THESIS

DEVELOPMENT OF TECHNOLOGY FOR LACTIC ACID PRODUCTION FROM CASSAVA STARCH

WALAIPORN TIMBUNTAM

GRADUATE SCHOOL, KASETSART UNIVERSITY
2008

THESIS APPROVAL

GRADUATE SCHOOL, KASETSART UNIVERSITY

Doctor of Philosophy (Biotechnology)
DEGREE

Biotechnology
FIELD

Biotechnology
DEPARTMENT

TITLE: Development of Technology for Lactic Acid Production from Cassava Starch

NAME: Miss Walaiporn Timbuntam

THIS THESIS HAS BEEN ACCEPTED BY

THESIS ADVISOR
(Associate Professor Klanarong Sriroth, Dr.Ing.)

THESIS CO-ADVISOR
(Miss Kuakoon Piyachomkwan, Ph.D.)

DEPARTMENT HEAD
(Associate Professor Sunee Nitisinprasert, Dr.Sci.)

APPROVED BY THE GRADUATE SCHOOL ON _____

DEAN
(Associate Professor Vinai Artkongharn, M.A.)

THESIS

DEVELOPMENT OF TECHNOLOGY FOR LACTIC ACID PRODUCTION FROM CASSAVA STARCH

WALAIPORN TIMBUNTAM

A Thesis Submitted in Partial Fulfillment of
the Requirements for the Degree of
Doctor of Philosophy (Biotechnology)
Graduate School, Kasetsart University
2008

Walaiporn Timbuntam 2008: Development of Technology for Lactic Acid Production from Cassava Starch. Doctor of Philosophy (Biotechnology), Major Field: Biotechnology, Department of Biotechnology. Thesis Advisor: Associate Professor Klanarong Sriroth, Dr.Ing. 178 pages.

Lactic acid is widely used in food, cosmetic, pharmaceutical and chemical industries and has received an increasing attention for used being as feedstock in the production of biodegradable poly lactic acid (PLA). Nowadays, refined sugars and high cost nitrogen sources are used in industrial production with costly and less environmentally friendly recovery and purification process of produced acid. Lactic acid production technologies need to be further advanced and implemented to become technically and economically feasible and environmentally sound. Moreover, the desirable characteristics for industrial lactic acid bacteria (LAB), i.e. the abilities of rapidly and completely converting cheap raw materials into lactic acid with minimal nutritional requirements and providing high yields of preferred stereoisomer are of great importance. In this study, high production yields of lactic acid (> 98%) from inexpensive raw materials including cassava starch, chips and roots, together with high product concentrations (140 – 150 g l^{-1}) and good productivity (4.69 g $l^{-1}h^{-1}$), were feasible by a stable strain (*Lactobacillus rhamnosus* DM3: the highest stereospecificity of L-type 92.8%) isolated from Thai fermented pork, using simultaneous saccharification and fermentation process (SSF) with low-cost fish waste hydrolysates (4% w/v) and yeast extract (0.5% w/v) as a nutrient source. Furthermore, biopolar electrodialysis (BED) was applied to effectively recover and purify lactic acid from the fermentation broth. By two-stage electrodialysis, i.e. monopolar and BED anion process, free lactic acids could be recovered from ammonium lactate salts in fermentation broth of which the final lactic acid concentration increased by 32 %.

_____ _____ ___/___/___

Student's signature Thesis Advisor's signature

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF ABBREVIATIONS

a_w	=	water activity
A	=	ampere
AAP	=	amino acid production
BED	=	bipolar electrodialysis
^{O}C	=	degree Celsius
CE	=	current efficiency
CFU	=	colony forming unit
CS	=	concentrating stream
CSL	=	corn steep liquor
dm^2	=	deci-metre square
DS	=	diluated steam
ED	=	electrodialysis
EPA	=	The Environmental Protection Agency
FAA	=	free factty acid
FDA	=	Food and Drug Administration
FWH	=	fish waste hydrolysates
g	=	gram
GMO	=	Genetically Modified Organism
GRAS	=	generally recognized as safe
h	=	hour
kg	=	kilogram
l	=	liter
L.	=	*Lactobacillus*
Lc.	=	*Lactococcus*
LAB	=	lactic acid bacteria
M	=	Molar
m^2	=	metre square
MED	=	mono-polar electrodialysis
mg	=	miligram

LIST OF ABBREVIATIONS (Continued)

mm Hg	=	millimeter of mercury
MRS	=	De Man Rogosa and Sharpe
MW	=	molecular weight
NFE	=	nitrogen-free extract
OAP	=	organic acid production
OAR	=	organic acid recovery
PLA	=	poly lactic acid
ppm	=	part per million
RHH	=	ram horn hydrolysates
SS	=	simple saccharification
SSF	=	simultaneous saccharification and fermentation
SWH	=	shrimp waste hydrolysates
TN	=	total nitrogen
USA	=	The United State of America
v	=	volume
V	=	Volt
w	=	weight
YE	=	yeast extract

DEVELOPMENT OF TECHNOLOGY FOR LACTIC ACID PRODUCTION FROM CASSAVA STARCH

INTRODUCTION

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.,* 2006a). 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 commercially either by chemical synthesis or microbial fermentation. The chemical synthesis of lactic acid from petroleum-based feedstock has a substantial disadvantage due to a racemic mixture of L- and D-isomers of produced lactic acid which, in some certain applications, cannot be applied economically, unless a costly separation process is employed. In contrast, microbial offers a significant advantage by producing optically pure lactic acid, either L- or D-isomeric type, of which the acid stereospecificity is strain – dependent (Datta *et al*., 1995). Moreover, microbial fermentation is well-recognized as a green technology, indicating the utilization of renewable biomaterials by an environmentally-friendly process, i.e. fermentation of sugar-based feedstock with lactic acid bacteria (LAB). LAB have been identified as the major lactic acid producing strains and most of them are considered as a GRAS (generally regarded as safe) substance (Hofvendahl and Hahn-Hagerdal, 2000). The LAB are facultative anaerobes or microaerophilic ones from the standpoint of oxygen requirement. The LAB are classified as homo- or hetero-fermentative based on product types. Homofermentative strains have produced mainly lactic acid without other metabolic products such as other organic acids, alcohol, aldehyes, ketones and carbon dioxide and are more preferred for industrial uses. The yield of lactic acid from glucose by homofermentative strains generally reaches 90% of the theoretical yield or even greater. Investigation of homofermentative strains with a high production yield is still of interest and the strain selection can be optimized by various concerns principally including the preferred

isomeric form of lactic acid, the tolerance to adverse growth conditions (substrate concentrations, temperature, pH, and acid), yields and productivities (g $l^{-1}h^{-1}$). Indeed, different LAB strains differ in their required growth conditions of which should be optimally undertaken to ensure the most efficiencies of lactic acid production.

In Thailand, there are much diversified sources of lactic acid fermentation being in naturally occurred and commercialized products, which can probably be a source of well-performing LAB such as fermented vegetable, fermented meat products, dairy products and industrial wastes (Christine *et al*., 1999; Noonpakdee *et al*., 2003). In this work, LAB were screened from fermented food products and fermented waste. Based on the yield and stereospecificity, the selected strain was identified and the optimal fermentation condition, (pH, temperature, agitation speed and initial substrate concentration) were investigated. The efficiency, i.e. yield and productivity, on lactic acid production was also reported when cassava starch, the cheapest commercially available feedstock in Thailand was applied.

Moreover, the recovery and purification of produced acids are very important and costly steps because they have significant impacts on quality of lactic acid and its final price. There are a number of processes for lactic acid recovery, such as crystallization, solvent extraction and direct distillation, which have been studied previously (Jong and Hong, 1999; Danner *et al*., 2000; Marinova *et al*., 2004), revealing certain limitations. The crystallization method is associated with a low yield, high costs of used chemicals and unfriendly process to the environment as it consumes lime and sulfuric acid, generating a large quantity of calcium sulfate sludge

as the solid waste. Solvent extraction is restricted by unfavorable distribution coefficients and environmental problems due to uses of various solvents. Direct distillation process is related to a high-energy consumption (Wang *et al*., 2006). Recently, there has been an increasing interest in applying the two-step electrodialysis including mono-polar and bipolar electrodialysis in chemical and agro-industrial process to directly acidify or basify the process streams without the addition of chemicals. Bipolar electrodialysis (BED) is another type of electrodialysis using a biopolar membrane, which consists of an anion and cation selective layer joined together. When an electrical current is applied, water splitting occurs at the junction of the biopolar membrane, thus generating cation and anion species, i.e. proton and hydroxyl ion, respectively. There are, in general, two types of BED systems which are two-compartment configuration, consisting of a biopolar membrane with cationic or anionic membrane, and three-compartment configuration, consisting of an anion, a bipolar and a cation exchange membrane. The advantage of biopolar membrane is to convert aqueous salt solutions into acids and bases, thus the generated base can be recycled for neutralizing the fermentation medium and negligible wastes are produced when the process is properly operated. (Habova *et al.,* 2004). A few studies (Lee *et al.,* 1998; Kim and Moon, 2001; Bailly, 2002; Habova *et al.,* 2004) have reported the promising application of BED in recovering lactic acids from sodium lactate salts using a bipolar-cation type. In this work, lactic acid recovery performance of various bipolar electrodialysis types including BED-anion, BED-cation and BED-anion-cation were evaluated using a model solution of ammonium lactate under various operating conditions (i.e. fixed voltage and fixed current). Furthermore, the effective system

was applied to recover free lactic acid from the fermentation broth obtained by lactic acid fermentation of cassava starch hydrolysates.

In fermentation process, LAB require carbon source, principally simple sugars and nitrogen source, as vital nutrients for their growths. Nutrient-enriched media containing pure sugar and nitrogen sources are not economically preferred. In fact, the raw materials for industrial lactic acid production should have several characteristics such as low cost, rapid fermentation rate, high lactic acid yields, little or no by-product formation and year-round availability (Oh *et al.,* 2005). The media used for lactic acid production are not economically feasible because they contain expensive nutrients, especially sugar and nitrogen sources. An economic analysis of lactic acid production has demonstrated that the nitrogen source, i.e. yeast extract (YE), can significantly contribute to the fermentation cost, which accounting for around 38% of the total production cost (Yoo *et al.,* 1997). Thus, the application of alternative low-priced nitrogen sources is of great concern in order to promote the economic feasibility of lactic acid in industrial uses. There have been a great number of studies that have employed some industrial by-products as a source of growth factors to achieve a partial or total replacement of YE such as ram horn hydrolysate (Kurbanoglu and Kurbanoglu, 2003), soybean hydrolysate (Kwon *et al*., 2000), corn steep liquor (Rivas *et al.,* 2004, Wee *et al.,* 2006b), fish waste and spent cells (Gao *et al*., 2006a, Gao *et al.,* 2006b). However, there are still other nitrogen-rich by-products that can be obtained immensely from large agro-industries such as waste water from monosodium glutamate industry, brewer's yeast autolysate from beverage industry and fish soluble waste from canning industry, such as tuna waste which has been

reported as a nutritional source for *Zymomonas mobilis* in ethanol production (Ruanglek *et al.,* 2006). Timbuntam *et al.* (2006) reported that in lactic acid production from sugar-cane juice, shrimp wastes could also be used as a YE substitute. The application of agro-industrial wastes as the nitrogen source to substitute YE in lactic acid production from cassava materials, i.e. dried chips and fresh roots were studied in this work.

OBJECTIVES

1. To screen effective lactic acid bacteria from Thai agricultural products and wastes and to evaluate their suitability for industrial lactic acid production.

2. To apply selected lactic acid bacteria strain in producing lactic acid from cassava materials.

3. To recover free lactic acid after simultaneous saccharification and fermentation of cassava starch using a two-stage electrodialysis.

4. To apply inexpensive nitrogen-containing agro-industrial wastes in lactic acid production.

LITERATURE REVIEW

Lactic Acid

Lactic acid (2-hydroxy propionic acid), known as milk acid, is a chemical compound that plays a role in several biochemical processes. It was first isolated in 1780 by a Swedish chemist and is a carboxylic acid with a chemical formula of $C_3H_6O_3$. It is a three carbon organic acid: one terminal carbon atom is part of an acid or carboxyl group; the other terminal carbon atom is part of a methyl or hydrocarbon group; and a central carbon atom having an alcohol carbon group. Lactic acid is chiral and has two optical isomers as shown below. One is known as L (+) lactic acid or (S) lactic acid and the other, its mirror image, is D (-) lactic acid or (R) lactic acid. L (+) lactic acid is the biologically important isomer (Figure 1).

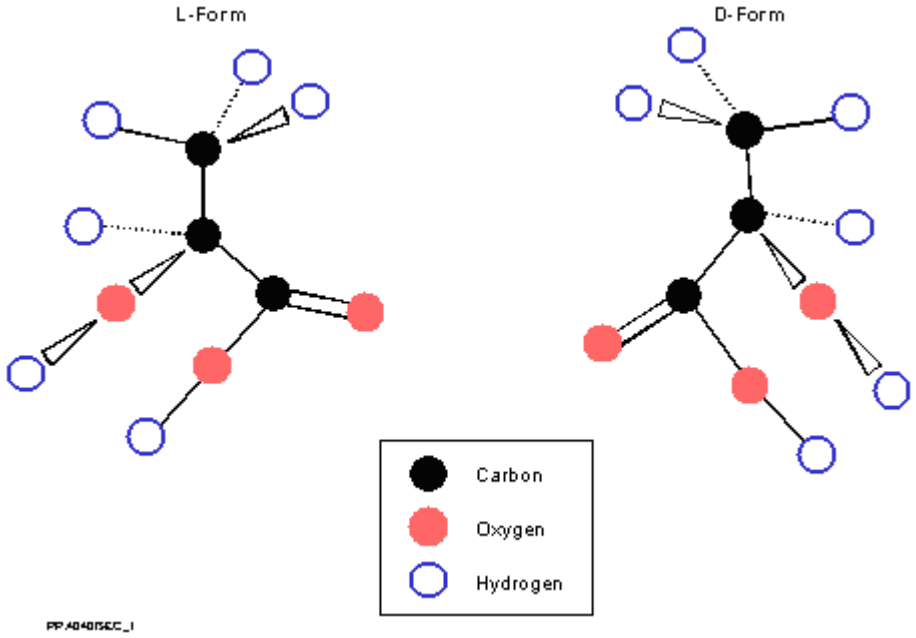


Figure 1 L-lactic acid and D-lactic acid

Source: Nexant (2008)

Lactic acid is soluble in water and water miscible organic solvents but insoluble in other organic solvents. It exhibits low volatility. Other properties of lactic acid are summarized in Table 1.

Table 1 Physical properties of lactic acid.

Parameter	
Chemical formula	$C_3H_6O_3$
Molecular weight	90.08
Acidity (pK_a)	3.85
Melting point	L: 53^OC
	D: 53^OC
	D/L: 16.8^OC
Boiling point	82^OC at 0.5 mm Hg
	122^OC at 14 mm Hg
Dissociation constant, K_a at 25^OC	1.37×10^{-4}
Heat of combustion, H_c	1361 KJ/mole
Specific Heat, C_p at 20^OC	190 J/mole/^OC

Source: Vick Roy (1985)

Lactic acid can be manufactured by (a) Chemical synthesis or (b) Fermentation (Figure 2).

1. Chemical synthesis

The chemical synthesis routes produce only the racemic lactic acid. The commercial process is based on lactonitrile, which used to be a by-product from acrylonitrile synthesis. It involves base catalyzed addition of hydrogen cyanide to acetaldehyde to produce lactonitrile. This is a liquid-phase reaction and occurs at an atmospheric pressure. The crude lactonitrile is then recovered and purified by distillation and is hydrolyzed to lactic acid by using either concentrated hydrochloric

or sulfuric acid, producing the corresponding ammonium salt as a by-product. This crude lactic acid is esterified with methanol, producing methyl lactate, which is recovered and purified by distillation and hydrolyzed by water with acid catalysts to produce lactic acid, which is further concentrated, purified, and shipped under different product classifications, and methanol, which is recycled (Datta *et al.*, 1995).

(a) Addition of Hydrogen Cyanide

$$\underset{\text{acetaldehyde}}{CH_3CHO} + \underset{\text{hydrogen cyanide}}{HCN} \xrightarrow{\text{catalyst}} \underset{\text{lactonitrile}}{CH_3CHOHCN}$$

(b) Hydrolysis by H_2SO_4

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

(c) Esterification

$$\underset{\text{lactic acid}}{CH_3CHOHCOOH} + \underset{\text{methanol}}{CH_3OH} \longrightarrow \underset{\text{methyl lactate}}{CH_3CHOHCOOCH_3} + H_2O$$

(d) Hydrolysis by H_2O

$$\underset{\text{methyl lactate}}{CH_3CHOHCOOCH_3} + H_2O \longrightarrow \underset{\text{lactic acid}}{CH_3CHOHCOOH} + \underset{\text{methanol}}{CH_3OH}$$

Other possible chemical synthesis routes for lactic acid include base catalyzed degradation of sugars; oxidation of propylene glycol; reaction of acetaldehyde, carbon monoxide, and water at elevated temperatures and pressures; hydrolysis of chloropropionic acid (prepared by chlorination of propionic acid), and nitric acid oxidation of propylene. None of these routes have led to technically and economically viable processes.

2. Fermentation

Chemical synthesis may be limited due to the shortage of naturally available raw materials in the future. While chemical synthesis produces a racemic mixture of D, L lactic acid, stereospecific acid can be made by carbohydrate fermentation and is depending on the strain being used. Of the 80,000 tons of lactic acid produced worldwide annually, about 90% are made by LAB fermentation and the rest is produced synthetically by the hydrolysis of lactonitrile. It is also possible to use renewable resources as substrates, such as starch and cellulose in fermentative production. Renewable resources do not give any net contribution of carbon dioxide to the atmosphere, as do the limited oil- and fossil-fuel-based sources. (Hofvendahl and Hahn-Hagerdal, 2000) The process can be described by

(a) Fermentation and neutralization

$$C_6H_{12}O_6 + Ca(OH)_2 \xrightarrow{\text{fermentation}} 2(CH_3CHOHCOO^-)\,Ca^{2+} + 2H_2O$$

carbohydrate — calcium hydroxide — calcium lactate

(b) Hydrolysis by H_2SO_4

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

calcium lactate — sulphuric acid — lactic acid — calcium sulphate

(c) Esterification

$$CH_3CHOHCOOH + CH_3OH \longrightarrow CH_3CHOHCOOCH_3 + H_2O$$

lactic acid — methanol — methyl lactate

(d) Hydrolysis by H_2O

$$CH_3CHOHCOOCH_3 + H_2O \longrightarrow CH_3CHOHCOOH + CH_3OH$$

methyl lactate — lactic acid — methanol

The broth containing calcium lactate is filtered to remove cells, carbon treated, evaporated and acidified with sulphuric acid to get lactic acid and calcium sulphate. The insoluble calcium sulphate is removed by filtration; lactic acid is obtained by hydrolysis, esterification, distillation and hydrolysis.

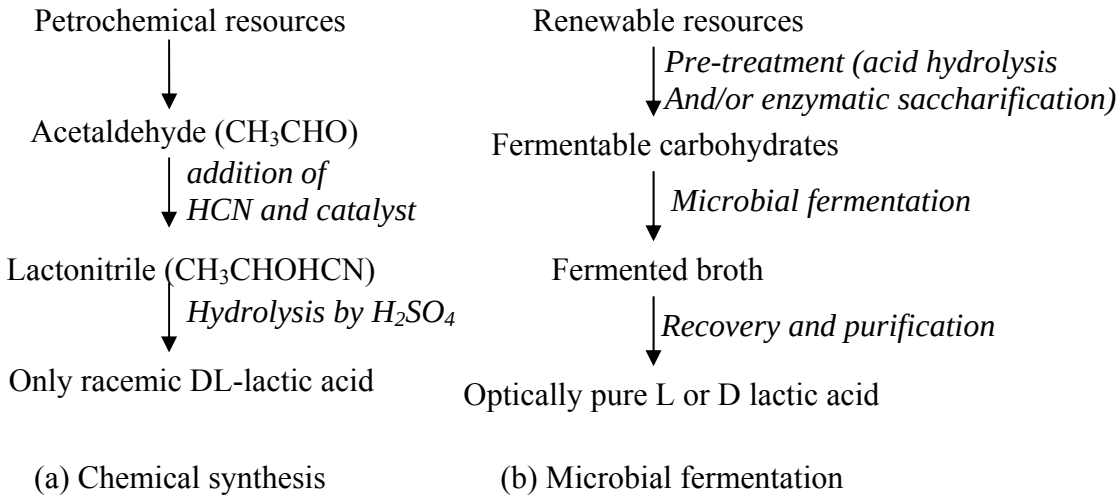


Figure 2 Overview of the two industrially manufacturing process of lactic acid; chemical synthesis (a) and microbial fermentation (b).

Source: Wee *et al.* (2006)

3. Current and novel application of lactic acid

In Figure 3 demonstrate the diagram of commercial uses and applications of lactic acid and its salt. Food and food-related applications account for approximately 85% of the total lactic acid demand, whereas the nonfood industrial applications account for only 15% of the demand. Lactic acid has been used as a preservative and acidulant in food and beverage sector for several decades. The esters of calcium and sodium salts of lactate with longer chain fatty acids have been used as very good dough conditioners and emulsifiers in bakery products. Lactic acid is considered as generally recognized as safe (GRAS) for use as food additive by the regulatory agencies like FDA in USA. It is used as an acidulant/flavorant/pH buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods, such as candy,

breads and bakery products, soft drinks, soups, sherbets, dairy products, beer, jams and jellies, mayonnaise, and processed eggs, often in conjunction with other acidulants. Lactic acid or its salts are now used in the disinfection and packaging of carcasses, particularly those of poultry and fish, where the addition of their aqueous solutions during processing increases shelf life and reduces microbial spoilage by *Clostridium botulinum* (Datta *et al.*, 1995).

