was found that increase ethanol concentration had a significant effect on the negative overall change of lactic acid component. This was the reason that concentrated lactic acid increased in distillation when water was removed, leading to oligomerization reactions as following in equation (6) and (7).

$$2L_1A \leftrightarrow L_2A + H_2O \qquad (6)$$

$$L_1A + L_2A \leftrightarrow L_3A + H_2O \qquad (7)$$

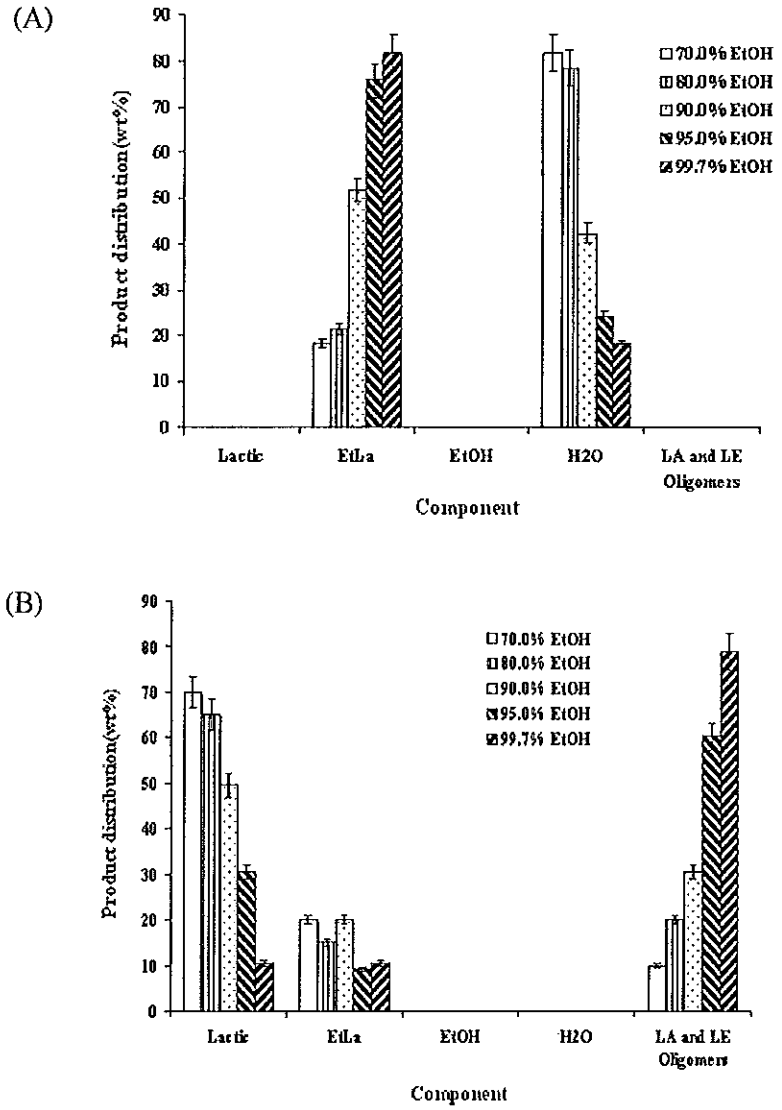


Figure 4.3.4 Product components obtained from (A) distillate stream and (B) bottom stream of second distillaton unit

From Figure 4.3.5, it was found that lactic acid component declined since it could be oligomerized and converted by itself to oligomer acid and water components in oligomerization. When lactic acid reacted with ethanol in esterification, ethanol component decreased and formed ethyl lactate and water components. This led to increase of ethyl lactate and water with the overall change of lactic acid, and lactic acid and ethyl lactate oligomer components. Moreover, water component was formed more than lactic acid and ethyl lactate oligomer component in lower concentration of

ethanol reactant. The water component decreased with increasing ethanol concentration because the overall change of lactic acid and ethyl lactate oligomer components increased highly, as clearly shown in Figure 4.3.5. Lactic acid and ethyl lactate oligomer components had a negative overall change in the range of 70-90 wt% ethanol concentration. The ethanol concentration of 95.0 wt% and 99.7wt% increased the positive overall changes of lactic acid and ethyl lactate oligomer components. The reason was that these components were obtained with the higher water content in ethanol concentration (70-90 wt%) and it led to hydrolysis reactions, as follow equation (4) and (5). Ethyl lactate component increases with ethanol concentration of 70 to 95 wt% since lactic acid reacted with ethanol to form ethyl lactate component in distillation step of ethanol and water (Figure 4.3.5 and 4.3.6)