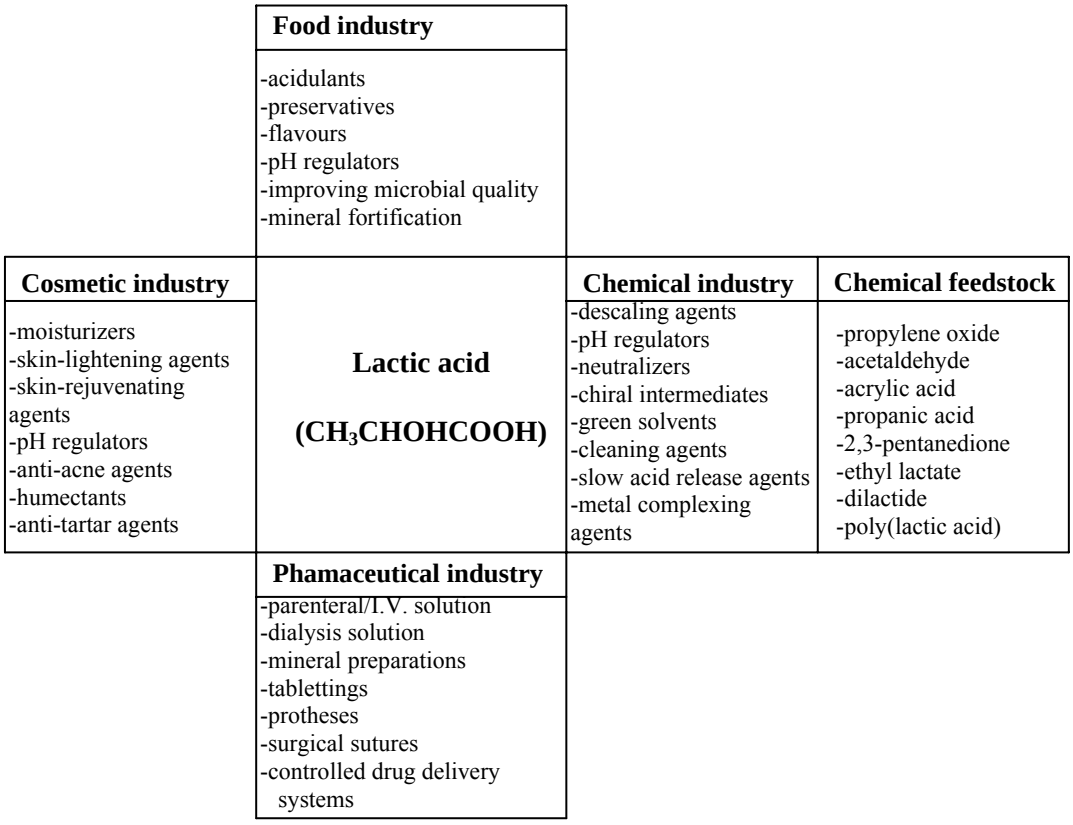


Figure 3 Diagram of the commercial uses and applications of lactic acid and its salt
Source: Wee *et al.* (2006)

The water-retaining capacity of lactic acid makes it suitable for use as moisturizing agents in cosmetic formulations. The ability of lactic acid to suppress the formation of tyrosinase is responsible for its effects like skin lightening and rejuvenation. As humectants, the lactates are often superior to natural products and more effective than polyols. Ethyl lactate is the active ingredient in many anti-acne

preparations. The natural occurrence of lactic acid in human body makes it very useful as an active ingredient in cosmetics (Wee *et al.,* 2006). Lactic acid has long been used in pharmaceutical formulations, mainly in tropical ointments, lotions, and parenteral solutions. It also finds applications in the preparation of biodegradable polymers for medical uses such as surgical sutures, prostheses, and controlled drug delivery systems. The presence of two reactive functional groups makes lactic acid the most potential feedstock monomer for chemical conversions to potentially useful chemicals such as propionic acid, acetic acid, acrylic acid etc (Dimerci *et al.,* 1993).

Technical-grade lactic acid is extensively used in leather tanning industries as an acidulant for deliming hides and in vegetable tanning. Lactic acid is used as descaling agent, solvent, cleaning agent, slow acid-releasing agent, and humectants in a variety of technical processes.

The demand for lactic acid has been increasing considerably, owing to the promising applications of its polymer, the PLA, as an environmentally-friendly alternative to plastics derived from petrochemicals. PLA has received considerable attention as the precursor for the synthesis of biodegradable plastic (Figure 4) (Senthuran *et al.,* 1997). The lactic acid polymers, with tremendous advantages like biodegradability, thermo plasticity, high strength etc., have potentially large markets in the packaging of goods, fabrication of prosthetic devices, and controlled delivery of drugs in humans. The substitution of existing synthetic polymers by biodegradable ones would also significantly alleviate waste disposal problems. As the physical properties of PLA depend on the isomeric composition of lactic acid, the production of optically pure lactic acid is essential for polymerization. L-Polylactide is a semicrystalline polymer exhibiting high tensile strength and low elongation with high modulus suitable for medical products in orthopedic fixation (pins, rods, ligaments etc.), cardiovascular applications (stents, grafts etc.), dental applications, intestinal applications, and sutures. L-Polylactic acid has a melting point of 175–178°C and slow degradation time.

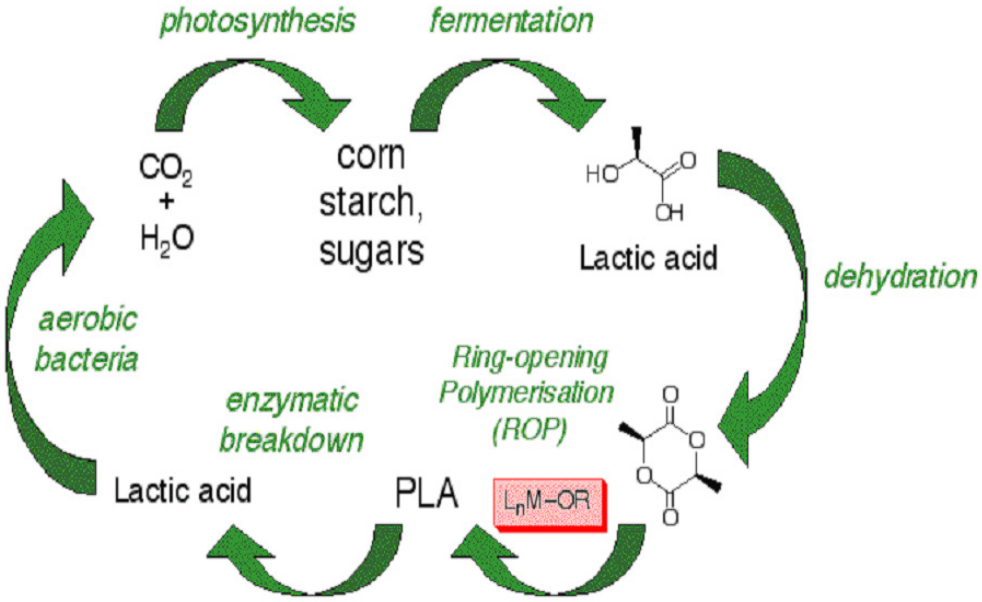


Figure 4 Cycle of PLA in environment

Source: Vernon C.G. (2006)

4. Future perspectives of lactic acid production

Lactic acid can be used for products that potentially have very large-volume uses in industrial applications and consumer products. The primary classes of such products are polymers for plastics and fibers, solvents for formulations and cleaning and oxygenated industrial chemicals. Cargrill Dow LLC, the current leader of lactic acid-based polymers and products, has stated publicly its belief that the PLA market will reach 500,000 (metric) tons per year worldwide by 2010, and the construction of two additional PLA plants are being considered presently (Wee *et al.*, 2006).

Environmentally-friendly, ‘green’ solvents are another potential area for lactic acid derivatives, particularly lactate esters of low-molecular-weight alcohols such as ethyl, propyl, and butyl lactate. Several specialized applications of lactate esters in electronics and precision cleaning were developed and commercialized by Purac. Blends of these esters with other biologically active solvents with a wide range of solvating and cleaning properties have been developed and commercialized very

recently by Vertec Biosolvents. The US Environmental Protection Agency (EPA) has approved the lactate ester solvents as inert ingredients with negligible toxicity and an excellent environmental profile. This has opened up a chance for the development and commercialization of a good range of specialty applications with these non-toxic, environmentally-friendly lactate ester solvents with other biologically derived solvents.

Lactic acid could be potentially used for the manufacturing of large-volume oxygenated chemicals, such as propylene glycol, propylene oxide, acrylic acid, and acrylate esters, and other chemical intermediates such as lactate ester plasticizers. The advances made in hydrogenolysis technology can be further developed and integrated to make propylene glycol from lactic acid in the future (Datta and Henry, 2006).

Previously, in various textile finishing operations and acid dying of wool, technical-grade lactic acid had been used extensively. Cheaper inorganic acids are now more commonly used in these applications. The future availability of lower cost lactic acid and increasing environmental restrictions on waste salt disposal may reopen these markets for lactic acid.

The use of chirality of lactic acid for the synthesis of drugs and agrichemicals is an opportunity for new applications of optically active lactic acid or its esters. Another use as an optically active liquid crystal whereby lactic acid is used as a chiral synthon has been recently described (Datta *et al.,* 1995). These advances can open new small-volume specialty chemical opportunities for optically active lactic acid and its derivatives.

The current worldwide demand of lactic acid is estimated to be 130,000–150,000 (metric) tons per year, and the commercial prices of food-grade lactic acid range between 1.38 US$/kg (for 50% purity) and 1.54 US$/kg (for 88% purity). Technical-grade lactic acid with 88% purity has been priced as much as 1.59 US$/kg. Lactic acid consumption in chemical applications, which include PLA polymer and

new green solvents, such as ethyl lactate, is expected to expand 19% per year (Wee *et al.,* 2006).

On an industrial scale, the manufacturing cost of lactic acid monomer will be targeted to be less than 0.8 US$/kg because the selling price of PLA should decrease roughly by half from its present price of 2.2 US$/kg. According to the cost analysis by Datta *et al.* (1995), the base manufacturing cost of lactic acid was estimated to be 0.55 US$/kg. There are several issues that need to be addressed for the biotechnological production of lactic acid, such as the development of high-performance lactic acid-producing microorganisms and the lowering of the costs of raw materials and fermentation processes. The biotechnological processes for the production of lactic acid from inexpensive raw materials should be improved continually to make them competitive with the chemically derived one.

Factors Affecting in Lactic Acid Production

1. Microorganism

Lactate is a very common end product of bacterial fermentations. Some genera, often referred to as LAB, form large amounts of this product. LAB have received wide interest because of their high growth rate and product yields.

LAB are a group of related bacteria that produce lactic acid as a major metabolic product. LAB have the property of producing lactic acid from carbohydrates through fermentation. LAB have been used to ferment or culture foods for at least 4,000 years. These organisms are heterotrophic and generally have complex nutritional requirements because they lack many biosynthetic capabilities. Most species have multiple requirements for amino acids and vitamins. Because of this, LAB are generally abundant only in communities where these requirements can be provided. LAB are used in the food industry for several reasons. Their growth lowers both the carbohydrate content of the foods that they ferment, and the pH due to lactic acid production. It is this acidification process which is one of the most desirable effects of their growth. The pH may drop to as low as 4.0, that is low enough to inhibit the growth of most other microorganisms including the most commonly found as human pathogens, thus allowing these foods have prolonged shelf life. LAB consist of bacterial genera within the phylum Firmicutes that are comprised of about 20 genera. The genera *Lactococcus*, *Lactobacillus*, *Streptococcus*, *Leuconostoc*, *Pediococcus*, *Aerococcus*, C*arnobacterium*, *Enterococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weisella* are the main members of the LAB (Axelsson, 2004; Davidson *et al.,* 1995; Ercolini *et al.,* 2001; Jay, 2000; Holzapfel *et al.,* 2001; Stiles and Holzapfel, 1997). *Lactobacillus* is largest of these genera, comprising around 80 recognized species. The taxonomy of LAB has been based on the Gram reaction and the production of lactic acid from various fermentable carbohydrates. Lactobacilli vary in morphology from long, slender rods to short coccobacilli, which frequently form chains. Typical LAB are gram-positive, nonsporing, catalase-negative, devoid of cytochromes, anaerobic but aerotolerant

cocci or rods that are acid-tolerant and produce lactic acid as the major end product during sugar fermentation. However, under certain conditions some LAB do not display all these characteristics. Thus, the most profound features of LAB are Gram positiveness and inability to synthesize porphyrin groups. The inability to synthesize porphyrin (e.g., heme) results in the LAB being devoid of catalase and cytochromes (without supplemented heme in the growth media). Therefore, the LAB do not possess an electron transport chain and rely on fermentation to generate energy (Axelsson, 2004). Since they do not use oxygen in their energy production, LAB grow under anaerobic conditions, but they can also grow in oxygen's presence. They are protected from oxygen by-products (e.g. H_2O_2) because they have peroxidases. These organisms are aerotolerant anaerobes. Because of the low energy yields, LAB often grow more slowly than microbes capable of respiration, and produce smaller colonies of 2-3 mm. LAB can grow at temperatures from 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 molecule of glucose metabolized. Lactic acid is the major product of this fermentation (Figure 5).

• Heterofermentative: produce only 50% lactic acid. These 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 metabolized (Figure 6).

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

In Table 2 species of the genera *Lactobacillus, Soprolactobacillus, Streptococcus*, *Lueconostoc, Pediococcus*, and *Bifidobacterium* are listed.

LAB are among the best studied microorganisms. Important new developments have been made in the research of LAB in the areas of multidrug resistance, bacteriocins, quorum sensing, osmoregulation, autolysins and bacteriophages. Progress has also been made in the construction of food grade genetically modified LAB. These have opened new potential applications for these microorganisms in various industries (Konings *et al.,* 2000). The desirable characteristics of industrial microorganisms are their ability to rapidly and completely ferment cheap raw materials, which requiring a minimal amount of nitrogenous substances, providing high yields of preferred stereospecific lactic acid under conditions of low pH and high temperature and producing low amounts of cell mass and negligible amounts of other by-products. The choice of an organism primarily depends on the carbohydrate to be fermented. *Lactobacillus delbreuckii* sp. *delbreuckii* are able to ferment sucrose. *L. delbreuckii* sp. *bulgaricus* is able to use lactose. *L. helveticus* is able to use both lactose and galactose. *L. amylophylus* and *L. amylovirus* is able to ferment starch. *L. lactis* can ferment glucose, sucrose and galactose. *L. pentosus* has been used to ferment sulfite waste liquor (Narayanan *et al.,* 2004).

Recently, strains used in the commercial production of lactic acid have become almost proprietary, and it is believed that most of the LAB used belong to the genus *Lactobacillus* (Datta *et al.,* 1995; Hofvendahl and Hahn-Hagerdal, 2000). Berry *et al.* (1999) attempted to produce lactic acid by batch culture of *L. rhamnosus* in a defined medium. Schepers *et al.* (2002) used *L. helveticus* for the production of lactic acid from lactose and concentrated cheese whey, and Burgos-Rubio *et al*. (2000) investigated the kinetic conversion of different substrates into lactic acid with the use of *L. bulgaricus*. Hujanen and Linko (1996) investigated the effects of culture temperature and nitrogen sources on lactic acid production by *L. casei*, and Roukas and Kotzekidou (1998) also used this strain for lactic acid production from deproteinized whey by mixed cultures of free and coimmobilized cells. Fu and

Mathews (1999) reported the kinetic model of lactic acid production from lactose by batch culture of *L. plantarum*, and Bustos *et al.* (2004) used *L. pentosus* for the production of lactic acid from vine-trimming wastes.

Table 2 List of homo and heterofermentative lactic acid bacteria and configuration of lactic acid produced

Genera and species	homofermentative	heterofermentative	Configuration of lactic acid
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(+)

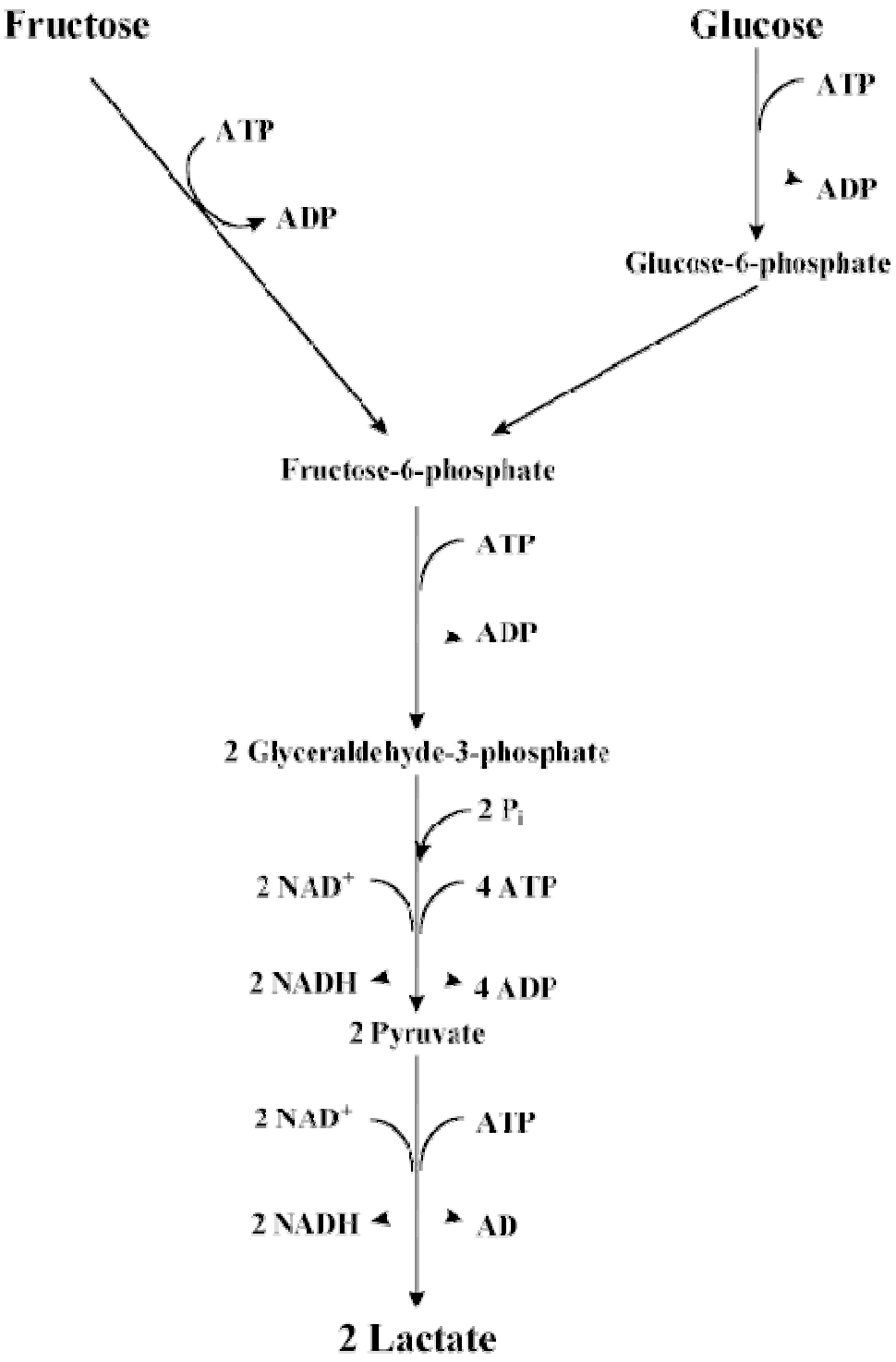


Figure 5 Homofermentative pathway of LAB

Source: Tomas J.R. (2006)

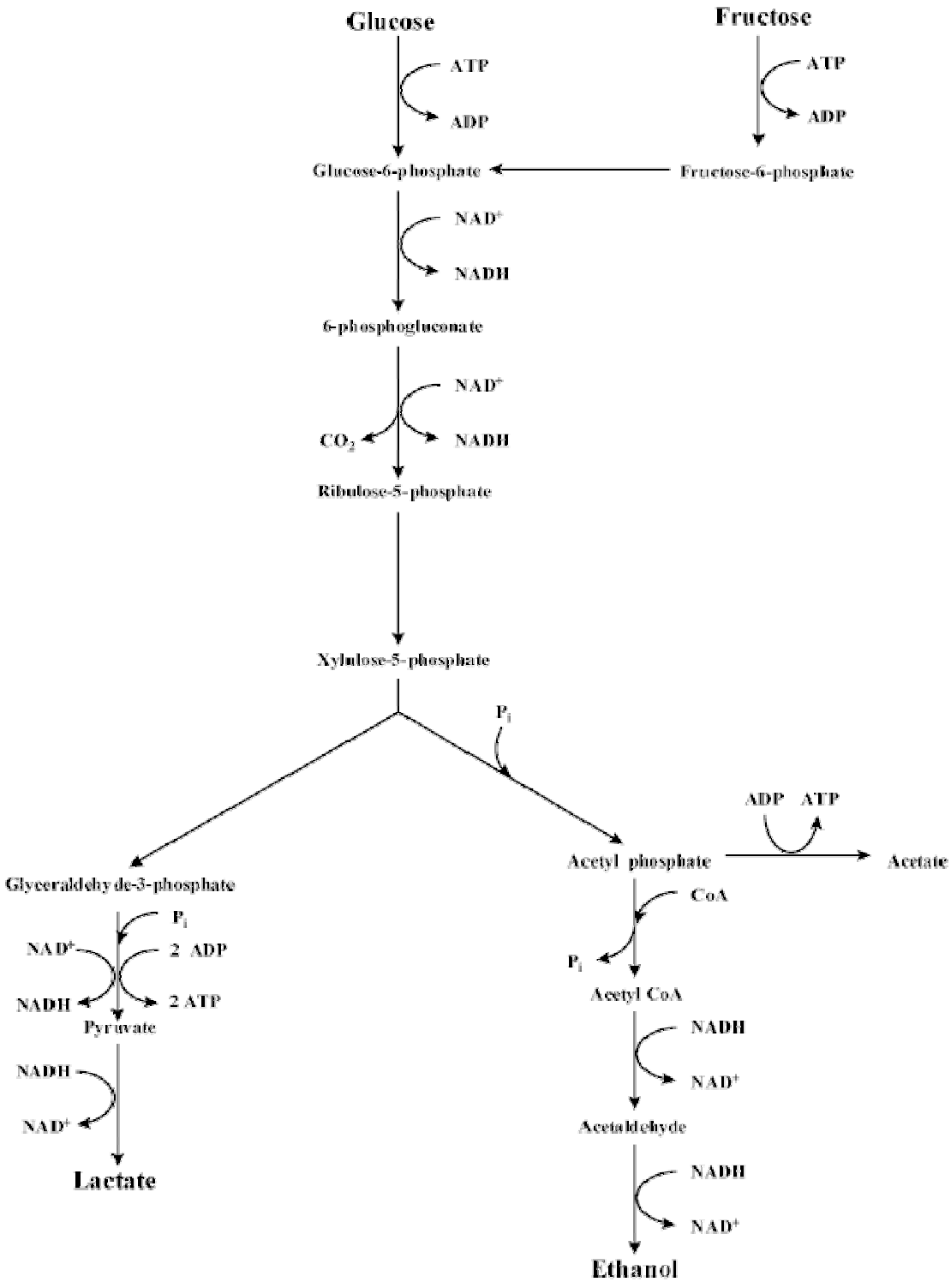


Figure 6 Heterofermentative pathway of LAB

Source: Tomas J.R. (2006)

2. pH

Wasewar *et al.* (2004) discussed that pH has been known to be the most important parameter related to the stability of the enzyme. When glucose is converted into lactic acid, the pH falls. As a result the rate of enzymatic reaction decreases and if the pH drops further, deactivation of enzyme is accelerated. It is essential to control the pH so that the rate of reaction is maintained at its maximum. The enzymatic reaction suffers not only from the substrate inhibition but also the competitive inhibition by lactic acid. Moreover, the pH effects on bacterial cell growth are in part due to the concentrations of dissociated and undissociated forms of lactic acid in the broth. Goncalves *et al.* (1997) have shown that both dissociated and undissociated lactic acid have inhibiting effects with the undissociated organic acids being more inhibitory than the dissociated acids.

Zhang *et al.* (2007) indicated that pH is 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 with no pH control. As confirmed by Hofvendahl and Hahn-Hagerdal (1997), 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 compared with 96 g l^{-1} when pH was held constant at 6.0. It is recognized that a favorable pH range is 5.0–6.0 (Huang *et al.,* 2003). Mulligan *et al*. (1991) reported the influence of pH on lactic acid production by *Streptococcus cremoris*; the result showed that decreasing the pH from 6.5 to 5.8 had little effect on growth but significantly reduced productivity. Akerberg *et al.* (1998) reported the initial glucose concentration did not influence which isomer of lactic acid was produced at pH 6.0. However, at pH 5.0 a lower initial glucose concentration resulted in increasing D-lactic acid formation. Additionally, the by-product formation (acetic acid, ethanol and formic acid) increased with decreasing pH. Tay and Yang (2002) found that production of lactic acid, ethanol and fumaric acid decreased as pH decreased from 6.0 to 4.0. Miura *et al*.'s (2003) results showed that the highest lactic acid yield (93 g l^{-1}) was achieved at

pH 6.0–6.5. Rosenberg and Kristofikova (1995) revealed that the variation in pH had no influence on the production of malic and fumaric acid by-products. A pH below 5.7 was only optimal for *Lactobacillus* strains, which are known to tolerate lower pH than lactococci (Kashket, 1987). Fu and Mathews (1999) reported lactic acid production from lactose with no pH control and indicated that lactose slightly inhibited cell growth in the exponential growth phase but there was no effect in the stationary and death phases.

To control the growth pH during the fermentation, removing lactic acid by electrodialysis and extraction, including aqueous two-phase systems, were successful in some studies (Kaufman *et al.,* 1996), whereas in others, the titration gave the same or better results (Ye *et al.,* 1996). Neutralizing agents such as calcium carbonate, sodium carbonate and sodium hydroxide need to be added into fermentation medium. Among these agents, calcium carbonate has been widely used in the shake flask investigations and bioreactor processes (Zhang *et al.,* 2007). But the regeneration of lactic acid results in the production of large amounts of solid calcium sulfate (Mattey, 1992). Better alternatives are ammonia or calcium hydroxide, leading to production of the fertilizer ammonium sulfate (Datta *et al.,* 1995) or gaseous carbon dioxide, respectively. Martak *et al.* (2003) found that calcium carbonate could be replaced by 25% (w/v) ammonia without a negative effect on lactic acid productivity, and the use of ammonia instead of calcium carbonate also eliminated wrapping of the neutralization agent by the fungal biomass. Therefore, calcium carbonate over-consumption and crystallization of calcium lactate can be avoided. The liquid ammonia (10% w/v) was added to 3, 100 and 5000 L air-lift reactors to control pH at 6.0, which led to a higher lactic acid yield (about 80%) than the system with addition of calcium carbonate by a ammonia-tolerant mutant strain-*Rhizopus* sp. MK-96-1196 (Liu *et al.,* 2006, Miura *et al.,* 2003).

3. Temperature

Temperature is also an important process operation factor that affects lactic acid production. During the production of ammonium lactate by *S. cremoris*, Mulligan

et al. (1991) found that increasing the temperature to 35^{O}C significantly increased the microbial growth by 34% and the acid productivity by almost three times. Temperatures below 30^{O}C and above 40^{O}C were very inhibitory, decreasing productivities by greater than 50%. Akerberg *et al.* (1998) had investigated the influence of temperature by *Lc. Lactis* and found that the maximal volumetric lactic acid productivity was highest at 33.5^{O}C: 2.8 g $l^{-1}h^{-1}$. At 33.5^{O}C and above, lactic acid formation decreased and the formation of equimolar amounts of acetic acid and ethanol increased. In addition, a number of unidentified compounds were detected in the chromatograms. Formic acid production, on the other hand, decreased with increasing temperature, and diminished as the fermentation continued. At higher temperatures, more of the lactic acid produced was in the D form. Payot *et al.* (1999) reported the thermopile character of *Bacillus coagulans* (growed at 52^{O}C) and proved that it was particularly adapted for industrial production of lactate without sterile conditions. However, the final concentration of lactic acid was low when compared with *L. rhamnosus* cultures because the high initial concentration of sugar could not be used with this strain. Hujanen *et al.* (2001) reported that the maximum lactic acid concentration of *L. casei* was reached at a temperature of 35^{O}C which was as Oh *et al.* (1995). In contrast, the results differed slightly from those by Rincon *et al*. (1993) who repeated the maximum lactic acid production from whey was at a temperature of 38^{O}C. They discussed that it was probably due to differences in the media and carbon sources used.

4. Carbon source

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, 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 they 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. The inexpensive carbon source for lactic acid fermentation in Thailand can be cassava. Cassava is a starch-accumulating crop. The annual root productivity in Thailand is about 18 to 20 million tons fresh root. The starch in root is initially converted to fermentable sugars namely glucose by the enzyme. The sugar is then fermented to lactic acid. Ghofar *et al.* (2005) investigated lactic acid production from fresh cassava root slurried with tofu liquid waste by *Streptococcus bovis,* the lactic acid concentration, productivity and yield were 6.7 % (w/w), 0.77 g $l^{-1}h^{-1}$ and 58.6 %, respectively.

4.1 Background of Thai Cassava

Cassava (*Manihot esculenta* Crantz) is the third most important crop in Thailand. *Manihot esculenta* is know by many names in Thailand, but “cassava” and “tapioca” are the most widely used terms for this root crop. Cassava was introduced into the southern part of Thailand from Malaysia during the period 1786-1840 and was gradually distributed throughout the entire country within a few years, the main concentration of this crop is the northeast of Thailand. The country has demonstrated the importance of cassava as more than a subsistence crop, and has developed a large and complex industrial system for processing and marketing of this crop. As a result of continuous growth of cassava industry, the expansion of cassava cultivation is highly expected. By 2008, with the national strategic planning of Ministry of Agriculture and Cooperatives, a total root production of 28.2 million tons is estimated.

4.1.1 Cassava Roots

Cassava roots have an average composition of 60 to 65% moisture, 30 to 35% carbohydrate, 0.2 to 0.6% ether extractives, 1 to 2% crude protein, and a

comparatively low content of vitamins and minerals. However, the roots are rich in calcium and vitamin C and contain a nutritionally significant quantity of thiamine, riboflavin, and nicotinic acid. The carbohydrate fraction contains 3.2 to 4.5% crude fiber and 95 to 97% nitrogen-free extract (NFE). The tuber NFE contains 80% starch and 20% sugars and amides. The starch content increases with the growth of the tubers and reaches a maximum between the 8^{th} and 12^{th} month after planting. Thereafter the starch decreases and the fiber content increases.

4.1.2 Cassava Chips

Cassava chip factories are small-scale enterprises and most have no official company registration. The manufacturing of cassava chips is recognized as an agricultural practice with many of the factories belonging to farmers or located in close proximity to the growing area. The chipping factories are installed with simple equipment, consisting mainly of a chopper. Roots are transferred to the chopper by tractor, after chopping into small pieces, the chips are sun-dried on a cement floor. The chips are spread to a specific thickness ensuring consisting in final moisture content. During drying, which is typically 2 to 3 days, a vehicle with a special tool for turning the chips is required to ensure uniform drying. Economic loss occurs as a result of weight loss of the chips, caused by dust that is formed from dry particulate matter, this is also a major problem leading to air pollution.

4.1.3 Cassava Starch

Starch is one of the most abundant substances in nature which is renewable and almost unlimited resource. It is formed by photosynthesis, i.e. the process of capturing and converting light into chemical energy, which is stored in the form of starch. Starch is mainly used as food, but is also readily converted chemically, physically and biologically into many other useful products. The development of both the food and non-food uses of starch has made much progress and continue to have a bright future. Important new products such as modified starches, starch sugars, starch-based plastics and fuel alcohol are now reviewed briefly.

Thailand is the world's largest exporter of cassava starch and starch derivatives, with annual production of over 2 million tons of starch. The attributes of Thai cassava starch include:

- *Year round supply of raw material*

Two crops of cassava can be grown each year, with almost complete flexibility of both planting and harvest schedule. Roots are harvested continuously throughout the year. Approximately one million hectares (2.5 million acres) of land are devoted to cassava, producing approximately 20 million tons of roots per annum. Cassava is one of the most important cash crops of Thailand.

- *New improved cassava varieties are GMO free*

Increasing market demand for cassava based products has spurred the development of improved varieties with the main purpose of improving economic return. New varieties are selected primarily for improved starch yield. Only the conventional breeding technique of hybridization is used to achieve this goal. No genetic modification techniques are used and thus Thai cassava starch and products are completely GMO free.

- *Best raw material for starch production*

With improved varieties and agricultural practice, up to 20 tons of high-quality roots can be produced per hectare. Unlike other starch sources, such as corn, rice and wheat, cassava roots contain high starch content and a very low quantity of impurities. Cassava is, therefore, an excellent material for starch production with respect to its availability, raw material cost, and ease of starch extraction.

- *Well-developed starch production technology*

The Thai cassava starch industry has over fifty years experience resulting in highly developed processing technology being used by most Thai manufacturers. The associated development of management systems assures a high production capacity of a high quality product.

Table 3 Composition of cassava materials.

	Roots		Chips	Starch
	Rodjanaridpiched (1989)	Sriroth *et al*., 1998		
Moisture content (%)	63.28	53.02	10.0	13.0
Carbohydrate (%)	29.73	25.00	76.6	85.0
Protein (%)	1.18	2.18	3.2	0.1
Lipid (%)	0.08	0.21	1.8	0.1
Ash (%)	0.85	-	2.0	0.2
Fiber (%)	0.99	1.71	2.1	-
Potassium (mg kg^{-1})	0.26	-	-	-
Phosphorus (mg kg^{-1})	0.04	-	0.07	0.01
Hydrocyanic acid (ppm)	173	110.40	-	-
Calcium	-	-	0.3	-

Source: Sriroth *et al.* (1998)

4.2 Fermentation process of starch

Starch has to be first hydrolyzed to glucose before it can be fermented. Conventionally, no process steps are integrated and the two hydrolysis steps, the liquefaction and the saccharification, as well as the fermentation step are performed as sequentially as batch processes. During the first hydrolysis step, starch is enzymatically degraded to oligosaccharides at a high temperature ($90^{O}C$) using the

commercial thermostable α-amylase. When this step is performed as batch type, a residence tank is required. The recommended reaction time for liquefaction is 60-120 min. In the saccharification step, the oligosaccharides produced in the liquefaction step are further hydrolysed to produce glucose using a commercial enzyme mixture consisting of an α-amylase and amyloglucosidase. In the fermentation step, the glucose produced in the saccharification step is converted to lactic acid (Akerberg and Zacchi, 2000). This two step process involving consecutive enzymic hydrolysis and microbial fermentation makes utilization of starch as a fermentation substrate economically unattractive.

Microbial conversion of starch to lactic acid can be made much more economical by coupling the enzymic hydrolysis of starchy substrates and microbial fermentation of the derived glucose, into a single step. A direct benefit of such a simultaneous saccharification and fermentation (SSF) process is a decrease in the inhibition caused by glucose accumulation, leading to an increase in saccharification rates and a consequent reduction in reactor volume and capital costs. Many factors, such as pH, temperature, substrates and product concentration of glucose and lactic acid can affect the efficiency of the SSF (Akerberg *et al.,* 1998; Jin *et al.,* 2003; Anuradha *et al.,* 1999; Linko and Javanainen, 1996; Moldes *et al.,* 2001). A disadvantage associated with SSF is the difference in cultivation conditions such as temperature and pH required for saccharification versus fermentation (Stenberg *et al.,* 2000). Therefore, characterization of the microbial and biochemical kinetics and determination of the optimal process conditions which enhances SSF is of importance for optimal lactic acid production.

SSF has been utilized for the conversion of cellulose to ethanol by yeast (Gauss *et al.,* 1976). Extensive studies on SSF have since been conducted focusing on the production of ethanol and single cell protein from cellulosic substrates. Phillipidis *et al.* (1993) have studied the enzymic hydrolysis of cellulose in an attempt to optimize SSF performance. Ghose *et al.* (1984) have increased ethanol productivity by employing a vacuum cycling in an SSF process using lignocellulosic substances. In addition to producing power fuels like ethanol from cellulobiomass, the

bioconversion of agricultural byproducts to single cell protein has attracted worldwide attention. Only a few studies have explored the possibility of producing lactic acid from agricultural biomass. Abe and Takagi (1991) conducted the SSF of cellulose powder to lactic acid in a media containing cellulases and *L. delbrueckii* cells. Venkatesh (1997) developed a kinetic model for SSF of cellulose to lactic at varying pH using *Trichoderma reesei* cellulases and *L. bulgaricus*. Luo *et al.* (1997) have investigated kinetics of SSF when the lignocellulosic residue was used as raw materials, the final concentration of lactic acid reached 33.97 g l^{-1} with a conversion ratio of 79% based on the consumed cellulose and a mathematic model to simulate SSF process with good agreement. Hassan *et al.* (2001) reported that the lactic acid production by SSF using various agriculture feedstock residues without any additional nutrients, was higher in alfalfa fiber and soya fiber compared to corncob and wheat straw. Yu and Hang (1989) have reported that *R. oryzae* is capable of SSF process with corn to L-lactic acid. Jin *et al.* (2003) investigated L (+) lactic acid production in SSF process using starch waste effluents by *R. arrhizus.* Lactic acid at 19.5 – 44.3 g l^{-1} with a yield of 0.85 – 0.96 g g^{-1} was produced in 40 h using 20-60 g starch l^{-1}. *L. amylovorus* and *L. casei* have been used in mixed culture with barley starch as substrate with no additional nutrients. Linko and Javanainen (1996) have reported the production of lactic acid from barley starch by simultaneous liquefaction and saccharification using α-amylase and glucoamylase enzymes and fermentation employing with *L. casei*. A lactic acid concentration as high as 162 g l^{-1} was obtained and yields were 90–98%.

Anuradha *et al.* (1999) investigated to establish optimum operating conditions for the SSF of starch to lactic acid using *L. delbrueckii*. Saccharification kinetics were determined through experiments on starch hydrolysis in which the effects of temperature, pH and different fermentation products as inhibitors were studied. The kinetic model was used to predict the performance SSF accurately. The saccharification rate was always higher in SSF than in simple saccharification (SS) at all substrate concentrations. Lactate productivity was 1.21 g l^{-1} h for SSF conducted under optimum conditions with 250 g potato starch l^{-1}, which was higher than that of lactic acid productivity by fermentation after saccharification. Potato tuber and pearl

tapioca are good raw materials for the production of lactic acid using SSF with yields up to 70%. Roy *et al.* (2001) studied optimal control strategies for SSF in fed-batch mode. The optimal control of starch additions in the fed-batch process gave improved performance of the SSF process with an increase of 20% in lactate productivity.

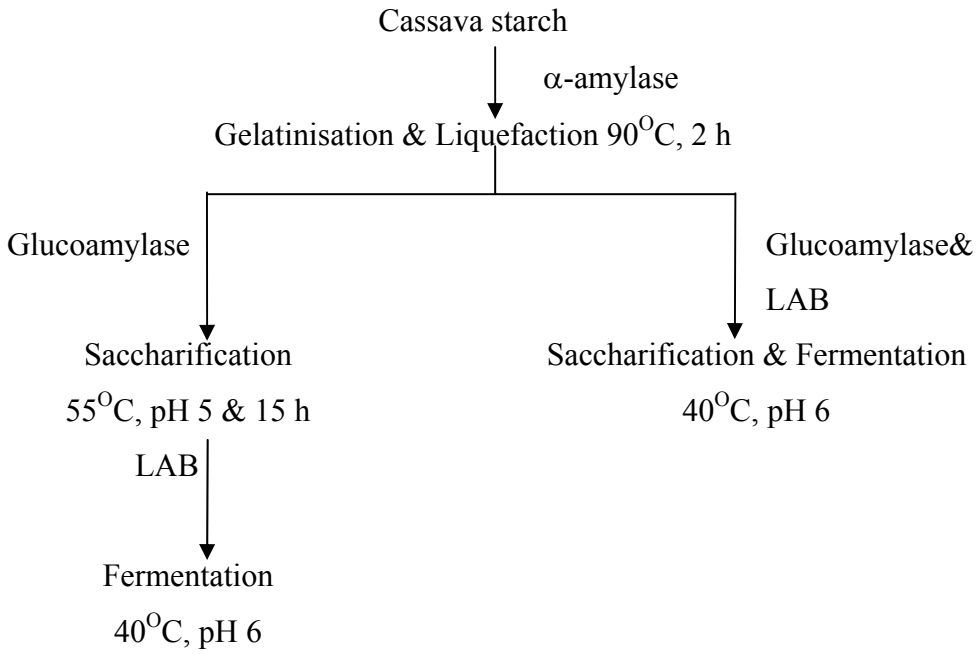


Figure 7 Typical process of using cassava materials as feedstock in LAB fermentativon

5. Nitrogen sources

LAB typically have complex nutritional requirements, due to their limited ability to synthesize their own growth factors such as B vitamins and amino acids. They require some elements for growth, such as carbon and nitrogen sources, in the form of carbohydrates, amino acids, vitamins, and minerals (Axelsson, 1993, Niel and Hahn-Hagerdal, 1999, Amrane, 2000). There are several growth-stimulation factors that have a considerable effect on the production rate of lactic acid. The mixture of amino acids, peptides, and amino acid amides usually stimulates the growth of LAB, and the resulting growth rates are much higher than those obtained with free amino acids (Niel and Hahn-Hagerdal, 1999). Fatty acids also influence LAB growth, and

phosphates are the most important salt in lactic acid fermentation. Ammonium ions cannot serve as the sole nitrogen source, but they seem to have some influence on the metabolism of certain amino acids.

As the LAB require high level of nutrient supplementation like amino acids, vitamins etc., yeast extract (YE) is supplemented as the best nutrient source (Stanier *et al*., 1986; Naveena, 2004; Rojan *et al*., 2005; John *et al*., 2006a). However, for the production of lactic acid as a source for commodity chemicals, YE is not cost effective. In the economic analyses for lactic acid production, the material cost of YE is estimated to contribute over 30% to the total production cost due to its high price ($3.3 – 3.85/ kg), which implies an obvious need for a cheaper alternative. To replace YE, some researchers have investigated on new nutrient sources. The addition of malt-combing nuts, waste from the industry of barley malting, were employed to reduce the high cost of such supplements (Pauli and Fitzpatrick, 2002). When the elemental nitrogen ratio of $(NH_4)_2SO_4$ to YE was 3:1, the substrate use and efficiency of lactic acid production were same as in whey supplemented with 20 g YE l^{-1} (Arasaratnam *et al.,* 1996). Whey protein hydrolyzate was supplemented for lactic acid production from whey (Fitzpatrick and Keeffe, 2001). According to Goksungur and Guvenc (1999), malt sprout was the second best nitrogen source. Soybean meal and cottonseed were used as the inexpensive nitrogen source (Sethi and Maini, 1999; Zhou *et al.,* 1995). Kwon *et al.,* (2000) reported 15 g YE l^{-1} could be successfully replaced with 19.3 g soybean hydrolyzate l^{-1} supplemented with vitamins, resulting in a production of 125 g lactic acid l^{-1} from 150 g glucose l^{-1}. Addition of mustard powder in pickle brine increases the rate of acid production (Sethi and Maini, 1999). Those nutrients could substitute partially for YE. However, large amount of their supplementation contributed to an increase in the concentration of impurities, corresponding to the increase in separation cost and the decrease in lactic acid recovery. Wheat bran hydrolyzate or wheat bran extract was also used as the nitrogen source (Krishnan *et al.,* 1998; Kotzamanidis *et al*., 2002). Nutrient supplementation raises the cost of production, as unutilized nutrients will raise the purification cost. Yun *et al.* (2004) used the amylase and amyloglucosidase treated rice bran and wheat bran for the DL lactic acid production by *Lactobacillu*s sp. RKY2, as they contained

several nutritional factors besides carbohydrate. The amount of lactic acid in fermentation broth reached 129 g l^{-1} by supplementation of rice bran with whole rice flour. Rivas *et al.* (2004) have reported the lactic acid production from corn cobs by SSF in media containing spent yeast cells and corn steep liquor, cellulolytic enzymes and *L. rhamnosus* CECT-288 cells. Nancib *et al.* (2005) tried the different nitrogen sources, both organic and inorganic nutrients, for the lactic acid using date juice. Their study revealed that YE gave the highest yield, but it could be replaced with ammonium sulfate when supplemented with vitamins. Hofvendahl *et al.* (1999) studied the lactic acid production with wheat starch. The addition of protease in the medium enhanced the lactic acid production; the productivity increased up to 1.5 g $l^{-1}h^{-1}$ from 0.23 g $l^{-1}h^{-1}$. When the protease, along with wheat starch, was supplemented with peptide, vitamins, and amino acids, the yield raised to 2.2, 2.4, and 2.8 g $l^{-1}h^{-1}$, respectively. Oh *et al.* (2005) reported, when the medium contained 200 g l^{-1} of whole wheat flour hydrolysate, 15 g l^{-1} of corn steep liquor, and 1.5 g l^{-1} of YE, 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. They showed an improvement of up to 106 % of lactic acid productivity and 138 % of maximal dry cell weight in comparison of the fermentation from whole wheat flour hydrolysate only. D-lactic acid production by *L. coryniformis* was studied, the models predicted a maximum lactic acid concentration (58.9 g l^{-1}) at 96 h using 5 g corn steep liquor l^{-1}, 3.6 g YE l^{-1} and 10 g peptone l^{-1} (Bustos *et al.,* 2004). Lee (2005) found 3.1 g YE l^{-1} and 5% corn steep liquor could be an effective substitute for 15 g l^{-1} yeast extract in 10% glucose medium. In pilot-scale, Wee *et al*. (2006) successfully conducted the production of lactic acid *via* the batch culturing of *Lactobacillus* sp. RKY2 using corn steep liquor as a nitrogen source. The fermentation parameters were not substantially altered by the scaling-up of the laboratory-scale fermentation to 30 and 300-litre scale fermentations. Interesting to note that John *et al*. (2006b) reported that by using protease-treated wheat bran, around 10-fold decrease in supplementation of the costly medium component, like YE, was achieved together with a considerable increase in the lactic acid production level. Maximum lactate yield after various process optimizations was 123 g l^{-1} with a productivity of 2.3 g $l^{-1}h^{-1}$ corresponding to a conversion of 0.95 g lactic acid per gram starch after 54 h at 37°C. Kurbanoglu and Kurbanoglu (2003) have investigated the

use of ram horn hydrolyzate (RHH) as a substrate for lactic acid production using *L. casei*. A concentration of 6% RHH was found to be optimal. The content of lactic acid in the culture broth containing 6% RHH (44 g l^{-1}) for 26 h was 22% higher than that of the control culture broth (36 g l^{-1}). Timbuntam *et al.* (2006) tried various nitrogen sources like silkworm larvae, yeast autolysate, dry yeast, and shrimp waste as a replacement of YE in cane juice medium (Table 4). At the same concentration of nitrogen sources (1% w/v), the addition of silkworm larvae, yeast autolysate, and shrimp waste all led to increases in lactic acid production more than that attained with YE. However the colony forming unit and cell dry weight were highest with YE. Gao *et al.* (2006a, 2006b and 2007) have investigated on acid-hydrolysis of spent cells and fish wastes for lactic acid fermentation. Only spent cells hydrolysate (SCH) did not show significant effect on lactic acid production. As the increase of fish wastes hydrolysate (FWH) amount added, fermentation efficiency increased with a result of increase of impurities in fermentation broth. When 1.7% FWH and SCH were used, the fermentation had a similar performance to that using 1.5% YE in lactic acid production and the impurities in fermentation broth were reduced to appropriate values.