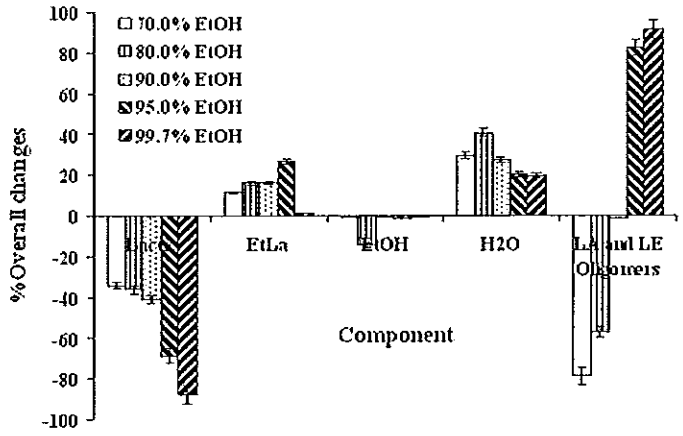


Figure 4.3.5 Mass balance of overall change for different components in two step distillation

Figure 4.3.6 showed mass balance of overall change for three components (lactic acid, ethyl lactate, and lactic acid and ethyl lactate oligomer components) in the two step distillations, which were calculated new composition before and after through the two step distillation based on lactic acid component. It was found that the negative overall change of lactic acid component increased with increasing ethanol concentration. Furthermore, ethyl lactate, and lactic acid and ethyl

lactate oligomer components increased with increasing ethanol concentration due to esterification and hydrolysis reactions.

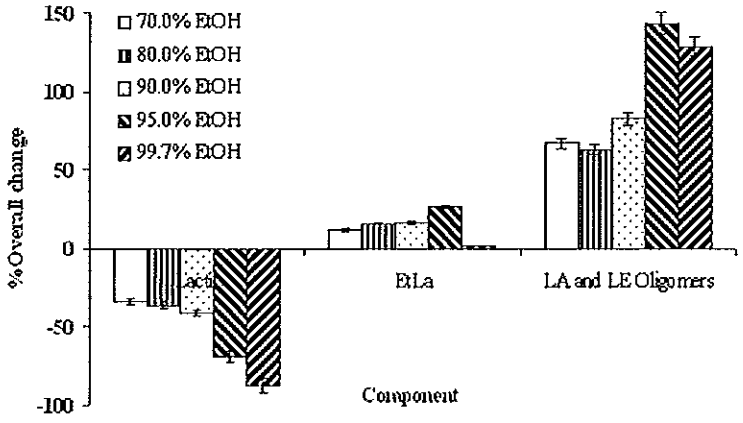


Figure 4.3.6 Mass balance of overall change for three components (LA, EtLA, and LA and LE Oligomer) in two step distillation

Mass balance of overall change for the three components (without distillate stream of first step distillation column) in the two step distillation was shown in Figure 4.3.7. Results showed that the negative overall change of lactic acid component increased with increasing ethanol concentration in the range of 70.0-99.7wt% from oligomerization reaction. Furthermore, lactate components increased with increasing the ethanol concentration of 70.0 to 95.0 wt% due to its formation in hydrolysis reaction.

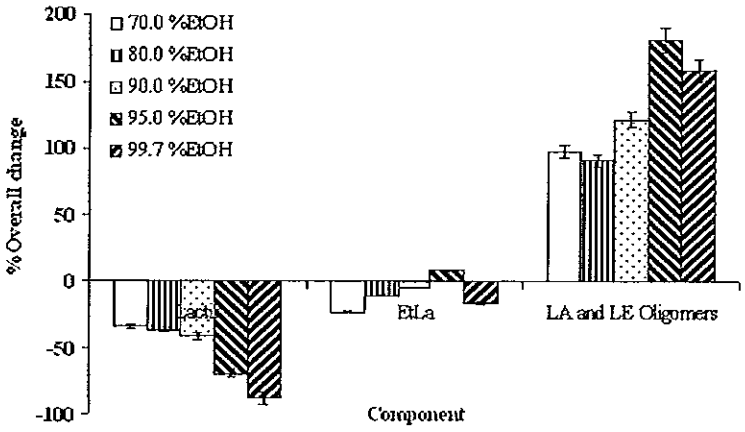


Figure 4.3.7 Mass balance of the overall change for three components (LA, EtLa, and LA and LE Oligomer) in the two step distillation (without distillate stream of first distillation)

CHAPTER 5

CONCLUSIONS AND SUGGESTIONS

5.1 Conclusion

This study showed the potential use of *Lactobacillus casei* TISTR 1500 for bioconversion of agricultural resources including palmyra sap and oil palm sap to reduce the manufacturing cost of lactic acid production. Lactic acid fermentation using palmyra sap was not significantly affected by acid hydrolysis and pH control. The final lactic acid concentration, dry cell weight and productivity increased with the increases of total sugar of palmyra sap concentrations up to 134.0 g L^{-1}. Improved biomass and lactic acid production of *L.casei* TISTR 1500 cultured in palmyra sap were achieved by MRS medium. The best bioconversion performance was attained under conditions; i.e., specific growth rate (μ), maximum productivity (R_M), cellular yield coefficient ($Y_{X/S}$) and lactic acid yield ($Y_{P/S}$) values of 0.05 h^{-1}, 2.02 g lactic acid L^{-1} h^{-1}, 0.20 g cell g^{-1} sugar, and 0.78 g g^{-1}, respectively. Lactic acid production by *L.casei* TISTR 1500 in oil palm sap was influenced by MRS supplementation. However, there was no significant increases in the amounts of biomass and product yield when pH of the oil palm sap with MRS medium was controlled. The highest values of production yield and maximal productivity were of 0.95 g g^{-1} and 0.55 g L^{-1} h^{-1}, respectively in a static flask at 37°C, pH 5.5 and 20 g L^{-1} of total sugars. Oil palm sap was proven to be a potential raw material for lactic acid production by *L.casei* TISTR 1500.

The maximal experimental adsorption capacity of lactic acid at 100 g L^{-1} onto Amberlite IRA402 and Dowex™ 66 were of 227.00 mg lactic acid g^{-1} dry resin and 148.60 mg lactic acid g^{-1} dry resin, respectively. Based on the fitted curves, the Freundlich model was found to provide a best-fit between 2-models (Langmuir and Dubinin-Radushkevich) tested. Moreover, the highest elution recovery of 85.07% was obtained by water. The recovery yield (%) of lactic acid adsorbed on Amberlite IRA402 and Dowex ™ 66 resin using water as eluant were of 34.35% and 46.75%, respectively. Thus, thus the recovery of lactic acid from Dowex ™ 66 resin was

higher than Amberlite IRA402 resin from synthetic mixtures. The recovery yields of lactic acid from synthetic and fermentation broths with adsorption column using water in the washing step were of 84.91% and 75.16%, respectively.

Production of ethyl lactate from concentrated lactic acid (88 wt%) and wet ethanol concentrations ranging between 70-99.7 wt% in a semi-batch catalytic distillation was investigated. The effect of ethanol concentration on esterification conversion and yield were increased with the increment of ethanol concentration. The two step distillation was used to purify ethyl lactate from the esterified products. This process was evaluated with overall mass balance from their components. Increasing of ethanol concentration had a significant effect of negative changing of lactic acid component and similar result of lactic acid and ethyl lactate oligomers components increased with increasing ethanol concentration (70.0-99.7 wt%) by oligomerization. Furthermore, lactic acid and ethyl lactate components increased with increasing ethanol concentration from 70.0 to 95.0 wt% for hydrolysis. Ethanol concentration at 95.0 wt% was proven to be potential reactant for ethyl lactate production.

5.2 Suggestions

The results of this work led to the following suggestions:

1. For the future work, the decrease addition of MRS media should be used for lactic acid production because the high cost of MRS media was considered to have a negative impact on its economic use in industrial scale process.