Table 4 Nutrient supplements used in the lactic acid production

Organism	Carbon source	Nitrogen source	Yield (g g^{-1}) or g l^{-1}/ Productivity (g $l^{-1}h^{-1}$)
L. delbrueckii subsp. *bulgaricus*	WFH	-	0.11 g g^{-1}
		YE	0.18 g g^{-1}
L. delbrueckii subsp. *delbrueckii*	WFH	-	0.82 g g^{-1}
		YE	0.91 g g^{-1}
L. delbrueckii subsp. *lactis*	Potato waste hydrolysates	-	1.0 g g^{-1}
		CSL	0.78 g g^{-1}
L. paracasei	Sweet sorghum	-	0.79 g g^{-1}
		YE+peptone	0.91 g g^{-1}
L. delbrueckii NRRL B-445	Molasses	-	0.81 g g^{-1}
		YE+peptone	0.70 g g^{-1}
L. salivarious NRRL B-1950	Soy molasses	-	0.76 g g^{-1}
		YE	0.85 g g^{-1}
L. lactis subsp. *lactis* AS211	WFH	-	0.77 g g^{-1}
		YE	0.91 g g^{-1}
L. lactis subsp. *lactis* ATCC 19435	WFH	-	0.76 g g^{-1}
		YE	0.88 g g^{-1}
L. lactis subsp. *Lactis* ATCC 19435	WFH+protease	-	1.5 g $l^{-1}h^{-1}$
		Vitamins	2.4 g $l^{-1}h^{-1}$
		Amino acids	2.8 g $l^{-1}h^{-1}$
		Peptides	2.2 g $l^{-1}h^{-1}$
	WFH	-	0.23 g $l^{-1}h^{-1}$
L. casei ATCC 10863	Glucose	RHH (1%)	0.08 g g^{-1}
		RHH (6%)	0.44 g g^{-1}
		RHH (7%)	0.28 g g^{-1}
Lactobacillus sp.	Cane juice	-	8.1 g l^{-1}
		1% YE	10.8 g l^{-1}
		1% SWL	13.5 g l^{-1}
		1% YA	15.3 g l^{-1}
		1% SW	12.6 g l^{-1}
L. delbrueckii	Alfalfa extract	-	0.55 g g^{-1}
		YE +PP	0.58 g g^{-1}
L. plantarum	Alfalfa extract	-	0.58 g g^{-1}
		YE +PP	0.62 g g^{-1}
L. amylovorus	Cassava strach	-	4.8 g l^{-1}
		Peptone	7.7 g l^{-1}

WFH: wheat flour hydrolysates, MS: malt sprout, YE: yeast extract, CSL: corn steep liquor, RHH: ram horn hydrolysates, SWL: silk worm larvae, YA: yeast autolysate, SW: shrimp waste, PP: polypeptone

Source: John *et al.* (2007)

Recovery and Purification

The recovery and purification are very important steps because they have significant influence on quality of lactic acid and its final price. Today, the conventional fermentation process produces calcium lactate precipitate, which must be concentrated by evaporation and reacidified by a strong acid. The disadvantages of the conventional fermentation process are a low reaction rate, an elaborate product recovery, a large amount of by-products (0.36×10^6 tons/acre crude calcium sulphate) and thereby negative impact on the environment. Recently, electrodialysis (ED) is considered as a rational technique to replace conventional method. ED is a process in which charged species are separated from an aqueous solution and other uncharged components by an applied electrical current. This is achieved inside a module equipped with ion-selective membrane, placed between two electrodes. ED is applied to remove salts from solutions or to concentrate ionic substances. (Li *et al.,* 2004, Gao *et al.,* 2004, Mauro and Sappino, 2000)

1. History of electrodialysis with bipolar membranes

Over the past 15 years, not more than 12 commercial plants, with totaling about 2,400 m^2 of Aqualytics and Tokuyama's bipolar membranes have been installed throughout the world (Bailly, 2002).

.

Table 5 reviews the different plants installed worldwide and the corresponding estimated membrane area.

The first bipolar system was delivered in 1986 to Washington Steel (USA) for the recovery of hydrofluorhydric and nitric acids from stainless steel pickling liquor, and the last one has been commissioned in Japan in 1999 for the production of amino acids.

In the last few years, some of the plants operating in the USA have been closed. Therefore, about 1,300 m^2 of bipolar membrane are currently in operation roughly equally shared between USA, Europe and Japan.

Table 5 Overview of bipolar ED plants installed worldwide.

Year	USA	Japan	Europe
1986	Pickling liquors recovery (stainless steel)		
1994	HF recovery (Chemical)		
1995	OAP (Agro industry) OAR (Inorganic acid production)		
1996		OAP (Specialty chemical)	Methane sulfonic acid (Italy)
1997	OAP (Specialty chemical)	OAP (Specialty chemical)	OAR (France) (Agroindustry)
1998			AAP (France) OAP(Czech Rep) (Agroindustry)
1999		AAP (Pharmaceutical)	
Total installed bipolar membrane area (estimated)	1500 m^2	400 m^2	500 m^2

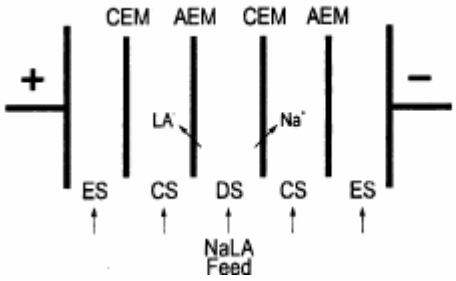
OAP: Organic acid production; OAR: Organic acid recovery; AAP: Amino acid production

Source: Bailly (2002)

2. Principles of electrodialysis

ED is an electro-membrane process in which ions are transported through ion exchange membranes from one solution to another under the influence of an electrical potential. The principle of mono-polar electrodialysis (MED) is depicted in Figure 8 Indicated is the arrangement of the ion exchange membranes to form distinct channels namely, dilution stream (DS) and concentrating stream (CS). In most applications, multiple anion and cation exchange membranes are arranged in an alternating pattern between an anode and a cathode to form a series of concentrating and diluting cells in the stack (between 5 and 500 cell pairs, typically more than 100). A third channel, the electrode rinse stream is located on either ends of the stack. During operation, an aqueous electrolyte solution is circulated in the electrode rinse stream to facilitate the transfering of the electric current and, to remove gases produced at the electrodes (Madzingaidzo *et al.,* 2002)

Mono-polar electrodialysis



CS : Concentrating Stream
DS: Diluting Stream
LAS: Concentrating Acid Stream (Free lactic acid)

Figure 8 Schematic diagram illustrating the main components of mono-polar electrodialysis stack.

Source: Madzingaidzo *et. al*. (2002)

The performance of an electrodialysis process can be determined by calculating the so called current efficiency (CE) of a given run. Current efficiency

gives the efficiency of current utilization in transporting salts from the diluting stream to the concentrate stream and is usually given as a percentage. Current efficiency is calculated based on the following relation:

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

Where i is the current density (A m^{-1}), F is the Faraday's constant and N is the mole flux (mol $s^{-1}m^{-2}$). The parameter N is calculated from $V_cA_m^{-1}dC_cdt^{-1}$, where V_c is the volume of concentrate solution, A_m is the total effective membrane area installed, Cc is the salt (sodium lactate) concentration in the concentrate solution and t is the time of operation.

Current efficiency is always less than 100%. Factors known to lower current utilization efficiency include, poor membrane selectivity, water transport by osmosis or with solvated ions, and loss of electric flows through the stack manifold.

3. Applications of electrodialysis

ED has found large scale application in the food and dairy industry; pharmaceutical, chemical, textile, and water treatment industries for purposes ranging from recovery, concentration, and purification, to brackish water desalination, and ground water denitrification. Integrating a microfiltration module between and electrodialysis unit and continuous immobilized cell fermentation, Von Eysmondt and Wandrey (1990) observed several fold increase in acetic acid productivity and concentration. Also working on acetic acid production, Chukwu and Cheryan (1999) described a continuous integrated process where the permeate from the membrane bioreactor was directly fed to the on-line MED unit where the acetic acid was recovered, concentrated (to 134 g l^{-1}) and purified simultaneously. The residual sugars and nutrients were retained in the diluting stream of the ED and recycled back to the bioreactor to facilitate optimum material utilization with concomitant reduction in waste generation.

Hongo *et al*. (1986) proposed to use ED for in situ lactate recovery to reduce the product inhibition. The obtained productivity was three times higher than that in non-pH controlled fermentation. However, fouling of anion exchange membranes by cells was observed in the ED fermentation. To solve this problem, Nomura *et al.* (1987) used immobilized growing cells entrapped in calcium alginate. The amount of lactic acid produced by semicontinuous ED fermentation using immobilized cells was 8-times higher than that produced by non-pH controlled fermentation, but some free cells were found in the solution. Czytko *et al*. (1987) found that the ED unit could only be operated with cell free solutions in order to prevent deposition of bacteria on the membranes. To increase the productivity of the lactic acid fermentation and to reduce the amounts of effluents, Boyaval *et al.* (1987) chose for the lactic acid fermentation in a bioreactor with total cell recycling, which was coupled with an ultrafiltration module and an ED unit. The outlet concentration of lactate was stabilized at 85 g l^{-1}. A similar system was studied by Yao and Toda (1990). H_2SO_4 was used as a donor of protons and lactic acid was the final product, reaching a concentration of 90 g l^{-1}. Choi *et al.* (2002) compared the conventional ED consisting of cation and anion exchange membranes and the ion substitution ED consisting of only cation exchange membranes. Both ED operations removed over 95% of sodium ions from the feed solution. Boniardi *et al.* (1997) found that lactic acid production by ED was possible to operate with high current efficiency values, while the overall recovery of sodium lactate depended on the current density. Electroosmotic water transport limits the maximum concentration value achievable using this technique. Gao *et al*. (2003) described the production of L-lactic acid by ED fermentation that the ED fermentation with continuous medium feed was the best and the productivity was 1.5 times higher than the conventional ED fermentation. The yield increased by above 30% and glucose transport was kept at a favorable value in intermittent ED fermentation.

Wang *et al.* (2006) reported to recover and concentrate lactic acid from kitchen garbage fermentation by four-compartment configuration electrodialyzer. The recovery ratio of lactic acid was over 89.5% in the case of 12 h ED under 2.0 A dm^{-2} current density, whereas the permeation ratios of reducing sugar and ammonia were

less than 0.97 and 0.15%, respectively. Under this condition, the average current efficiency was over 93%, and the concentration of lactic acid in permeate solutions after ED increased by about 2.2-folds than that in feed solutions. When lactic acid was fed at intervals, the concentration of lactic acid in permeate solutions was increased from 10.0 to 162.8 g dm^{-3}. It was 5.1-folds higher than the lactic acid concentration in the initial feed solution.

4. Principles of bipolar membranes

The conventional method for generating H^+ and OH^- ions from water was achieved by ED. ED also generates O_2 and H_2, and the production of these gases consumes about a half of the electrical energy of the process. Nowadays, special ion-exchange membranes are available for splitting water directly into H^+ and OH^- ions without generating oxygen and hydrogen. This process used bipolar membranes (Franken, 2000).

.

In its simplest form, a bipolar membrane is a cation-exchange membrane laminated together with an anion-exchange membrane. An intermediate layer (the "junction layer") is the heart and the most important part of the membrane. The principle of "splitting" water by using a bipolar membrane is illustrated in Figure 9. The diffusion of water from both sides of the bipolar membrane allows its dissociation under the electric field to generate protons and hydroxyl ions which further migrate from the junction layer through the cation-exchange and anion-exchange layers of the bipolar membrane.

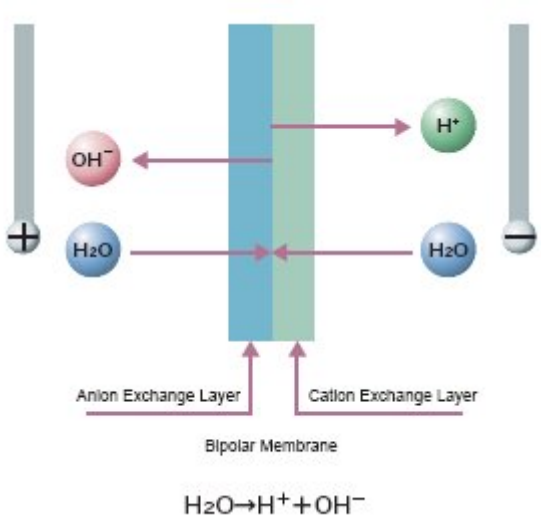


Figure 9 Illustration of water splitting by bipolar membrane.
Source: ASTOM Corporation (2007)

The application of a bipolar membrane reduces the energy costs associated with electrode polarization in the more conventional electrolytic approach. The theoretical potential to achieve the water splitting capability is 0.83 V at 25^{O}C. The actual potential drop across a bipolar membrane is quite close to this, being in the range of 0.9-1.1 V for current densities between 500 and 1500 A m^{-2}, which is the general region of practical interest. The value of the membrane potential drops equates to the theoretical energy consumption of the order of 600 – 700 kW.h per tone of NaOH. It has to be realized that the actual energy consumption in practical operating units is significantly higher because of the ohmic resistances in the other cell stack components.

4.1 Electrodialytic water splitting

The nature of the water splitting phenomenon, which is manifested by the change in the pH of the solutions adjacent to the membrane, is still under discussion. As has been revealed in many experiments, two main factors appear to determine the behavior of bipolar membranes. These are the structure of the bipolar junction

between the two ion-exchange layers, and the nature of the charged groups which are attached to the polymeric matrix.

The bipolar junction is responsible for the capacitive properties of bipolar membranes, which otherwise are purely dissipative. For reverse polarization conditions ($V < 0$), the asymmetric behavior of the membrane becomes apparent. The penetration of co-ions from the solution to the ion-exchange layers is highly impeded because of the electrostatic repulsion with the fixed-charge groups, while the counter-ions are extracted from these layers. Thus, a narrow region almost devoid of mobile ions appears between the ion-exchange layers. The charge separation over this layer produces an extremely high electric field; $E=10^8$ V m^{-1}.

However, when the applied voltage is high enough, an over-limiting current arises. The significant changes observed in the pH of the solutions close to the membrane reveal the presence of water-splitting products separated by the bipolar membrane. Since those pH changes are only observed under reverse polarization, it is generally believed that H^+ and OH^- are generated through protonation-deprotonation reactions between membrane fixed-charge groups and water molecules at the space-charge region forming the bipolar junction. The high electric field over this region is believed to significantly enhance the dissociation rate of water molecules (water splitting) in these chemical reactions.

An understanding of the electric field-enhanced (EFE) water dissociation occurring in bipolar membranes, as well as its coupling with ion transport, is essential for the development of new bipolar membranes. Early approaches considered the theory of Onsager for the EFE dissociation of weak electrolytes (the so-called second Wien effect), and preliminary results indicated that an electric field of about 10^9 V m^{-1} would produce an increase of 10^7 in the dissociation constant.

Other theories propose another mechanism to explain the water-splitting phenomenon, and that is the protonation and deprotonation reactions which involve the fixed-charged groups and the water molecules that exist in the bipolar junction.

These catalytic water dissociation reactions should be enhanced by the effect of the bipolar-junction electric field in order to account for the high dissociation rates observed.

4.2 Bipolar process configurations

The configuration of the bipolar process depends on the application. Typical use of bipolar membrane is in the treatment of concentrated salt solutions ,MX, to produce HX and MOH (for example Na_2SO_4 from the chemical industry to produce H_2SO_4 and NaOH). A cell system consists of an anion, a bipolar and a cation-exchange membrane as a repeating unit. In this case, the configuration is a three compartment (Figure 10a). This elementary cell is repeated and placed between two electrodes. The MX solution flows between the CEM and AEM. When a direct current is applied, water will dissociate in bipolar membrane to form equivalent amounts of H^+ and OH^- ions. The H^+ ions permeate through the cation-exchange side of the bipolar membrane and form HX with the sulfate ions provided by the MX solution from the adjacent cell. The OH^- ions permeate the anion-exchange side of the bipolar membrane and form MOH with the sodium ions permeating into the cell from the MX solution through adjacent CEM. The final result is the production of MOH (NaOH) and HX (H_2SO_4) from MX (Na_2SO_4) at a significantly lower cost than by other methods. There are applications where high purity of both acid and base is not possible to obtain or even may generate problems during the process, e.g. when producing organic acids or bases from the corresponding salts that reveal low dissociation tendency and therefore low conductivity in the protonated form. In such cases, a two compartment cell is recommended as depicted in Figure 10b (for acidification) or in Figure 10c (for the generation of the corresponding base). Moreover, when a higher ratio of acid and base with respect to the product salt content is required, a configuration with two homo polar membranes of the same type can be applied. The outlet of the middle loop is recycled again into the acidic loop next to the BPM for a more efficient exchange between protons and cations (Figure 10d), or between OH- ions and anions (Figure 10e) (Pourcelly, 2002).

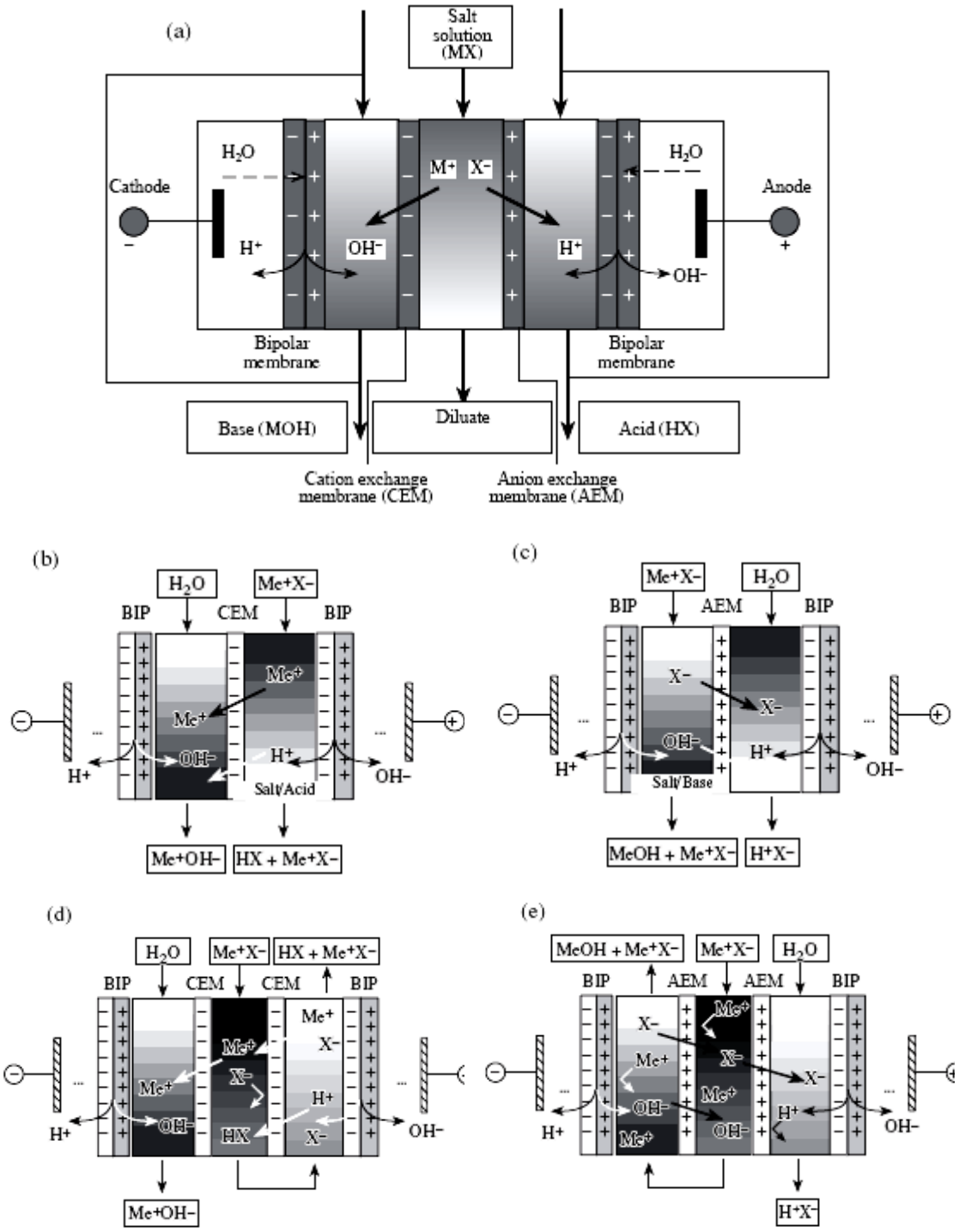


Figure 10 Different cell configurations for bi-polar membrane applications.

Source: Pourcelly, G. (2002)

5. Applications of bi-polar electrodialysis

The two-stage electrodialysis method (mono and bi-polar electrodialysis) for purification of lactic acid was described by Glassner and Datta (1990). Lee *et al.* (1998) studied the two-stage electrodialysis method for lactic acid recovery, too. In the desalting electrodialysis, 115 g lactate l^{-1} was obtained in the concentrate, only 1 g lactate l^{-1} remained in the diluate, the current efficiency was about 90% and the energy consumption for the lactate transfer from the diluate to the concentrate was 0.25 $kW.h.kg^{-1}$. In the second step, 88-93% of the total amount of lactate was converted to lactic acid, and the current efficiency was about 80%. The total energy consumption for lactic acid recovery was in the range of 0.78- 0.97 $kW.h.kg^{-1}$. Heriban *et al.* (1993) dealt with electrodialysis with double exchange. A 4-times higher lactic acid concentration in the continuous mode compared to the lactic acid concentration was reached in the processed solution. A concentration of 236.8 g lactic acid l^{-1} was obtained in the course of the experiment with model solutions, and the energy consumption was in the range of 1.3-2.3 $kW.h.kg^{-1}$. Kim and Moon (2001) studied two- and three-compartment bi-polar electrodialysis for lactic acid recovery. High volumetric productivity (71.7 g $l^{-1}h^{-1}$) was reached.

Madzingaidzo *et al.* (2002) tried to develop and optimize lactic acid purification using two-stage electrodialysis. Lactate concentration in the product stream increased to a maximum of 15% during MED. Stack energy consumption averaged 0.6 $kW.h.kg^{-1}$ and lactate transported at current efficiencies in the 90% range.

Glucose in the concentrate stream solutions was reduced to < 2 g l^{-1}. Acetate impurities enriched from about 0.5 g l^{-1} in the feed stream to 1.5 g l^{-1} in the concentrate stream solutions. After MED, the concentrated sodium lactate solutions were further purified using BED. Free lactic acid concentration reached 16% with lactate flux of up to 300 g $m^{-2}h^{-1}$. Stack energy consumption ranged from 0.6 to 1 $kW.h.kg^{-1}$ lactate. Under optimized process conditions, current efficiency during BED was consistently around 90%. Glucose was further reduced from 2 to < 1 g l^{-1} in the

free lactic acid solution. Acetic acid impurity remained at around 1 g l^{-1} and significant reduction in colour and minerals in the product streams were observed during ED purification. Habova *et al.* (2004) tried to pretreated fermentation broth before ED which consisted of ultrafiltration, decolourisation and removing of multivalent metal ions. In the MED step the final lactate concentration of 175 g l^{-1} was obtained and afterwards the final lactic acid concentration of 151 g l^{-1} was reached in the BED step.