2. Since the pH control in fermentation was not significantly affected, it should be optimized in which pH value would be the best for the production of lactic acid.

3. To further improve the lactic acid recovery by ion-exchange, multiple columns would be investigated in order to improve the percentage recovery of lactic acid.

4. Ethyl lactate production from fermentation broth, which was treated by ion-exchange to obtain lactic acid, should be determined.

5. Process scaling up of fermentation and separation process should be studied for large scale fermentation broth and purification.

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APPENDIX

Appendix A

Analytical Method

1. Determine of organic acids (lactic acid and acetic acid), ethyl lactate and ethanol by GC analysis

Organic acids (lactic acid and acetic acid), ethyl lactate and ethanol were determined by gas chromatography (GC, Shimadzu 14A) using a capillary column (BP-20) with flame ionization detector with helium as the carrier to separate and determine amount of volatile components of a very small sample. The GC temperature program was initiated at 40°C and was ramped at 10°C min^{-1} to 110°C. Then, the temperature was heated from 110°C to 180°C and was ramped at 12°C min^{-1}. The temperature of the detector and injector were maintained at 250°C and 230°C, respectively. The sample was loaded at the injection port (via a manual syringe) which was heated in order to volatilize the sample. Once in the gas phase, the sample was carried onto the column by the carrier gas. After samples were measured organic acids and ethyl lactate concentration were calculated by comparing to standard curve.

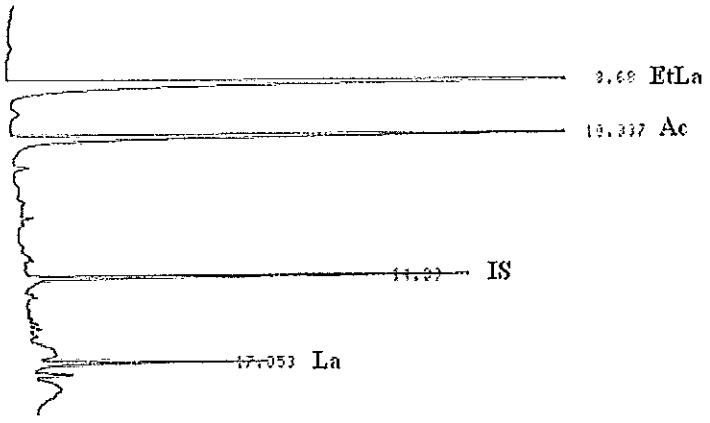


Figure 1A GC chromatogram of ethyl lactate, acetic acid and lactic acid

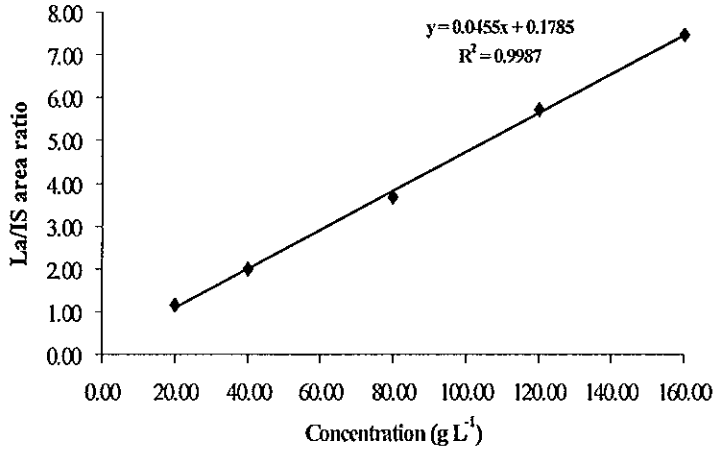


Figure 2A Lactic acid standard curve

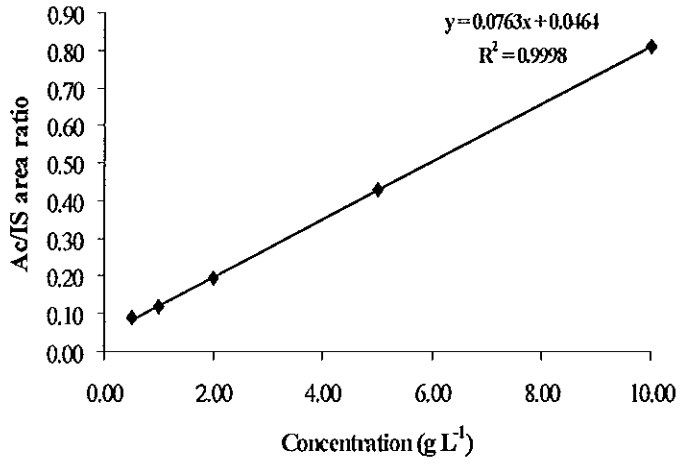


Figure 3A Acetic acid standard curve

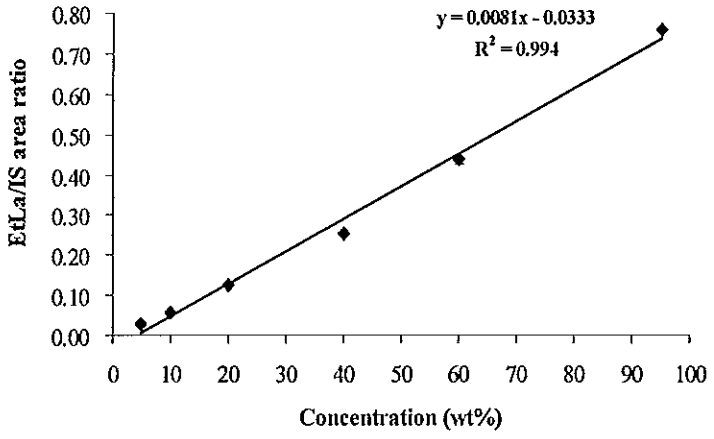


Figure 4A Ethyl lactate standard curve

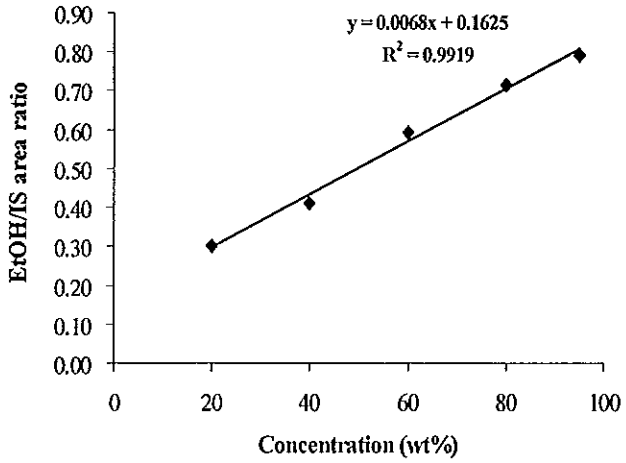


Figure 5A Ethanol standard curve

2. Determine of sucrose, fructose and glucose by HPLC

The supernatant was used to analyze for sucrose, fructose and glucose concentrations. These sugars were measured by high performance liquid chromatography (HPLC, SHIMADZU). The apparatus included a quaternary pump, a manual injector, a compartment, and NH_2 column operating conditions were: sample volume 10 μL; mobile phase a 75:25 mixture of filtrated HPLC-grade acetonitrile and deionized water; flow rate of 1.5 mL min^{-1} and column temperature 40°C. After samples were measured organic acids and ethyl lactate concentration were calculated by comparing to standard curve.