Li *et al.* (2004) have investigated to combine the advantages of both conventional electrodialysis and bipolar membrane electrodialysis within the bioreactor configuration for lactic acid fermentation (Figure 11). The electrokinetic process enabled removal of the biocatalytic product (lactic acid) directly from the bioreactor system, in a concentrated form, as well as enabling good pH control without generation of troublesome salts. Moreover, end product inhibition of glucose catabolism was reduced, resulting in a greater generation of the end-product lactic acid. An automatic pH sensor and current application system was developed and successfully implemented for lactic acid fermentation in the electrokinetic bioreactor.

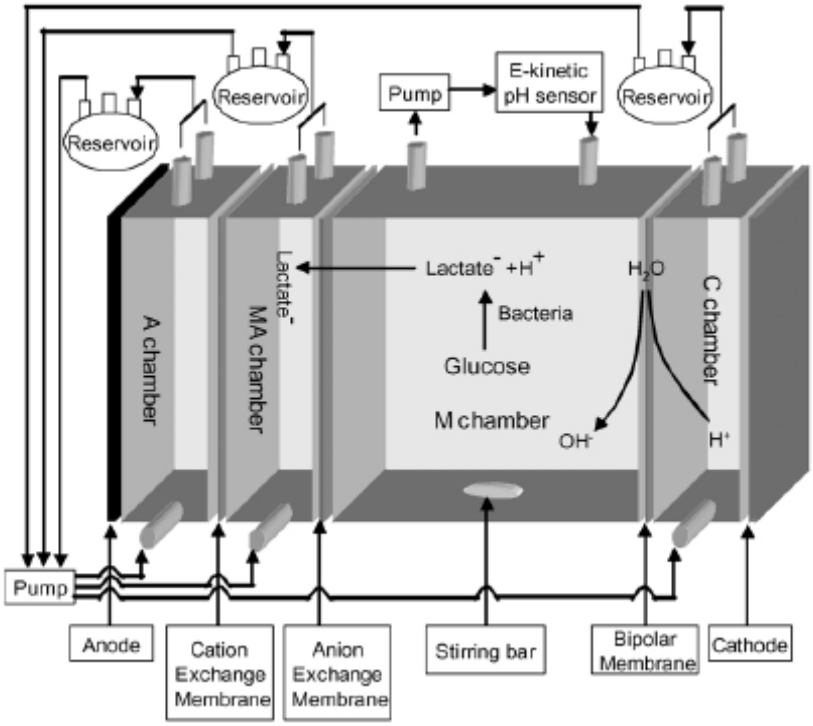


Figure 11 Details of electrokinetic bioreactor. Each compartment has a circulation loop and reservoir for pH control, sampling and product recovery. Chambers A, MA, C and M are recirculated at 120 ml/min using a peristaltic pump with Norprene tubing (Cole-Parmer Instrument Co. Ltd, London, UK). The anode and cathode are connected to a computer-controlled DC power supply, with a pH sensor in chamber M connected to a computer interface.

Source: Li *et al.* (2004)

MATERIALS AND METHODS

Materials

1. Equipments

- Fermenter (MDL B.E.Marubishi with pH controller: LABO Controller Model MDS-2C, Japan)
- Electrodialysis unit ("ACILYZER 02B", TOKUYAMA Corporation, Japan)
- Membrane sheets (NEOSEPTA ion exchange membrane, Tokuyama Corporation, Japan)
- Micro centrifuge (Hettich Zentrifugen Rotina 35R, Germany)
- High Performance Liquid Chromatography, HPLC (System controller: SCL-10A *VP*, Liquid chromatograph: LC-10AD *VP*, Degasser: DGU-12A, RI detector: RID-10A, UV-Vis detector: SPD-10A *VP*, Auto injector: SIL-10AD *VP*, Column oven: CTO-10AS *VP*, Shimadzu, Japan)
- Chromatography columns (Aminex HPX-87H, Bio-Rad, U.S.A.)
- Spectrophotometer (Model Genesys 10 UV: Thermospectronic, U.S.A.)
- Water bath (Memmert WBU 45. Germany)
- pH meter (Horiba F-21, Japan)
- Incubator with R3-controller (Binder, Germany)
- Laminar flow (Microflow Advanced BIO Safety Cabinet-class 2, England)
- Balance (Precisa XB 620C and XB 320M, Switzerland)
- Autoclave (Hiclave HVA-85, Japan)
- Glasswares and chemicals

2. Enzymes

- α-amylase (Liquazyme Supra, Novozymes, Denmark)
- Glucoamylase (OPTIDEX L-400, Genencor International Inc. U.S.A.)

Methods

1. Screening of lactic acid bacteria from various fermented samples for industrial lactic acid fermentation

1.1 Scheme of LAB screening

Lactic acid bacterial strains
⇓
High lactic acid production —no→ reject
⇓ yes
Homofermentative —no→ reject
⇓ yes
To study the acid and substrate tolerance of LAB
that being suitable for lactic acid production
⇓
Product stereospecificity
⇓
Selection of strains for identification
and maintenance

1.2 Screening of LAB from various fermented sources

The LAB were isolated from various sources including fermented food products from dairy, vegetable and meat and fermented waste water and solid waste from cassava starch factories, by a serial dilution method using 0.85% (w/v) NaCl. The samples were incubated in MRS agar (Table 6) containing bromocresol purple blue and $CaCO_3$ as an indicator by a pour plate method, at $37^{O}C$ under an anaerobic condition for 48 h. When the LAB grows, the colors of medium change from purple to yellow and the clear zone around the colony appears. The LAB were selected for the next experiment.

Table 6 Concentration of *Lactobacillus* MRS broth.

Component	Amount (per liter)	
Peptone	10	g
Yeast extract	5	g
Glucose	20	g
Tween 80	1	ml
Ammonium citrate	2	g
Sodium acetate	5	g
Magnesium sulfate	0.1	g
Manganese sulfate	0.04	g

Adjust to pH ~ 7.2, sterilize at 121^{O}C for 15 min, adding agar 15 g l^{-1} for MRS agar.

1.3 Selection of high lactic acid production

The selected LAB were then cultivated in 5 ml basal medium (Table 7) for 48 h. The culture broth was collected and then assayed for lactic acid concentrations by a titration method.

Table 7 Concentration of the Basal medium for LAB fermentation.

Component	Amount (per liter)	
Peptone	5	g
Yeast extract	10	g
Glucose	20	g
Sodium acetate	1.5	g
Magnesium sulfate	0.2	g
Manganese sulfate	0.05	g
Potassium hydrogen phosphate	1.5	g
Di-potassium hydrogen phosphate	1.5	g

1.4 Homofermentative test

The homofermentative identification of purified colonies was carried out using an inverted-tube technique. The strains were cultivated in a 10 ml screw cap culture tube in which a small inverted tube was immersed in the medium. Heterofermentative lactic acid strains were identified by a production of CO_2 as a by product, which was trapped inside the inverted tube.

1.5 Studying of acid and substrate tolerance of LAB for lactic acid production

The potential high-yielded and homofermentative strains were selected and subsequently evaluated for their tolerance to high acid (3-20% v/v sodium lactate, pH (2-8) and substrate concentration (3-30% w/v glucose). The strains having potential tolerance to adverse environment, i.e. high lactic acid, low pH and high glucose concentration were then selected for product's stereospecificity identification (L- or D-isomer).

1.6 Product stereospecificity test

For this purpose, the selected strains were cultivated at $37^{O}C$ for 48 h in 10 ml of MRS medium. The D- and L-lactate concentrations were quantified enzymatically using the test kit of combination "D-lactic acid/L-lactic acid" (Cat. No. 112821m, Boehringer Mannheim, Germany), to calculate the percent D- and L-isomer of the total lactic acid.

1.7 Identify of LAB

Subsequently, the strain identification of selected stereospecific strains, cultivated in 10 ml of MRS medium, was performed, based on their abilities to ferment 49 types of carbohydrate, using API 50 CHL test kit (BioMerck, France). During incubation, the carbohydrates were fermented to acids which caused a

decrease in pH as detected by a color change of an indicator. The results of their biochemical profiles of each strain were then used for strain identification.

1.8 Maintenance of culture

The stock cultures were prepared in 10% (v/v) glycerol by placing 0.7 ml of a culture broth into a sterile tube containing 0.7 ml of 20% (v/v) glycerol solution. The 10% (v/v) glycerol stock cultures were then stored at $-20^{O}C$.

2. Fermentation

2.1 Optimal fermentation conditions of selected strains

The optimal conditions for lactic acid production by selected strains were performed at various conditions including pH 5-7, temperature 30-45^{O}C, the agitation speed (100-200 rpm) and initial substrate concentrations (30-200 g l^{-1}). To conduct the study, the media were prepared by mixing sterile glucose solutions (30-200 g l^{-1}) with 10g yeast extract, 5g peptone, 0.2g $MgSO_4.7H_2O$, 0.05g $MnSO_4.4H_2O$, 1.5g each of sodium acetate, KH_2PO_4 and K_2HPO_4, which were autoclaved at 121^{O}C for 20 min prior to uses. The batch fermentation of selected strain conducted at 2.5 L working volume in a 5 L fermentor and were carried out at various condition including pH (5,6 and 7), temperature (30, 37, 40 and 45^{O}C), the agitation speed (100, 150 and 200 rpm) and initial substrate concentrations (30, 50, 100, 150 and 200 g l^{-1}). The fed-batch fermentation was performed at optimum condition. The seed cultures (5% v/v) were prepared by cultivating in the same fermentation medium at 37^{O}C, 18 h.

2.2 Application of selected LAB strain in producing lactic acid from cassava starch by Simple Saccharification (SS) and Simultaneous Saccharification and Fermentation (SSF) process

Cassava starch were obtained commercially from Sanguan Wongse Industries Co., Ltd (Thailand). A slurry of cassava starch (30% DS dry solid) was

initially liquefied by α-amylase (0.1% v/w of Liquazyme Supra) in a presence of 40 ppm of Ca^{2+} as a cofactor at $80^{O}C$ for 120 min. The liquefied starch hydrolysates were used as the initial substrate for SS and SSF processes of which the solid contents were adjusted as specified in each experiments.

2.2.1 Simple Saccharification (SS)

The liquefied starch hydrolysates, diluted to the required concentration of 100 g l^{-1}, were adjusted to the optimum pH at 4-4.5 for further hydrolysis by glucoamylase (0.1% v/w OPTIDEX L-400) at $60^{O}C$ for 8 h. Then, the obtained glucose syrup was fermented by a selected strain in a batch type using the 5% (v/v) seed cultures prepared by cultivating in the fermentation medium described previously (in section 2.1) at $40^{O}C$, 18 h. The fermentation was accomplished at an optimal condition as determined in a previous study (in section 2.1).

2.2.2 Simultaneous Saccharification and Fermentation (SSF)

The liquefied starch hydrolysates were used as the initial substrate at various concentrations (100, 150 and 200 g l^{-1}). In a batch-type mode, glucoamylase enzymes (0.5% v/w OPTIDEX L-400) were added simultaneously to the samples with the 5% (v/v) seed cultures prepared by cultivating in the fermentation medium described previously (in section 2.1) at $40^{O}C$, 18 h. The fermentation was accomplished at an optimal condition as determined in a previous study (in section 2.1).

3. Application of bipolar electrodialysis (BED) on recovery of free lactic acid

3.1 Lactate solutions

The recovery of lactic acid by BED was demonstrated by using a model solution and the actual fermentation broth. For a model solution, the ammonium

lactate solution was prepared by using an aqueous solution of 85% (v/v) lactic acid (APS Finechem, Australia) adjusted to pH 6 by 25% (v/v) NH_4OH.

The actual fermentation broth was accomplished at an optimal condition as determined in a previous study (in section 2.2.2). At the end of fermentation, the collected broth was centrifuged at 10,000 rpm for 10 min and subjected to ultrafiltration (UF: MW cut-off 100,000, diameter 76 mm {Cat. No. PBHK07610, Millipore Corporation, USA} and the operating controlled pressure of 0.09 MPa) to remove the suspended particles.

3.2 Lactic acid recovery by BED

3.2.1 General experimental condition

The bipolar electrodialysis (BED) unit was equipped with 6-cation, 5-anion and 5-bipolar membrane sheets having 2 dm^2 area. The direct current (DC) generator was used to supply the voltage and current in the following ranges: 0-40 V and 0-27 A, respectively. Ammonium sulphate solutions were circulated in the electrolyte tank at the concentration of 0.25 M. The liquid flow rates in the stream were 1.6 l min^{-1} and the experiments were conducted at an ambient temperature under the non-sterile condition. The temperature, current load of rectifier, voltage of rectifier, flow rate and tank liquid level, were recorded during operating. Samples were taken from the feed stream and product stream and the lactic acid concentrations were quantified by High Performance Liquid Chromatography (HPLC) analysis.

3.2.2 Comparison of different BED systems

The recovery performance of three BED systems including BED-anion, BED-cation and BED-anion-cation, was evaluated at a fixed voltage by using a model solution of ammonium lactate. The salt solution was run in the feed stream. At the beginning of BED, ultra-pure water was filled in the product stream. Liquid flow rates in the feed and product streams were set at 1.6 l min^{-1}. The voltage was regulated

at 20 V. Samples were taken from the feed and product stream during the run for lactic acid analysis by HPLC. The electrode-rinsed electrolyte solution (0.25 M $(NH_4)_2SO_4$) was freshly prepared for each batch experiment.

3.2.3 Comparison of operating conditions

The recovery of lactic acid by a practical BED system identified in a comparison of different BED system experiment (section 3.2.2) was conducted at a fixed voltage (20 V) or a fixed current (5 A) by using a model solution of ammonium lactate. Similarly, the salt solution was run in the feed stream and ultra-pure water was filled in the product stream. Liquid flow rates in the feed and product streams were set at 1.6 l min^{-1}. Samples were taken from the feed and product stream during the run for lactic acid analysis by HPLC. The electrode-rinsed electrolyte solution (0.25 M $(NH_4)_2SO_4$) was freshly prepared for each batch experiment.

3.2.4 BED trial with actual fermented broth

The recovery of lactic acid from actual fermented broth by a practical BED system identified in a comparison of different BED system experiment (3.2.2) was conducted in a condition obtained in comparison of operating conditions, at a fixed voltage or current (section 3.2.3). The fermentation broth after suspended particle removal was exposed to the mono-polar electrodialysis (MED) prior to operate with BED, in order to separate the ammonium lactate salts from other organic acids in fermentation broth. The MED was operated with 7-cation and 5-anion exchange sheets, each sheet had a working area of 2 dm^2 and the voltage was regulated at 20 V. The fermentation broth and ultra-pure water were filled in the feed stream and product stream, respectively. Subsequently, the sample was preceded to BED experiment as previously described in comparison of operating conditions.

The performance of an electrodialysis process was reported as a percentage of current efficiency (CE), i.e. the efficiency of current utilized in transporting salts as following:

$$\text{Current efficiency (\%)} = 100 \times \frac{(C_F V_F - C_I V_I) \times 26.8}{(i_{TOTAL} \times N_M)}$$

Where C_F and C_I = the final and initial concentration (unit in Normal, N)

V_F and V_I = the final and initial volume (unit in liter, l)

26.8 = the Faraday's constant (96500/3600sec)

i_{TOTAL} = the total current (A.h)

N_M = the number of membrane installed (N_M in this case was 5).

The recovery ratio (R) of lactic acid was defined by the following equation: (Wang *et al*. 2006)

$$R\ (\%) = \frac{W_F - W_I}{W_{I,feed}} \times 100$$

Where R = the recovery ratio of lactic acid

W_F and W_I = the final and initial amount (unit in gram, g) of lactic acid in the product stream.

$W_{I,feed}$ = the initial amount (unit in gram, g) of lactic acid in the feed stream.

4. Nitrogen sources

4.1 Preparation and characterization of agro-industrial wastes

4.1.1 Preparation of agro-industrial wastes

Glutamic acid-producing waste: The liquid waste water from glutamic acid production, locally named as GM1, was kindly obtained from Thai Fermentation Industry Co., Ltd., Thailand. The solid particles were removed through centrifugation (at 10,000 rpm, 10 min, at 4°C) and the supernatant (GM) was used.

Fish waste: Fish extracts (FE) were obtained commercially from Siam Victory Co., Ltd (Bangkok, Thailand). In addition, the other fish waste from canning industry was kindly supported by I.S.A Value Co., Ltd., Thailand and then subjected to acid hydrolysis process, according to the method described by Gao *et al.* (2006b) as summarized in Figure 12. The fish wastes were initially minced and mixed with water with a ratio of 1:1 (by wet weight). Subsequently, the initial pH of the waste slurry was adjusted to 1 by the addition of 6 M HCl and the slurry was hydrolyzed at 121^{O}C for 40 min. After acid hydrolysis, insoluble particles were separated by filter paper No.1 and the filtrate, namely fish waste hydrolysates (FWH) were used for fermentation of cassava materials.

Shrimp wastes: Shrimp wastes were obtained from Marine Gold Products Ltd., Thailand. Similar to fish wastes, shrimp wastes were acid-hydrolyzed prior to use under the same condition as previously described for preparing FWH. The liquid obtained after acid hydrolysis was named as shrimp waste hydrolysates (SWH).

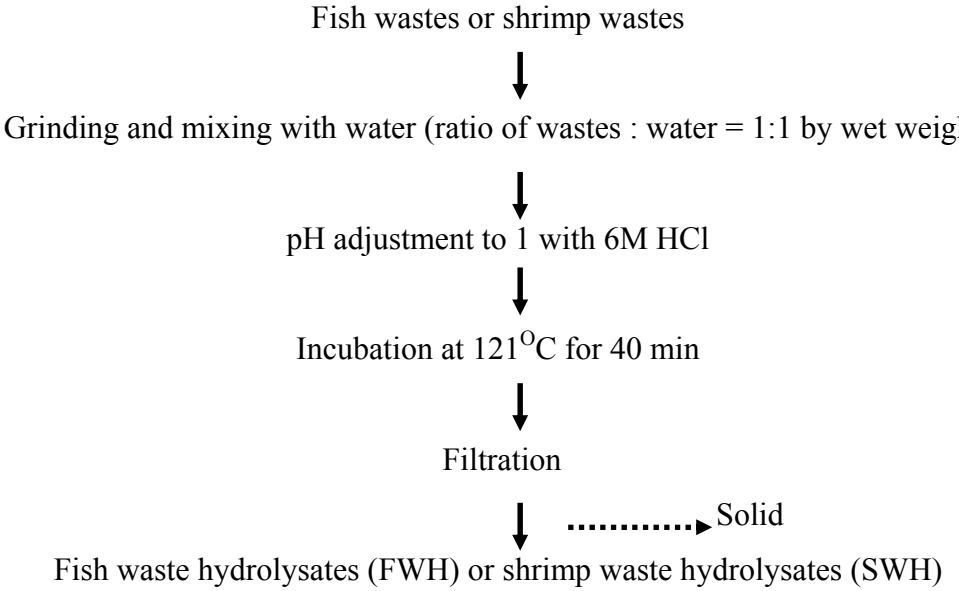


Figure 12 Procedure of preparing fish waste hydrolysates (FWH) and shrimp waste hydrolysates (SWH).

4.1.2 Characterization of agro-industrial wastes

The total nitrogen (TN) contents of waste samples including FE, GM, FWH and SWH and yeast extract (YE; Merck, Germany) were evaluated by the Kjeldahl method (A.O.A.C, 1980) and used to estimate the protein content by multiplying with a factor of 6.25. The contents of free amino acid (FAA) were determined by Ninhydrin method (Rosen, 1957) and the ash was quantified according to a standard method described by AOAC (1980).

4.2 Lactic acid fermentation of cassava starch by Simultaneous Saccharification and Fermentation (SSF) process with the substitution of yeast extract (YE) by agro-industrial wastes

4.2.1 Complete substitution of yeast extract (YE)

A slurry of cassava starch (300g dry solid l^{-1}) was initially liquefied by α-amylase (0.1% v/w of Liquazyme Supra) in a presence of 40 ppm of Ca^{2+} as a cofactor at $80^{O}C$ for 120 min. The liquefied starch hydrolysates were used as the initial substrate for SSF processes of which the solid contents were adjusted to 150 g dry solid l^{-1}. The fermentation was accomplished at an optimal condition as determined in a previous study (in section 2.2.2). Various agro-industrial wastes including FE, GM, FWH and SWH were added to completely substitute YE at an equivalent total nitrogen in the media, i.e. 1 g l^{-1}.

4.2.2 Partial substitution of yeast extract (YE)

A potential low-price nitrogen source was selected based on the result of work in section 4.2.1 and was used in a combination with YE, at a 50% replacement. For this purpose, the total nitrogen content in the fermentation media was still adjusted to 1 g l^{-1} and the fermentation with the addition of a mixture of YE and potential nitrogen source was carried out using cassava starch as previously described in section 2.2.2.

4.3 Lactic acid fermentation of cassava materials by Simultaneous Saccharification and Fermentation (SSF) process with the substitution of yeast extract (YE) by agro-industrial wastes

A potential low-price nitrogen source was used to substitute yeast extract (YE), partially or completely, depending on the work in section 4.2. In this experiment, the lactic acid production was performing by using dried chips and fresh roots (150 g dry solid l^{-1}) which were obtained locally and initially liquefied by α-amylase and then simultaneously saccharified and fermented (SSF) as described in section 2.2.2. The total nitrogen content in the fermentation media was still controlled to be at 1 g l^{-1}.

5. Analytical assays

The glucose concentrations in liquefied and glucose syrups were measured by Somogyi-Nelson method (Somogyi, 1952). The starch content in raw materials was quantified by Polarimetric method-the European Economic Communities, EC 79, 1999. The density of microbial cells in the medium was monitored by measuring the absorbance at 620 nm using a spectrophotometer. The viable cell counts (CFU) were carried out by the dilution spread plate method using MRS agar. The supernatant was removed by centrifugation (at 10,000 rpm for 10 min) and kept for further determination of lactic acid concentrations by simple titration and chromatographic method.

The amount of total acidity, reported as lactic acid equivalent, was determined by titrating the broth against 0.1 M standardized NaOH. The percentage by weight (% w/w) of acid in the sample was calculated by multiplying the volume of alkali (ml) by the factor of 0.09. Moreover, lactic acid and residual sugar contents were quantified by High Performance Liquid Chromatography (HPLC) equipped with an Aminex HPX-87H column incubated at $40^{O}C$, using 5mM H_2SO_4 solution as the mobile phase at an elution rate of 0.6 ml min^{-1} and the Ultraviolet-Visible (UV-Vis) detector was

employed. The collected culture broth samples were centrifuged at 10,000 rpm, 10 min and the supernatant was then diluted and filtered before injection.

Place

At laboratory of Cassava and Starch Technology Research Unit, Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University.

Duration

From June 2005 to September 2007

RESULTS AND DISCUSSION

Part 1: Screening of lactic acid bacteria from various fermented samples for industrial lactic acid fermentation

The inspection of LAB from natural resources has been recognized as the most powerful approach for obtaining useful and genetically-stable microorganisms being suitable for industrial applications. Various concerns should have been taken into consideration when the screening process is employed. In case of LAB, the homofermentative strains having stereospecificity, either L- or D-isomer types are of great interest in term of the economical point of view. The homofermentative strains tend to give a high product yield whereas the strains producing only one desirable optical isomer of lactic acid or stereospecificity are beneficial for downstream processing since a costly separation process of these two L- and D-isomer is not required. Moreover, strains with tolerance to the adverse environment are preferred to the industrial uses.

1. Screening of LAB from various fermented sources

In this study, LAB were isolated from various samples. The total plate counts on MRS agar were somewhat varied, depending on the sources. The result shown in Appendix Table 1, waste samples, i.e. cassava pulp and liquid waste water gave a high total plate count in the range of $10^6 – 10^7$ CFU/g whereas a high variation in the total plate count was observed in fermented food products, ranging from $10^2 – 10^8$ CFU/g. The great difference in microbial counts of these commercialized products produced by naturally occurring microbial or added inoculums was presumably accounted by typical attributes of each food product which relied on raw material types, food processing and consumer preferences. Noticeably, the total plate count of fermented pork (Nham) was higher than others sample varied at the level of $10^8 – 10^9$ CFU/g. Nham is a Thai traditional fermented sausage made from ground pork with garlic, pepper, salt and cooked rice. It can be consumed raw or cooked. Nowadays, the manufactures of Thai fermented pork sausage is preferably achieved by using a

starter culture for direct inoculation and the sized of the starter inoculums from an initial of 10^6-10^7 CFU/g have to be applied to obtain safe dominance in the ranging of 10^8-10^9 CFU/g (Fadda *et al*., 2002, Hammes *et al*., 1990). In the starter-inoculated treatments, the LAB were approximately 100 times more numerous than the native flora. Thus, the evolution of microorganisms in this product had high possibility to increase cells more than other sources.

2. Selection of high lactic acid production and homofermentative test

After counting, colonies on different plates were randomly picked from plates and transferred into MRS agar. The isolates were purified by successive streaking on the appropriate agar about twice. The high lactic acid production test was investigated in basal medium and homofermentative test in MRS broth. The results were summarize as shown in Appendix Table 3. From a total of 57 screened samples, one hundred and fifty six strains were isolated of which 94 strains exhibited homofermentative characteristics with a production yield of lactic acid greater than 10 g l^{-1} when glucose concentration of 20 g l^{-1} was fermented at pH 6 and temperature of 37 °C for 48 h.

Besides, it could be noticed that the strains with high cell density were able to produced high lactic acid concentrations and almost heterofermentative strains gave lower lactic acid concentrations. As the theory, heterolactic fermentation of glucose through the 6- PG/PK pathway gives 1 mole each of lactic acid, ethanol, acetate, CO_2 and 1 ATP, in contrast, homolactic fermentation of glucose results in 2 moles of lactic acid and a net gain of 2 ATP per mole glucose consumed via the Embden-Mayerhof-Parnas pathway (Hofvendahl and Hahn-Hagerdal, 2000). Hence, it is markedly that only homofermentative strains were used for further study.

3. Studying of acid and substrate tolerance of LAB for lactic acid production

The 94 homofermentative and high-yielded strains were further evaluated for their fermentation efficiencies under the stress of high lactate concentration (3-20%

w/v of sodium lactate) and acidic condition (pH 2-8) and the production yields with various initial glucose concentrations (3-30% w/v) were also evaluated.

3.1 Various sodium lactate

All selected homofermentative strains could not grow in a presence of high lactate concentration (>10 % w/v) and only 3 strains did not grow in a media containing 3 % w/v sodium lactate (Appendix Table 4). A total of 41 strains were able to grow at 5 % w/v lactate. As observed, it could be presumed that the inhibitory effect on bacterial growth of the lactate anion was significant at the concentration above 10 % (w/v) of lactate initially added. Although, these strains could grow under lactate at 5 % (w/v) but lactic acid production was lower and varied in ranging of 8 – 15 g lactic acid l^{-1}. In agreement with results obtained for *Lactobacillus casei* (Senthuran *et al*., 1999), a decrease in productivity as well as biomass could be seen in medium containing 40-50 g lactate l^{-1}. Anuradha *et al.* (1999) studied the inhibitory effect of lactate on cell growth at lactate concentrations ranging from 10 to 250 g l^{-1}. *L. delbrueckii* was able to survive in lactate concentrations as high as 250 g l^{-1}, although its growth rate decreased. A probable reason for this capability to withstand such high concentrations of lactate, was that *L. delbrueckii* could with stand lower intracellular pH without depending on the energy consuming by proton pumps.

The above result could be attributed to the effect of undissociated lactic acid that can cause severe inhibition rather than the gradual production of acid during fermentation. Moreover, the pH during conducting this experiment was not controlled, therefore, acidic pH supported non-dissociated form of lactic acid and played the product inhibition role (Hofvendahl and Hagerdal, 1997. Senthuran *et al*.,1999).

3.2 Various pH

No selected homofermentative strains was able to grow at a very acidic condition (pH =2). However, some strains (n = 42) were capable to grow in an acidic

environment (pH = 3) with a low yield. The cultivation of most strains was very efficient at a slight acidic condition (pH 5-7) with high yields of lactic acid (12-18 g l^{-1} at glucose = 20 g l^{-1}). These result agreed with the experiments by Hofvendahl and Hahn-Hagerdal (2000) that described the optimal pH for lactic acid production varied between 5 - 7. From the result at pH lower than 3, it could be assumed that pH had more effect on lactic acid production than on cell growth. There have been many reports deduced that the effect of pH is very strong on lactic acid productivity and yields are considerably inhibited at low pH values (Goncalves *et al*., 1997, Akerberg *et al*., 1998). As evident from Appendix Table 5, at pH 3 and whatever pH lower than 5 of the highly acidic environment, glucose consumption was very low and then led to small amount of lactic acid production. By the way, the inhibitory effects from dissociated and undissociated lactic acid can be related with pH variation (Fu and Mathews, 1999).

3.3 Various substrate concentrations

There was one strain isolated from fermented pork (being coded as CH6-2) that could not grow when the initial substrate concentration was greater than 20% (w/v). The rest could grow in all evaluated substrate concentrations (3-30% w/v), yielding the lactic acid concentration of 10 – 20 g l^{-1} (Appendix Table 6). The results indicated that initial glucose concentration did not have the effect on cell growth and lactic acid production of almost strains. Using of a high sugar concentration for lactic acid production is desirable from an economical viewpoint. As investigated, all of initial glucose concentration showed the conversion to lactic acid in somewhat similar values. Increasing initial glucose concentration did not result in significantly higher lactic acid concentrations at the end of the cultivation time.

In batch fermentation, the initial substrate may affect the lag phase of cell growth (Fu and Mathrews, 1999). When glucose concentration is higher than 10 % (w/v) and extended lag phase is observed due to a decrease in a_w in the system promoted by large amounts of water-binding substance, such as a sugar (Shirai *et al*.,

2001). However, the optimum initial glucose concentration for high lactic acid yield depends on the used strain.

The selected homofermentative strains with potential tolerance to lactate, acid and high substrate concentrations (n = 53) were subsequently evaluated for their stereospecificity.

4. Product stereospecificity test

Results of the product stereospecificity screening are summarized in Appendix Table 7. Most strains demonstrated the ability to produce an equivalent quantity of L- and D-isomer. Only 4 strains (being coded as DY, DM3, CB(R2)-bf and FCP2) produced lactic acid with highly L-isomer specific (>90%), but none were found to be D-isomer specific.

As compared to the chemical synthesis, fermentative production has the advantage that by choosing a strain of lactic acid bacteria producing only one isomers, an optical pure product can be obtained. Although most lactic acid bacteria produced only isomeric form of lactic acid, but sometimes there is a slight production of the other isomer. The lactate dehydrogenase (LDH) is stereospecific, giving either D- or L-lactic acid. Types of isomeric form of the enzyme present in LAB mainly determine the isomery of the produced lactic acid. The L-lactic acid-producing LAB are *L. amylophilus*, *L. delbrueckii* and *L. rhamnosus* (Hofvendahl and Hahn-Hagerdal, 2000). It is well known that the D-isomer is harmful to humans and only the L-isomer low molecular weight polymers stimulate growth in plants, therefore, the production of the L-isomer is favored (Hofvendahl and Hahn-Hagerdal, 1997). In addition, optically pure L-lactic acid is polymerized to a high crystal polymer suitable for fiber and oriented film production and is expected to be useful in the production of liquid crystal as well (Amass *et al.,* 1998). Consequently, four strains that mention above were identification and were employed for studying in the next title.

5. Identification of LAB

The identification of these highly L-isomer specific strains based on their biochemical profiles of carbohydrate fermentation capability suggested that DY and FCP2 were *Lactobacillus para parcasei* and, DM3 and CB(R2)-bf were *Lactobacillus rhamnosus*. All results were illustrated in Table 8 and Table 9. Among 4 selected strains, the strain DM3 which being screened from fermented pork was the most interesting one for further conducting the application trial with cassava starch since it yielded the highest L-specific (92.8%) and the total lactic acid concentration (16.6 g l^{-1}). Moreover, many studies report that *L. rhamnosus* is probiotic bacteria (Hilde *et al*., 2005 and Liew *et al*., 2005) that can produce exopolysaccharides (Macedo *et al*., 2002; Tomasz *et al*., 2003) and lactic acid (Sunhoon *et al*., 2000; Beatriz *et al*., 2004; Gao *et al*., 2005).

The identification of DM3 strain was achieved by 16S rDNA gene sequence method, a well-known method, typically used to identify bacteria strain. Experimentally, the extracted DNA of bacterial strain DM3 (coded as SIID2549-02) was amplified by PCR (polymerase chain reaction) method. The PCR products were, then, cloned and purified and 16S rDNA gene sequence (having 1,500 bases) of the strain DM3 fragment (Figure 13) was aligned with the sequence of *Lactobacillus* sp. using BLAST search (Basic Local Alignment Search Tool, an algorithm for comparing primary biological sequence information, in this case, the nucleotides of DNA sequences) (Figure 14). The phylogenetic placement of DM3 (as shown in Figure 15) showed the highest level of similarity to *Lactobacillus rhamnosus* (99.94%).

Table 8 Identification of selected homofermentation LAB screened from fermented products by carbohydrate fermentation ability using API 50 CHL kit.

	Code			
	DY	**DM3**	**CB(R2)-(bf)**	**FCP2**
Control	-	-	-	-
Glycerol	-	-	-	-
Erythritol	-	-	-	-
D-Arabinose	-	+	+	-
L-Arabinose	-	+	+	-
Ribose	+	+	+	+
D-Xylose	-	-	-	-
L-Xylose	-	-	-	-
Adonitol	+	-	-	-
β Methyl-xyloside	-	-	-	-
Galactose	+	+	+	+
D-Glucose	+	+	+	+
D-Fructose	+	+	+	+
D-Mannose	+	+	+	+
L-Sorbose	+	-	-	+
Rhamnose	-	+	+	-
Dulcitol	-	+	+	-
Inositol	-	+	+	+
Mannitol	+	+	+	+
Sorbitol	+	+	+	-
α Methyl-D-mannoside	-	-	-	-
α Methyl-D-glucoside	+	+	+	+
N Acetyl glucosamine	+	+	+	+
Amygdaline	+	+	+	+
Arbutine	+	+	+	+
Esculine	+	+	+	+

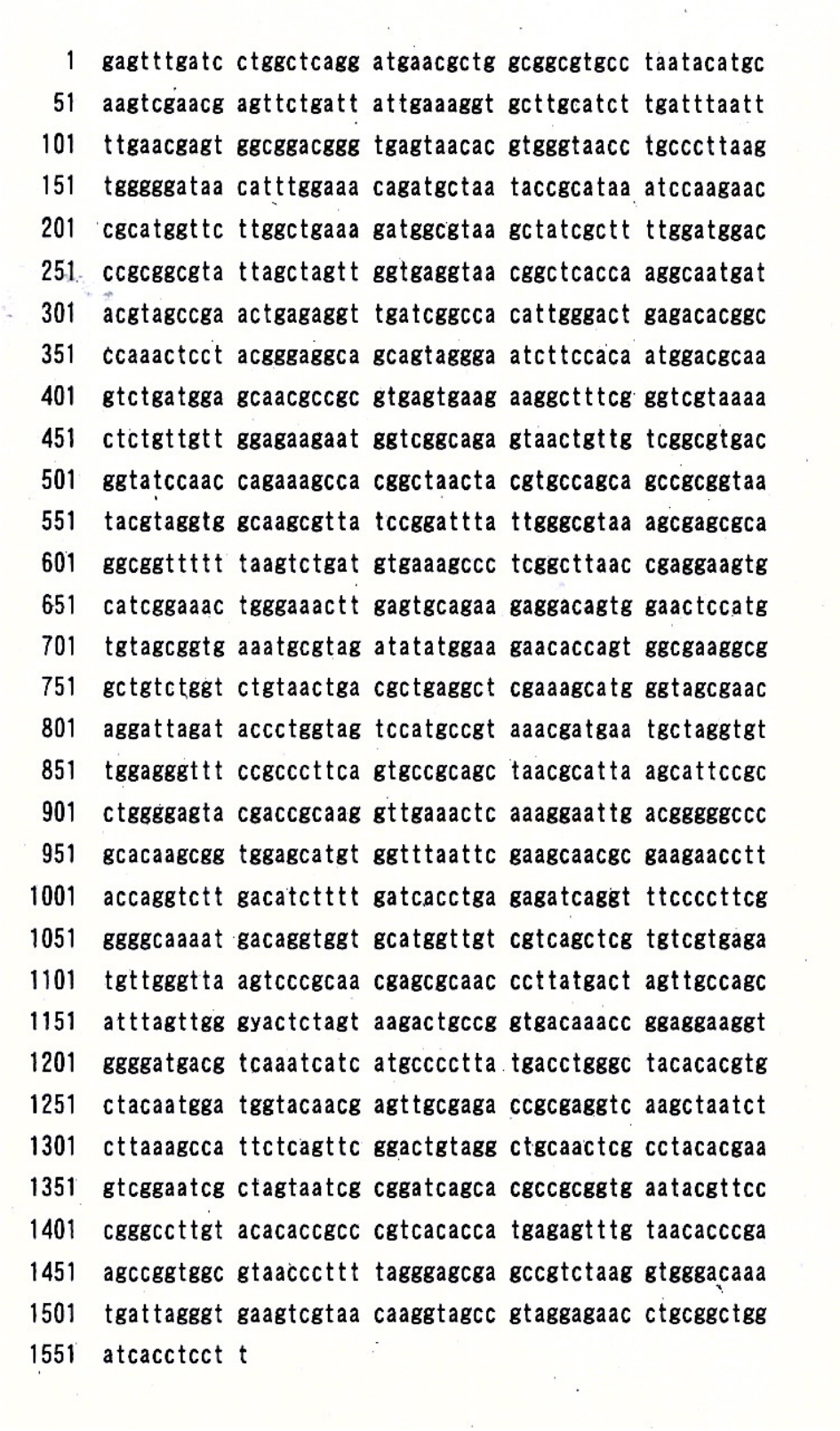
Table 8 (Continued)

	Code			
	DY	**DM3**	**CB1(R2)-bf**	**FCP2**
Salicine	+	+	+	+
Cellobiose	+	+	+	+
Maltose	+	+	+	+
Lactose	+	+	+	-
Melibiose	-	-	-	-
Saccharose	+	+	+	+
Trehalose	+	+	+	+
Inuline	-	-	-	+
Melezitose	-	+	+	+
D-Raffinose	-	-	-	-
Amidon	-	-	-	+
Glycogene	-	-	-	-
Xylitol	-	-	-	-
β Gentiobiose	+	+	+	+
D-Turanose	+	-	-	+
D-Lyxose	-	-	-	-
D-Tagatose	+	+	+	+
D-Fucose	-	-	-	-
L-Fucose	-	+	+	-
D-Arabitol	-	-	-	-
L-Arabitol	-	-	-	-
Gluconate	+	+	+	+
2 ceto-gluconate	-	-	-	-
5 ceto-gluconate	-	-	w	w

Table 9 Summarized characteristics of potential selected strains of LAB screened from various fermented sources with high stereospecificity and acid production.

Selected strain	**Source**	**Type**	**Lactic acid Stereopecificity**	**Lactic acid concentration* ($g\ l^{-1}$)**
DY	Drinking yoghurt	*Lactobacillus para parcasei*	L-type (89.9%)	16.9
FCP2	Cassava pulp	*Lactobacillus para parcasei*	L-type (91.2%)	13.7
DM3	Fermented pork	*Lactobacillus rhamnosus*	L-type (92.8%)	16.6
CB(R2)-bf	Cassava pulp	*Lactobacillus rhamnosus*	L-type (90.2%)	16.4

* Fermentation was carried out at pH 6 and $37^{O}C$ with initial glucose concentration of 20 $g\ l^{-1}$.



```
   1  gagtttgatc ctggctcagg atgaacgctg gcggcgtgcc taatacatgc
  51  aagtcgaacg agttctgatt attgaaaggt gcttgcatct tgatttaatt
 101  ttgaacgagt ggcggacggg tgagtaacac gtgggtaacc tgcccttaag
 151  tgggggataa catttggaaa cagatgctaa taccgcataa atccaagaac
 201  cgcatggttc ttggctgaaa gatggcgtaa gctatcgctt ttggatggac
 251  ccgcggcgta ttagctagtt ggtgaggtaa cggctcacca aggcaatgat
 301  acgtagccga actgagaggt tgatcggcca cattgggact gagacacggc
 351  ccaaactcct acgggaggca gcagtaggga atcttccaca atggacgcaa
 401  gtctgatgga gcaacgccgc gtgagtgaag aaggctttcg ggtcgtaaaa
 451  ctctgttgtt ggagaagaat ggtcggcaga gtaactgttg tcggcgtgac
 501  ggtatccaac cagaaagcca cggctaacta cgtgccagca gccgcggtaa
 551  tacgtaggtg gcaagcgtta tccggattta ttgggcgtaa agcgagcgca
 601  ggcggttttt taagtctgat gtgaaagccc tcggcttaac cgaggaagtg
 651  catcggaaac tgggaaactt gagtgcagaa gaggacagtg gaactccatg
 701  tgtagcggtg aaatgcgtag atatatggaa gaacaccagt ggcgaaggcg
 751  gctgtctggt ctgtaactga cgctgaggct cgaaagcatg ggtagcgaac
 801  aggattagat accctggtag tccatgccgt aaacgatgaa tgctaggtgt
 851  tggagggttt ccgcccttca gtgccgcagc taacgcatta agcattccgc
 901  ctggggagta cgaccgcaag gttgaaactc aaaggaattg acgggggccc
 951  gcacaagcgg tggagcatgt ggtttaattc gaagcaacgc gaagaacctt
1001  accaggtctt gacatctttt gatcacctga gagatcaggt ttcccccttcg
1051  ggggcaaaat gacaggtggt gcatggttgt cgtcagctcg tgtcgtgaga
1101  tgttgggtta agtcccgcaa cgagcgcaac ccttatgact agttgccagc
1151  atttagttgg gyactctagt aagactgccg gtgacaaacc ggaggaaggt
1201  ggggatgacg tcaaatcatc atgccccttа tgacctgggc tacacacgtg
1251  ctacaatgga tggtacaacg agttgcgaga ccgcgaggtc aagctaatct
1301  cttaaagcca ttctcagttc ggactgtagg ctgcaactcg cctacacgaa
1351  gtcggaatcg ctagtaatcg cggatcagca cgccgcggtg aatacgttcc
1401  cgggccttgt acacaccgcc cgtcacacca tgagagtttg taacacccga
1451  agccggtggc gtaaccctтt tagggagcga gccgtctaag gtgggacaaa
1501  tgattagggt gaagtcgtaa caaggtagcc gtaggagaac ctgcggctgg
1551  atcacctcct t
```

Figure 13 Sequence of 16S rDNA of bacterial strain DM3 (SIID2549-02)

Sequences producing significant alignments:	Score (bits)	E Value
gi|27464420|gb|AF526388.1| Lactobacillus casei 16S ribosoma...	3076	0.00
gi|34305690|gb|AY363376.1| Lactobacillus sp. rennanqilfy10 ...	3076	0.00
gi|20562986|gb|AY094065.1| Lactobacillus sp. IDLAc 16S ribo...	2993	0.00
gi|1848056|dbj|D16552.1| Lactobacillus casei gene for 16S r...	2969	0.00
gi|34493781|gb|AY369076.1| Lactobacillus paracasei subsp. p...	2965	0.00
gi|7621503|gb|AF243146.1| Lactobacillus rhamnosus strain F1...	2957	0.00
gi|31323375|gb|AY299488.1| Lactobacillus rhamnosus strain M...	2931	0.00
gi|31323373|gb|AY299486.1| Lactobacillus rhamnosus strain M...	2931	0.00
gi|1843426|dbj|D86516.1| Lactobacillus zeae gene for 16S ri...	2907	0.00
gi|1808583|dbj|D79212.1|LBA16SRRNA Lactobacillus paracasei ...	2880	0.00
gi|1843427|dbj|D86517.1| Lactobacillus casei gene for 16S r...	2880	0.00
gi|1843428|dbj|D86518.1| Lactobacillus casei gene for 16S r...	2864	0.00
gi|6996495|emb|AJ272201.1|LCA272201 Lactobacillus casei sub...	2858	0.00
gi|14583099|gb|AF385770.1| Lactobacillus casei strain BL23 ...	2850	0.00
gi|175023|gb|M58815.1|LBARR16SN Lactobacillus casei 16S rib...	2850	0.00
gi|1848055|dbj|D16551.1| Lactobacillus casei gene for 16S r...	2850	0.00
gi|1848052|dbj|D16548.1| Lactobacillus casei gene for 16S r...	2848	0.00
gi|28194135|gb|AF469172.1| Lactobacillus casei strain ATCC ...	2846	0.00
gi|20257220|gb|AY091596.1| Lactobacillus casei strain KH-1 ...	2842	0.00
gi|30519702|emb|AJ558112.1|LCA558112 Lactobacillus casei 16...	2840	0.00

Figure 14 Alignment of 16S rDNA gene sequence of the strain DM3 (SIID2549-02) with the sequence of other *Lactobacillus* sp. using BLAST.