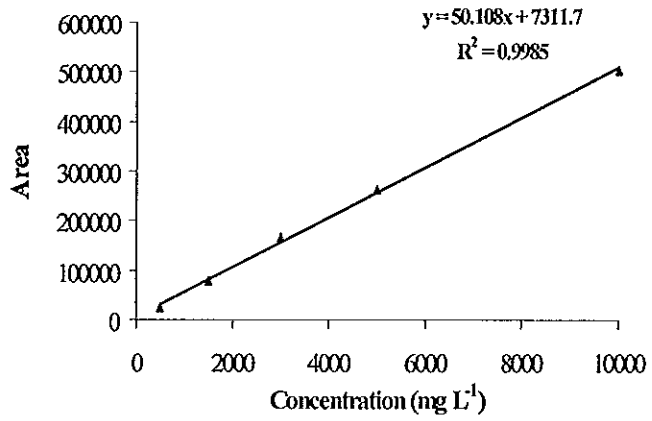


Figure 6A Sucrose standard curve

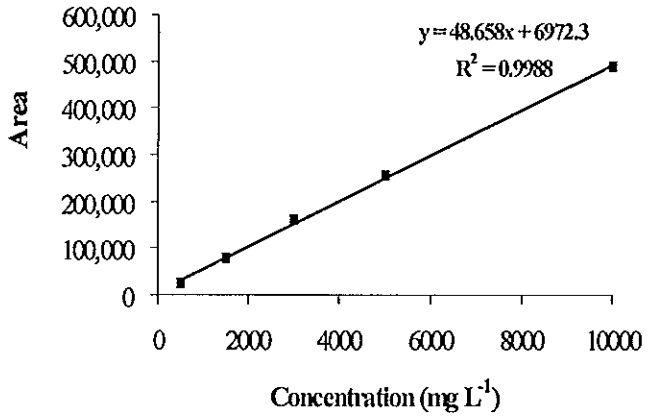


Figure 7A Glucose standard curve

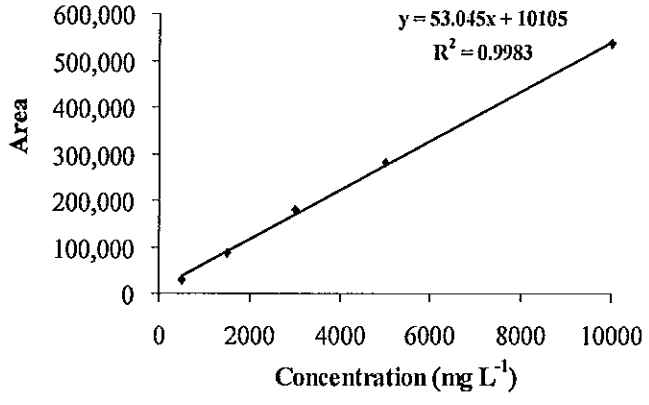


Figure 8A Fructose standard curve

3. Total sugars determination by phenol-sulphuric method

Standard curve of sugar was prepared using the serial concentration of glucose solution (0.2-1.0 mg mL^{-1}) in distilled water. The 500 µL of each concentration was transferred to test tube and added with 500 µL of 5% phenol solution. The mixtures were shaken and followed by the addition of 2.5 mL conc. sulphuric acid. All mixtures were homogenized by vortex and stand for 15 min. The absorbance (550 nm) of the reaction mixture was measured. Finally, the relation between absorbance (550nm) and glucose concentration was plotted.

Determination of total sugar in samples, sugar concentration in sample solution was determined as the method described above. The reaction mixture composed with 500 µL of 5% phenol solution and 2.5 mL conc. sulphuric acid solution.

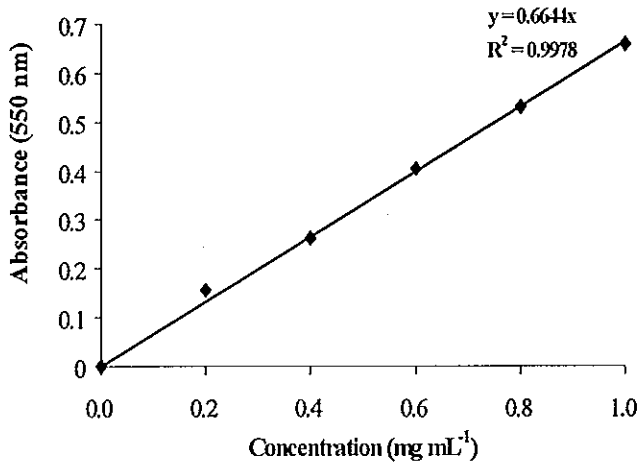


Figure 9A Standard curve of total sugars by phenol-sulphuric method using glucose as standard curve

4. Dry cell weight

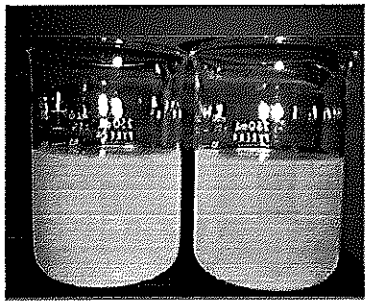
Dry cell weight was determined by centrifugation of culture broth (2 mL) at 8,000 rpm for 15 min. The cell sediments were dried for 24 h at 105°C and then weighted to constant weight after cooling in a desiccator.