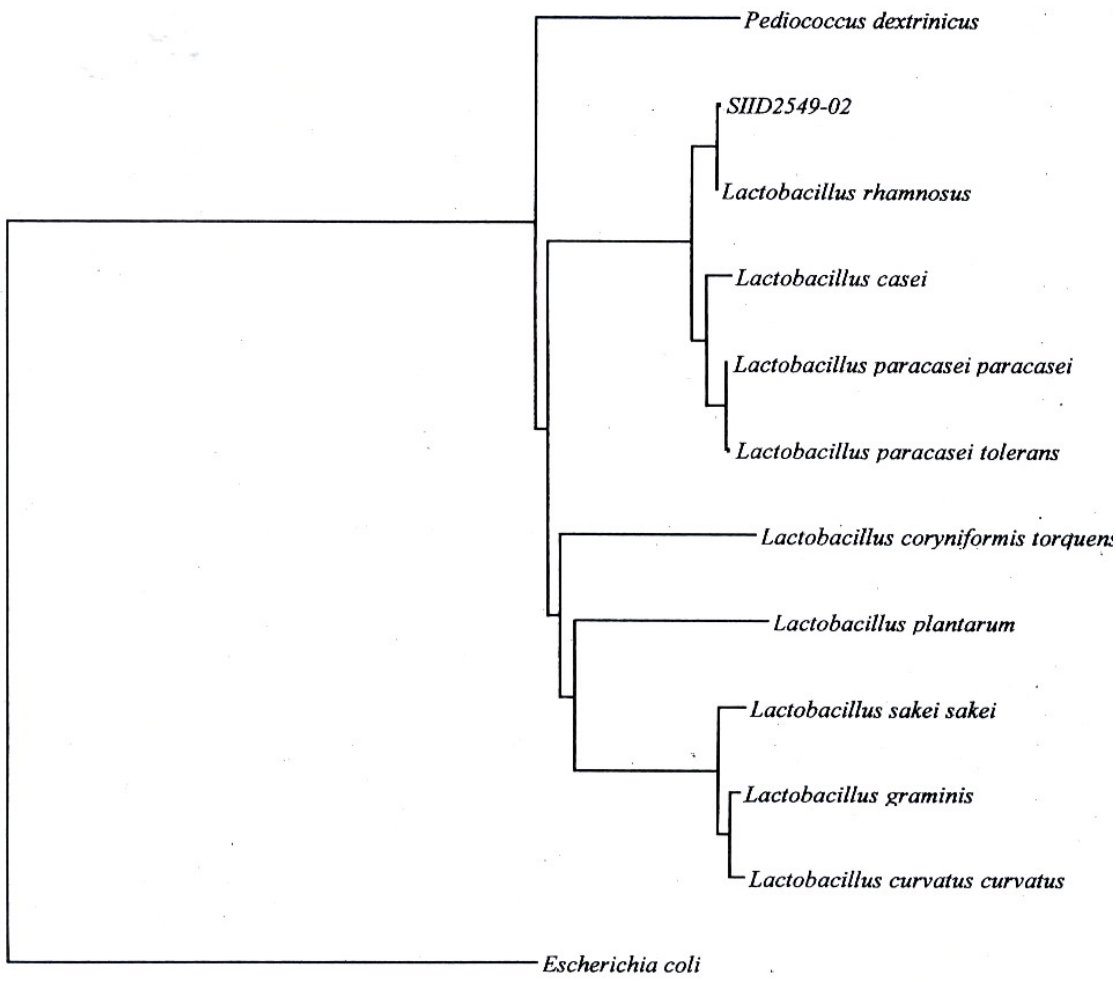


Figure 15 Phylogenetic tree showing the relationship of bacterial strain DM3 (SIID2549-02) with 16S rDNA of *Lactobacillus rhamnosus.*

Part 2: Optimal fermentation conditions and application of selected LAB strain in producing lactic acid from cassava starch

The efficiency of lactic acid production by microbial fermentation method can be influenced by many factors. The important ones include pH, temperature and the media nutrients. In this section, DM3 was used to produce lactic acid under various conditions.

1. Optimal fermentation conditions of selected strain

1.1 pH

In order to study the effect of pH, lactic acid fermentation of the strain DM3 was carried out at 37^{O}C with the continuous agitation at 150 rpm. Figure 16 demonstrates changes in parameters during fermentation without and with controlled pH (at 5, 6 and 7). Without the pH-control system, the strain DM3 could grow upon 8 h after inoculation whereas the pH continually decreased, proportionally to produced lactic acid concentrations. When the pH was dropped to 3, the cell growth was not further increased (the highest cell density and cell counts were 3.98 and 3.5 x 10^{9} CFU/ml, respectively).

In addition, the accumulation of free lactic acid, which produced a proportional decrease in pH, also inhibits an inhibition of glucose consumption and lactic acid production. The optimum pH range for lactic acid production by the stain DM3 was 5 to 7 of which pH 6 gave the shortest time of microbial lag phase and the glucose was completely consumed within 10 h, as compared to 12 h for fermentation at pH 5 and 7, resulting in the highest productivity (2.90 g $l^{-1}h^{-1}$) (Table 10). Therefore, fermentation at pH 6 was considered as the best condition for this strain.

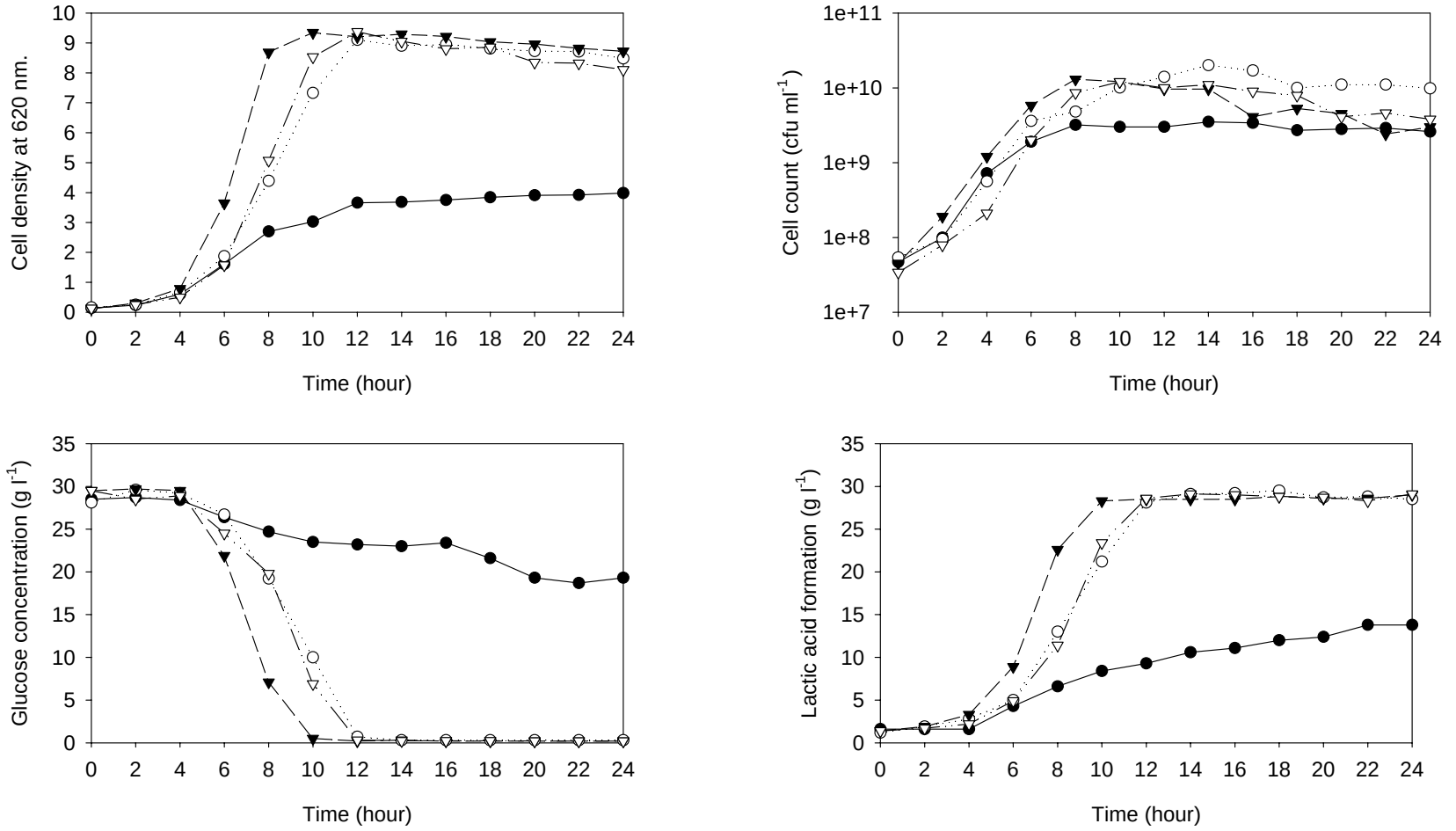


Figure 16 Effect of pH on cell growth and lactic acid production during fermentation of DM3, at glucose concentration 30 g l^{-1}, agitation speed of 150 rpm and temperature of 37^{O}C, (●) no control pH, (○) pH 5.0, (▼) pH 6.0, (▽) pH 7.0.

Table 10 Lactic acid fermentation of DM3, *Lactobacillus rhamnosus* screened from Thai fermented pork, at various pH.

Parameter	No control	pH 5	pH 6	pH 7
Cell density at 620 nm.	3.98	9.10	9.34	9.37
Glucose residual (g l^{-1})	19.3	0.3	0.2	0.2
Glucose consumption (g l^{-1})	10.7	30	30	30
Glucose consumption (g)	26.8	75	75	75
Substrate utilization (%)	64.3	100	100	100
Total lactic acid formation (g l^{-1})	13.8	29.5	29.0	29.1
Total lactic acid formation (g)	33.1	73.8	72.5	69.8
$\%Yield_{P/S}$	123.5	98.4	96.7	93.1
Fermentation time (h)	24	12	10	12
Productivity (g $l^{-1}h^{-1}$)	0.58	2.46	2.90	2.43

* The lactic acid production was performed at glucose concentration 30 g l^{-1}, agitation speed of 150 rpm and temperature of $37^{O}C$.

1.2 Temperature

Figure 17 and Table 11 shows the effect of temperature at 30, 37, 40 and 45^{O}C on lactic acid fermentation by the strain DM3, when the pH was regulated constantly at 6. The optimal temperature was in the range of 30 to 45^{O}C. No significant difference in lactic acid concentrations and yields was observed, indicating a good adaptability of this microbial strain to a wide temperature range. At 40^{O}C, the lag phase of cell growth was the shortest (4 h) and the glucose consumption was the fastest when compared to 30 and 37^{O}C. The fermentation periods were 12, 10 and 8 h for 30, 37 and 40^{O}C, respectively. As a result, the fermentation temperature at 40^{O}C provided the highest productivity and yield (3.73 g $l^{-1}h^{-1}$ and 97.3%). One property for industrially useful LAB is the tolerance to high temperature as these external conditions themselves would be useful to minimize contamination of the culture by other microorganisms (Mohd Adnan and Tan, 2007). For that reason, fermentation at 40^{O}C was the best temperature for lactic acid production of this strain.

Table 11 Lactic acid fermentation of DM3, *Lactobacillus rhamnosus* screened from Thai fermented pork, at various pH.

Parameter	**30^{O}C**	**37^{O}C**	**40^{O}C**	**45^{O}C**
Cell density at 620 nm.	10.81	9.34	10.79	7.51
Glucose residual (g l^{-1})	0.2	0.2	0.2	0.2
Glucose consumption (g l^{-1})	30	30	30	30
Glucose consumption (g)	75	75	75	75
Substrate utilization (%)	100	100	100	100
Total lactic acid formation (g l^{-1})	29.6	29.0	29.8	29.1
Total lactic acid formation (g)	71.0	72.5	73.0	72.8
%$Yield_{P/S}$	94.7	96.7	97.3	97.1
Fermentation time (h)	12	10	8	12
Productivity (g $l^{-1}h^{-1}$)	2.47	2.90	3.73	2.43

* The lactic acid production was performed at glucose concentration 30 g l^{-1}, pH 6 and agitation speed of 150 rpm.

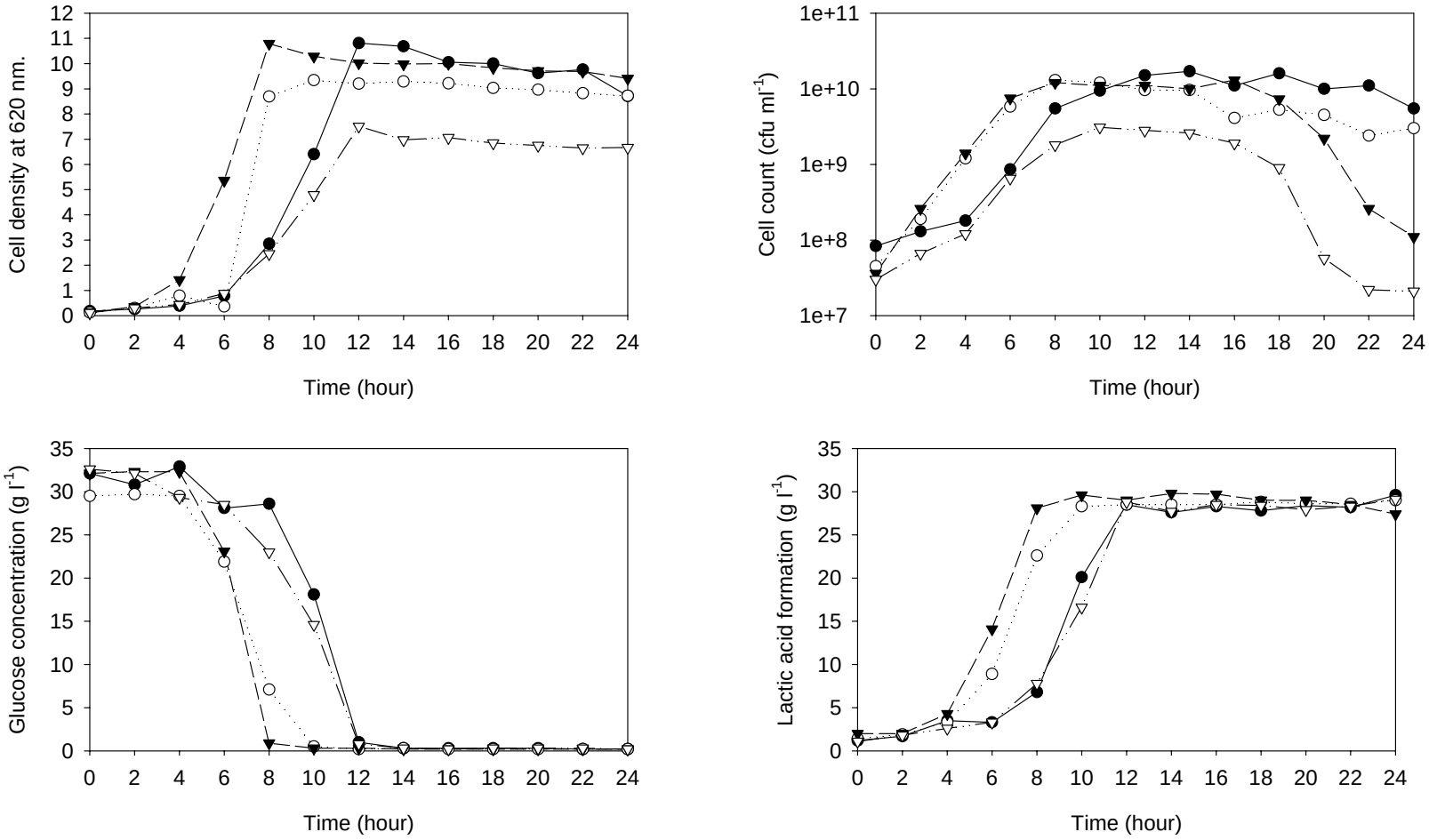


Figure 17 Effect of various temperatures on cell growth and lactic acid production during fermentation of DM3, at glucose concentration 30 g l^{-1}, pH 6 and agitation speed of 150 rpm, (●) 30^{O}C, (○) 37^{O}C, (▼) 40^{O}C and (▽) 45^{O}C.

1.3 Agitation speed

The consequence of agitation speed on lactic acid fermentation efficiency was carried out at a controlled temperature and pH, i.e. 40^{O}C and pH 6. When the agitation speed of 150 rpm was employed, the highest lactic acid concentration, yield and productivity were achieved (29.8 g l^{-1}, 97.3% of yield and 3.73 g $l^{-1}h^{-1}$, respectively; Figure 18 and Table 12). It could be somehow related to the amount of dissolved oxygen due to stirring of the media.

Table 12 Lactic acid fermentation of DM3, *Lactobacillus rhamnosus* screened from Thai fermented pork, at various agitation speed.

Parameter	**100 rpm**	**150 rpm**	**200 rpm**
Cell density at 620 nm.	10.8	10.79	11.22
Glucose residual (g l^{-1})	0.4	0.2	0.4
Glucose consumption (g l^{-1})	30	30	30
Glucose consumption (g)	75	75	75
Substrate utilization (%)	100	100	100
Total lactic acid formation (g l^{-1})	28.7	29.8	29.0
Total lactic acid formation (g)	71.8	73.0	69.6
$\%Yield_{P/S}$	95.7	97.3	92.8
Fermentation time (h)	10	8	10
Productivity (g $l^{-1}h^{-1}$)	2.87	3.73	2.90

* The lactic acid production was performed at glucose concentration 30 g l^{-1}, pH 6 and temperature of 40^{O}C.

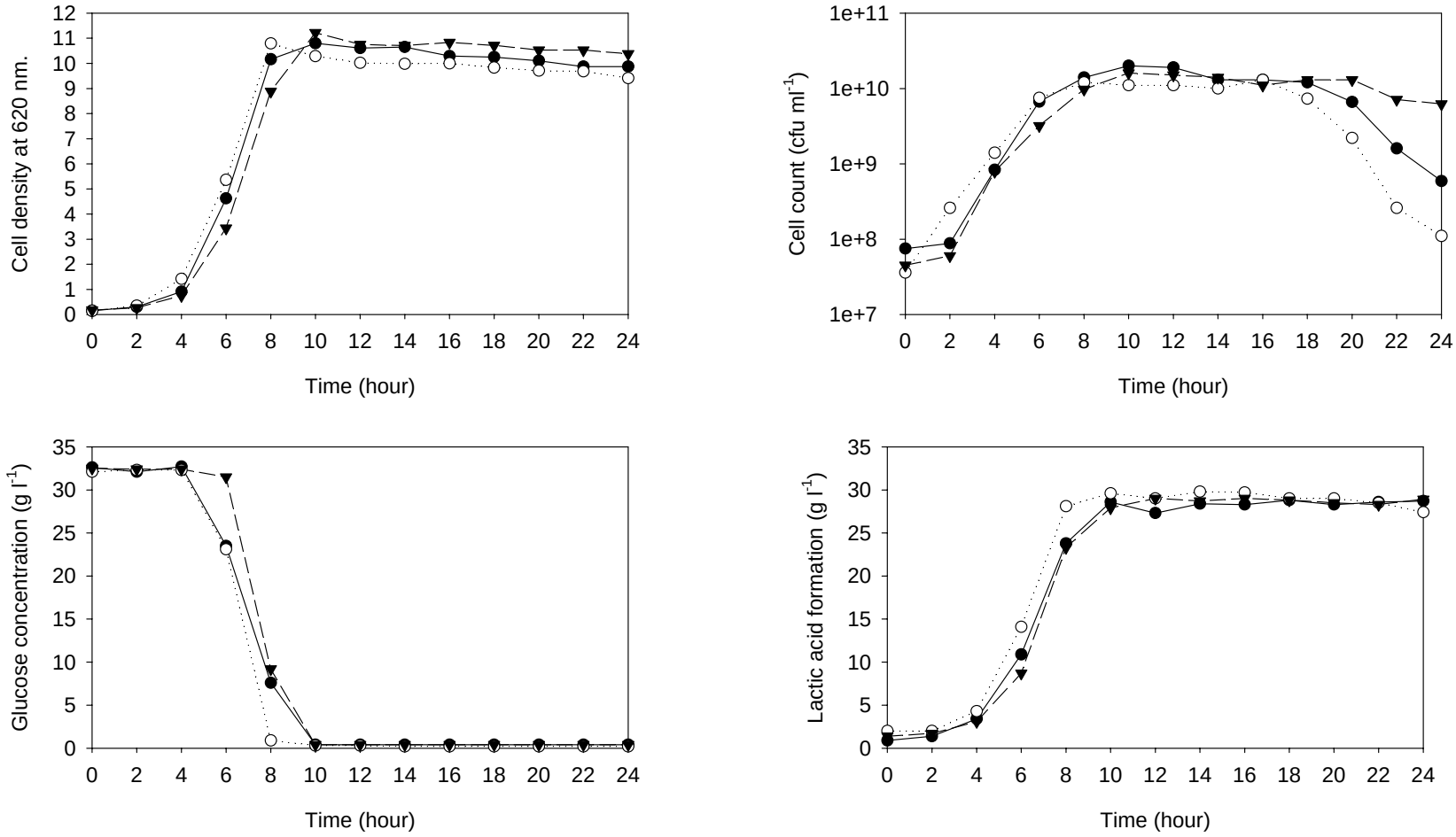


Figure 18 Effect of various agitation speed on cell growth and lactic acid production during fermentation of DM3, at glucose concentration 30 g l^{-1}, pH 6 and temperature of 40^{O}C, (●) 100 rpm, (○) 150 rpm and (▼) 200 rpm.

Different LAB strains differed in their requirement for growth conditions. For the strain DM3, the maximum lactic acid concentrations could be achieved when fermentation was carried out at pH 6.0, temperature of 40^{O}C and agitation speed of 150 rpm, which was in accordance with a previous report (Hofvendahl and Hahn-Hagerdal, 2000), suggesting the optimal condition for lactic acid at pH 5-6.8, temperature 30-45^{O}C with continuously agitating of 100-200 rpm. Among these three factors, it was obvious that pH of the medium had the greatest effect on lactic acid production. Kumar Dutta *et al.* (1996) studied on lactic acid production from *L. delbrueckii* at various pH (5.0-6.0) and reported that pH 5.3 gave the best result. Goncalves *et al*. (1997) reported that a mechanism of lactic acid inhibition on microbial growth and cell metabolism, which related to lactic acid production, was a complex process. The inhibition mechanism could not be simply described exclusively as an effect of lactic acid/lactate equilibrium, nor could it be generalized for a wide rang of extra-cellular pH values. Nevertheless, their studies suggested that the growth inhibition by lactic acid was a function of pH. At high pH, the intracellular dissociated or ionized lactic acid concentration was the inhibitory species whereas, at low pH, the pH effect on bacteria cell growth was, in part, due to the concentrations of undissociated forms of lactic acid in the broth. Some researchers have demonstrated that both dissociated and undissociated lactic acids have inhibitory effects with the undissociated organic acids being more inhibiting than the dissociated one. The use of an organism having an optimum temperature above 40^{O}C considerably reduces the cooling costs of a large-scale fermentation. Moreover, Wouters *et al.* (2000) noted the reduced glycolytic activity, leading to reduced production of lactic acid in *L. lactis* at low temperature. The ability to grow at a high temperature is a desirable trait as it can translate to increase the rate of growth and lactic acid production. At the same time, a high fermentation temperature reduces the risk of contamination by other microorganisms.

1.4 Influence of initial substrate concentration

The effect of the initial substrate concentration was carried out by varying the initial glucose concentrations at 30, 50, 100, 150 and 200 g l^{-1}. At a low glucose

concentration, the lag phase of microbial cell was shortest and the cell growth was higher, when compared to high glucose concentrations (150 and 200 g l^{-1}) (Figure 19). The maximum cell counts were obtained at different fermentation periods; $1.3X10^{10}$ CFU/ml at 16 h, $1.5X10^{10}$ CFU/ml at 12 h, $1.5X10^{10}$ CFU/ml at 12 h, $9.2X10^{9}$ CFU/ml at 22 h and $5.8X10^{9}$ CFU/ml at 20 h for the trials with the initial glucose concentration of 30, 50, 100, 150 and 200 g l^{-1}, respectively. Furthermore, at the lowest glucose concentration of 30 g l^{-1}, microbial cell counts started to decline after 16 h, presumably due to the lack of major nutrient, i.e. glucose.