APPENDIX B

Raw Materials and Apparatus

1. Raw Materials

(A)



(B)

Figure 1B Raw material of palmyra sap (A) and oil palm sap (B)

2. Apparatus

Figure 2B Gas Chromatography (GC)

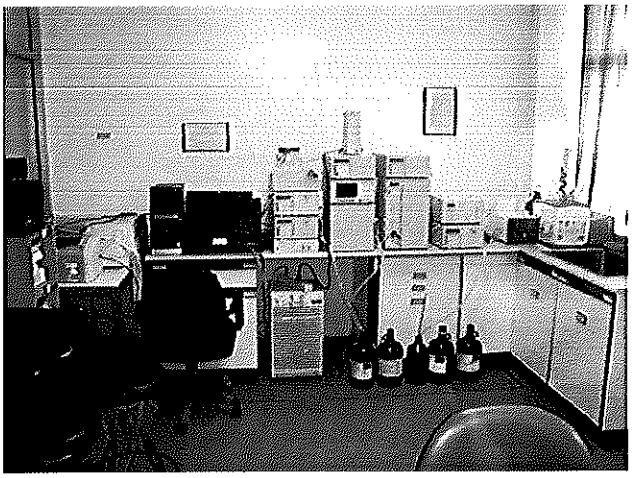


Figure 3B High Performance Liquid Chromatography (HPLC)

Figure 4B Autoclave

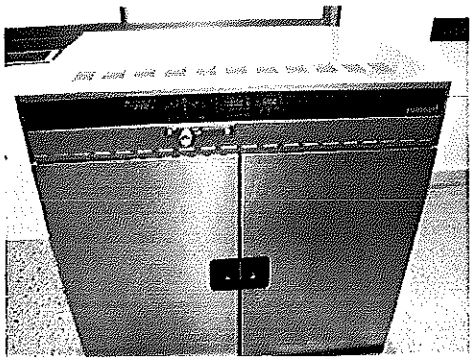


Figure 5B Incubator

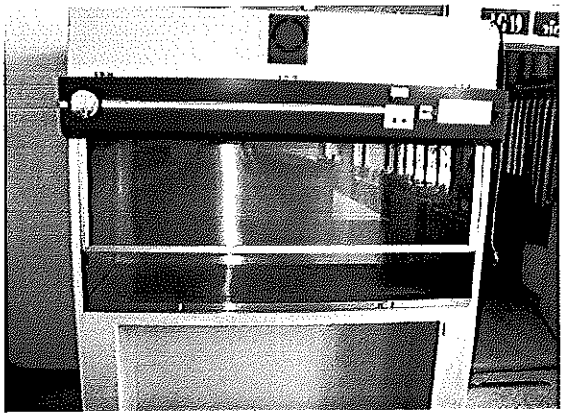


Figure 6B Laminar Flow

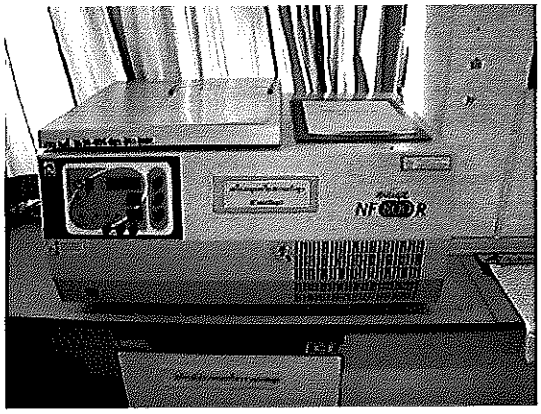


Figure 7B Centrifuge

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List of Publications and Proceedings

Publications

Chooklin, S., Kaewsichan, L. and Kaewsrichan, J. 2011. Potential Use of *Lactobacillus casei* for the Bioconversion from Palmyra Sap to Lactic acid. J. Sustain. Energ. Environ. (Accepted)

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