When the initial glucose concentration increased (30-200 g l^{-1}), the amount of lactic acid produced also increased, except at 200 g l^{-1} (Table 13). In addition, the glucose consumption during fermentation by DM3 was completed at different time intervals, regarding to the initial glucose concentration. The glucose was completely consumed faster at a low initial substrate concentration (the completion periods were 8, 12, 18, and 36 h for the initial glucose concentration of 30, 50, 100 and 150 g l^{-1}, respectively). At a very high glucose concentration of 200 g l^{-1}, prolonged fermentation time (greater than 120 h) still did not allow a completion of glucose consumption, implying a low production yield (79.2 %). The optimal initial glucose concentration for lactic acid production by DM3 was 150 g l^{-1}, concerning the lactic acid concentration in the final medium (130.8 g l^{-1}) which was beneficial to subsequent process of purification. However, the highest productivity of lactic acid was achieved when glucose concentration was 100 g l^{-1} (4.79 g $l^{-1}h^{-1}$) (Table 13).

The results of this work clearly demonstrate that the high initial substrate concentration could have profound effects on cell growths. Similar results about substrate inhibition in lactic acid fermentation were also reported by Akerberg *et al.* (1998) for *Lc. lactis* ssp. *lactis* on glucose, Wee *et al.* (2006b) for *Lactobacillus* sp. RKY2 on glucose, Mehaia and Cheryan (1987) for *L. bulgaricus* on lactose, Monteagudo *et al.* (1994) and Kotzamanidis *et al.* (2002) for *L. delbrueckii* on sucrose. Bibal *et al.* (1988) and Giraud *et al.* (1991) showed at an initial glucose concentration of 100 g l^{-1} the cell growth rate decreased by 37% compared with fermentation without substrate inhibition. Lactic acid concentrations above 70 g l^{-1} and 110 g l^{-1}

inhibited the growth of *Streptococcus cremoris* and *L. plantarum.* Evidently, Goncalves *et al.* (1991) reported the kinetics of substrate inhibition on lactic acid production by using glucose concentrations in the range of 50 – 340 g l^{-1}. A maximum of 140 g lactic acid l^{-1} was reached when 200 g l^{-1} of glucose was used, resulting in a 70% yield. Gao *et al.* (2005) reported the total productivity at glucose concentration of 200 g l^{-1} was about four times lower than that at glucose concentration of 100 g l^{-1} which the production rates of lactic acid at the two glucose concentrations were similar within 40 h, but the production rate of lactic acid at 200 g l^{-1} became slow after 50 h. This result support that the initial substrate concentration may affect the lag phase of cell growth but substrate inhibitory effects in lactic acid fermentation may be negligible. Maybe, the decrease in productivity at high initial glucose concentrations could partly be attributed to be a result of increased accumulation of lactate.

Table 13 Lactic acid fermentation of DM3, *Lactobacillus rhamnosus* screened from Thai fermented pork, at various initial substrate concentration.

Parameter	**30** g l^{-1}	**50** g l^{-1}	**100** g l^{-1}	**150** g l^{-1}	**200** g l^{-1}
Cell density at 620 nm.	10.79	13.97	14.54	14.70	12.94
Glucose residual(g l^{-1})	0.2	0.4	0.4	3.3	25.4
Glucose consumption(g l^{-1})	30	50	100	146.7	175
Glucose consumption(g)	75	125	250	366.8	437.5
Substrate utilization(%)	100	100	100	97.8	87.5
Total lactic acid formation(g l^{-1})	29.8	46.6	86.2	130.8	130.8
Total lactic acid formation(g)	73.0	111.8	224.1	340.0	346.6
%$Yield_{P/S}$	97.3	89.4	89.6	92.7	79.2
Fermentation time (h)	8	12	18	36	120
Productivity(g $l^{-1}h^{-1}$)	3.73	3.88	4.79	3.63	1.09

* The lactic acid production was performed at pH 6, temperature of 40^{O}C and agitation speed of 150 rpm.

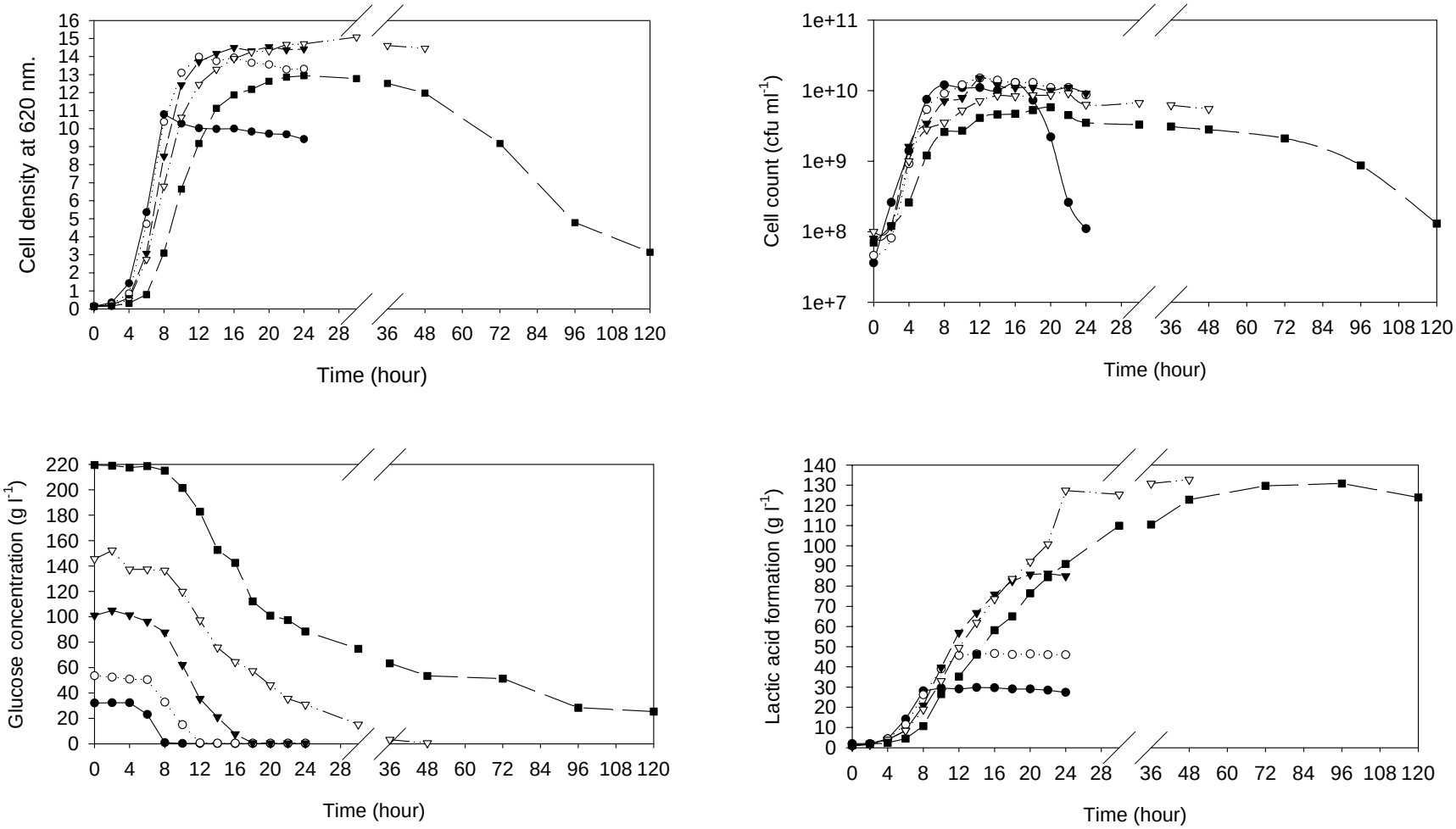


Figure 19 Effect of various initial substrate concentration on cell growth and lactic acid production during fermentation of DM3, at pH 6, temperature of 40^{O}C and agitation speed of 150 rpm, (●) 30 g l^{-1}, (○) 50 g l^{-1}, (▼) 100 g l^{-1}, (▽) 150 g l^{-1} and (■) 200 g l^{-1}

1.5 Influence of feeding substrate concentration of fed-batch mode

For fed-batch culture, the initial substrate concentration at 30 g l^{-1} was the best condition because of the incubation time was rapidly completed. The potential of fed-batch culture was investigated by feeding glucose at 100 g l^{-1}, 100 g l^{-1} with additional yeast extract, 150 g l^{-1} and 200 g l^{-1} after inoculation at 8 h. The results are shown in Figure 20.

Cell density and cell count gave similar result, after inoculation it started to grow and increased to its maximum at 10 h. For the case of feeding 200 g l^{-1}, after 72 h, it started to decline. The similar patterns of glucose consumption for each experiment were observed between 0 to 8 h. The initial glucose concentration at the time of inoculation was approximately 30-32 g l^{-1} and rapidly decreased close to zero at 8 h and increased once more after feeding. The feeding of 200 g l^{-1} had effect on glucose consumption as it used longer time and still had glucose residuals. The lactic acid formation, at 0 to 24 h of all experiments gave similar results. They were increased rapidly during the 24 h until lacking glucose. The feeding of 200 g l^{-1} gave the highest lactic acid concentration only 128.4 g l^{-1} even though more glucose residuals were available. From Table 14, the results suggested that the glucose concentration at feeding of 100 g l^{-1} without adding more yeast extract was the best process for lactic acid production, because of the highest yield, productivity and no glucose residual, without more nutrient, i.e. nitrogen required.

Since, batch culture fermentation is limited to relatively low productivity due to high initial substrate inhibition; a lot of efforts have gone into developing fed-batch culture. Fed-batch fermentation is widely used for the production of microbial biomass, ethanol, organic acid, antibiotics, vitamins, enzyme and other compounds (Borzani *et al*., 1993). Some works have been done with lactic acid fermentation in fed-batch culture (Roukas and Kotzekidou, 1998; Chen and Lee, 1997). Bai *et al.* (2003) reported the comparison of batch culture and fed-batch culture under the optimum feeding rate for lactic acid production when the total glucose concentration was the same (217 g l^{-1}). In the case of fed-batch culture, although the final L-lactic

acid concentration (210 g l^{-1}), yield (97%) and residual glucose (< 0.5 g l^{-1}) were almost the same to those in batch culture, the cell growth (2.7 g l^{-1}) was 64% higher than that in batch process and the L-lactic acid productivity (2.2 g $l^{-1}h^{-1}$) was 82% higher than the batch productivity. In contrast, in this study the fed-batch culture of *L. rhamnosus* DM3 was not experimentally to be a better system for lactic acid production. Feeding of 100 g l^{-1} gave higher lactic acid concentration than batch culture but longer fermentation time so productivity was much lower. Besides, feeding at 150 g l^{-1} and 200 g l^{-1} gave similar results as batch culture.

Table 14 Lactic acid fermentation of DM3, *Lactobacillus rhamnosus* screened from Thai fermented pork, with the influence of feeding substrate concentration of fed-batch mode (initial substrate concentration : 30 g l^{-1})

Parameter	**Feeding 100** g l^{-1}	**Feeding 100** g l^{-1} + **Yeast extract**	**Feeding 150** g l^{-1}	**Feeding 200** g l^{-1}
Cell density at 620 nm.	16.52	15.27	14.61	14.22
Glucose residual(g l^{-1})	0.3	0.5	10.2	63.5
Glucose consumption(g l^{-1})	130	130	170	166
Glucose consumption(g)	307	307	425	415
Substrate utilization(%)	100	100	98.4	74.5
Total lactic acid formation(g l^{-1})	118.8	110.4	130.8	128.4
Total lactic acid formation(g)	308.9	287.0	340.1	333.8
%$Yield_{P/S}$	100.6	93.5	80.0	80.4
Fermentation time (h)	30	30	48	120
Productivity(g $l^{-1}h^{-1}$)	3.96	3.68	2.73	1.07

* The lactic acid production was performed at pH 6, temperature of $40^{O}C$ and agitation speed of 150 rpm.

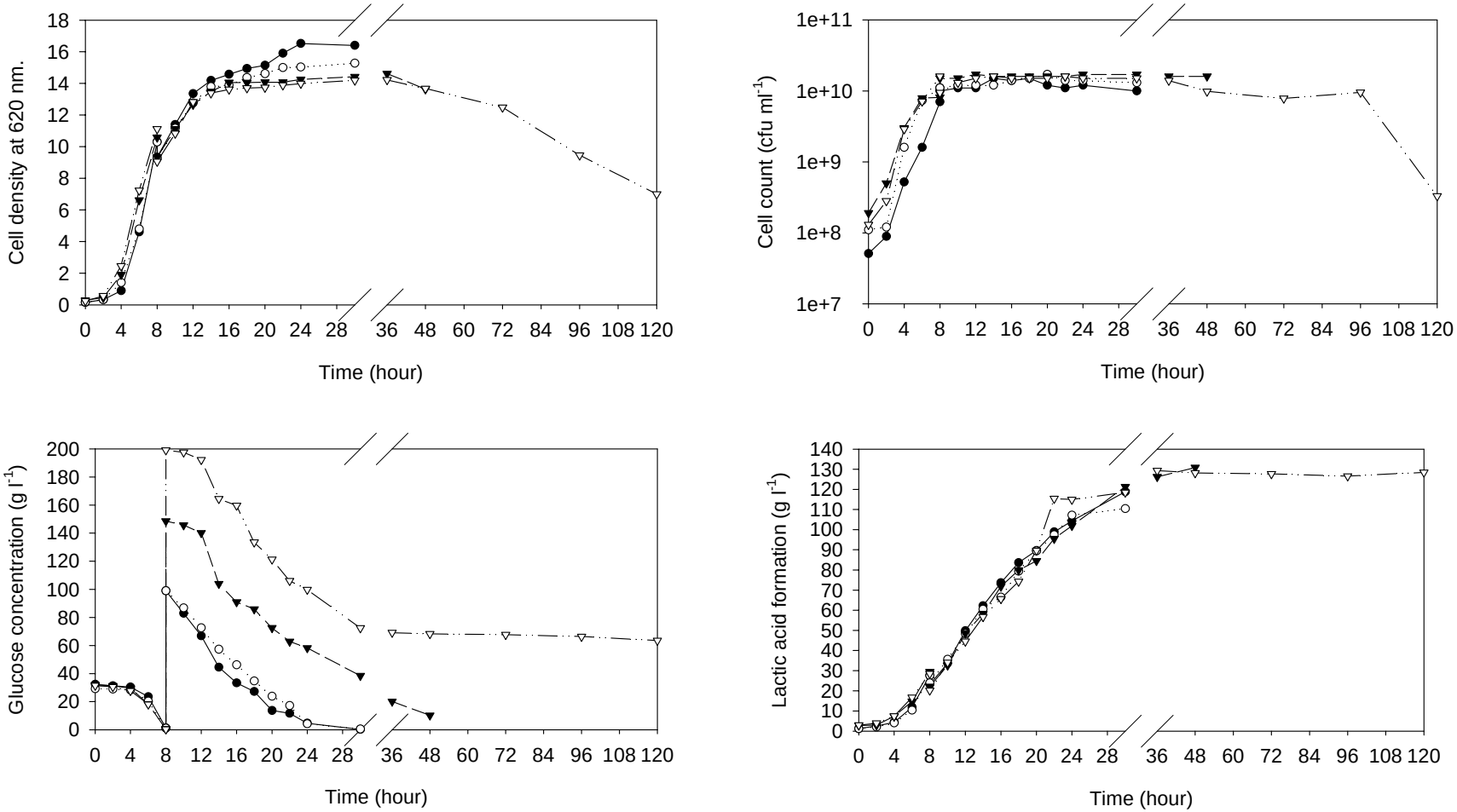


Figure 20 Fed-batch fermentation during lactic acid production by DM3, at pH 6, temperature of 40^{O}C and agitation speed of 150 rpm, (●) feeding 100 g l^{-1}, (○) feeding 100 g l^{-1} with additional yeast extract, (▼) feeding 150 g l^{-1} and (▽) feeding 200 g l^{-1}.

2. Application of selected LAB strain in producing lactic acid from cassava starch by Simple Saccharification (SS) and Simultaneous Saccharification and fermentation (SSF) process

A potential use of the strain DM 3 in lactic acid production for industrial uses was demonstrated by using cassava starch which is a promising feedstock in fermentation industry. Cassava starch, one of the cheapest carbon sources, currently has been gained a lot of industrial interest since it can lower overall production cost in fermentation process. Typically, the raw material cost is very significant, accounting up to 30-40 % of total production costs (Nolasco-Hipolito *et al.,* 2002; Akerberg and Zacchi, 2000). Thailand is the world's largest exporter of cassava starch and starch derivatives, with the annual production of over 2 million tons of starch. The starch, in general, possesses 13 % moisture, 85 % starch and a few amount of ash (0.2 %). The starch is very pure and can be readily converted to sugar, mainly glucose by a sequential hydrolysis of α-amylase and glucoamylase. The glucose syrup is then used for microbial fermentation. Recently, the process called SSF or Simultaneous Saccharification and Fermentation is developed as it is much more economically practical, not only in terms of the total process time reduction, but also a decrease in a reactor volume. Only one vessel is needed, and only one temperature and pH has to be adjusted. However, the disadvantage associated with SSF process is the different cultivation condition, important ones are the temperature and pH which, in simple or conventional process, must be separately adjusted to the optimum for each step of saccharification and fermentation.

In this work, a comparison on lactic acid production of the strain DM3 using cassava starch hydrolysates between SS and SSF process was investigated at the initial substrate concentration of 100 g starch l^{-1}. In SS process, a high yield (97 %) of lactic acid was obtained in 16 h, exhibiting a high productivity (6.08 g $l^{-1}h^{-1}$). Yet the productivity decreased if the saccharification time was also considered (productivity = 4.05 g $l^{-1}h^{-1}$). In SSF process, no separate process of saccharification was employed. The liquefied syrup was directly subjected to saccharification by glucoamylase simultaneously with fermentation by LAB. A slight modification in saccharification

process, i.e. an increase of enzyme concentration was carried out to optimize and facilitate the microbial growth in SSF process (Table 15). As a result, SSF process could provide comparable fermentation efficiency as indicated by % yield (97 and 92 % for SS and SSF process, respectively) (Table 16). A slight reduction of lactic acid productivity by SSF process was evident (5.43 g $l^{-1}h^{-1}$), but if the saccharification time in SS process was concerned (productivity = 4.05 g $l^{-1}h^{-1}$), a productivity of lactic acid could be much improved by SSF process.

The effect of the initial starch concentration in SSF process was further carried out using the initial liquefied starch concentrations of 100, 150 and 200 g sugar equivalent l^{-1}. The glucose amount was negligible at the initial time of SSF process (< 2% of theoretical total sugar content). The amounts of glucose at the time of inoculation were approximately 6.8 g l^{-1} and increased to their maximum of 59.7 g l^{-1} at 8 h, 87.5 g l^{-1} at 6 h and 136.7 g l^{-1} at 6 h for the initial liquefied starch concentration at 100, 150 and 200 g sugar equivalent l^{-1}, respectively. After that, the glucose concentration slowly declined (Figure 21). Similar to Anuradha *et al.* (1999), the experimental results indicated that there was an initial phase, where the saccharification rate, i.e. glucose production, was greater than the fermentation rate, i.e. glucose consumption, wherein the glucose was accumulated. Therefore, a lag period in the fermentation was introduced in the simulation. This might be due to the effect of high starch concentrations in the broth. During this period, the cells did not grow, but used glucose for cell maintenance only. Furthermore, initially glucose in the broth was accumulated as the fermentation was the rate limiting step. Once the cells adjusted to the medium and started to grow, the saccharification became the rate limiting step and the glucose concentration decreased. The glucose concentration gradually decreased to zero where the saccharification rate was equivalent to the fermentation rate. At this stage, the cells were in the stationary phase and produced lactic acid mainly through maintenance process. With this SSF process of starch, the strain DM3 could overcome the limitation of high initial substrate concentrations which had profoundly affected cell growths, as evidenced by the higher cell growths, when compared to fermentation of glucose (> 10 folds). SSF, therefore, has an advantage that the cells are capable to be subjected to the environment with a high

substrate concentration, but less glucose accumulation, leading to a lower cell growth inhibition. This benefit was more pronounced when a high substrate concentration, i.e. 200 g l^{-1} was used (the maximum lactic acid concentrations was 166.2 and 130.8 g l^{-1} when SSF and simple fermentation of derived glucose to lactic acid process were compared, respectively which were approximately 1.27-folds greater). However, the suitable of initial starch concentration in SSF process of this strain was 150 g liquefied starch l^{-1} because this concentration gave the best result of productivity (5.70 g $l^{-1}h^{-1}$). Also it gave a better result on lactic acid concentration than at 100 g liquefied starch l^{-1} and less glucose residual than at 200 g liquefied starch l^{-1}(Table 15).

The lactic acid production was operated successfully when commercial cassava starch was used. To decide the most practical process for industrial purposes, the optimal fermentation conditions (i.e. high percentage of yield and/or high lactic acid concentration and/or high productivity) and are not always the most preferred from an economical point of view. In fact, the cost of substrate and downstream processing are proportionally high and must be also taken into consideration. The substrate is usually an issue of geographic availability. If the substrate is expensive, the yield should be maximized, whereas the productivity is maximized if investment costs are high. This work had explored the potential use of selected LAB strain and fermentation condition on a cheap resource like cassava starch that can be used further to develop lactic acid production industrially.

Table 15 Some parameters during lactic acid fermentation by a selected strain DM 3 (*L. rhamnosus*) screened from Thai fermented pork using cassava starch as a substrate by Simple Saccharification (SS) and Simultaneous Saccharification and Fermentation (SSF) process at various substrate concentrations**.**

Parameter	**SS**	**SSF**	**SSF (150 g l^{-1})**		**SSF**
	(100 g l^{-1})	**(100 g l^{-1})**	**0.1 %****	**0.5 %****	**(200 g l^{-1})**
Cell density at 620 nm	19.73	18.69	21.82	21.77	23.62
Glucose residual (g l^{-1})	0.5	0.8	8.2	2.0	10.5
Starch consumption (g l^{-1})	100	100	141.8	148	189.5
Starch consumption(g)	250	250	355.7	370	473.8
Substrate utilization (%)	100	100	94.9	98.7	94.8
Total lactic acid formation (g l^{-1})	97.2	97.7	134.4	136.8	166.2
Total lactic acid formation (g)	243.0	230.0	315.8	342.0	390.6
$\%Yield_{P/S}$	97	92	88.8	92	82
Fermentation time (h)	16	18	48	24	120
Productivity(g $l^{-1}h^{-1}$)	6.08 (4.05*)	5.43	2.80	5.70	1.39

*Using a total time of fermentation and Saccharification (24 h).

**Glucoamylase concentration (% v/w)

The lactic acid production was performed at pH 6, temperature of $40^{O}C$ and agitation speed of 150 rpm.

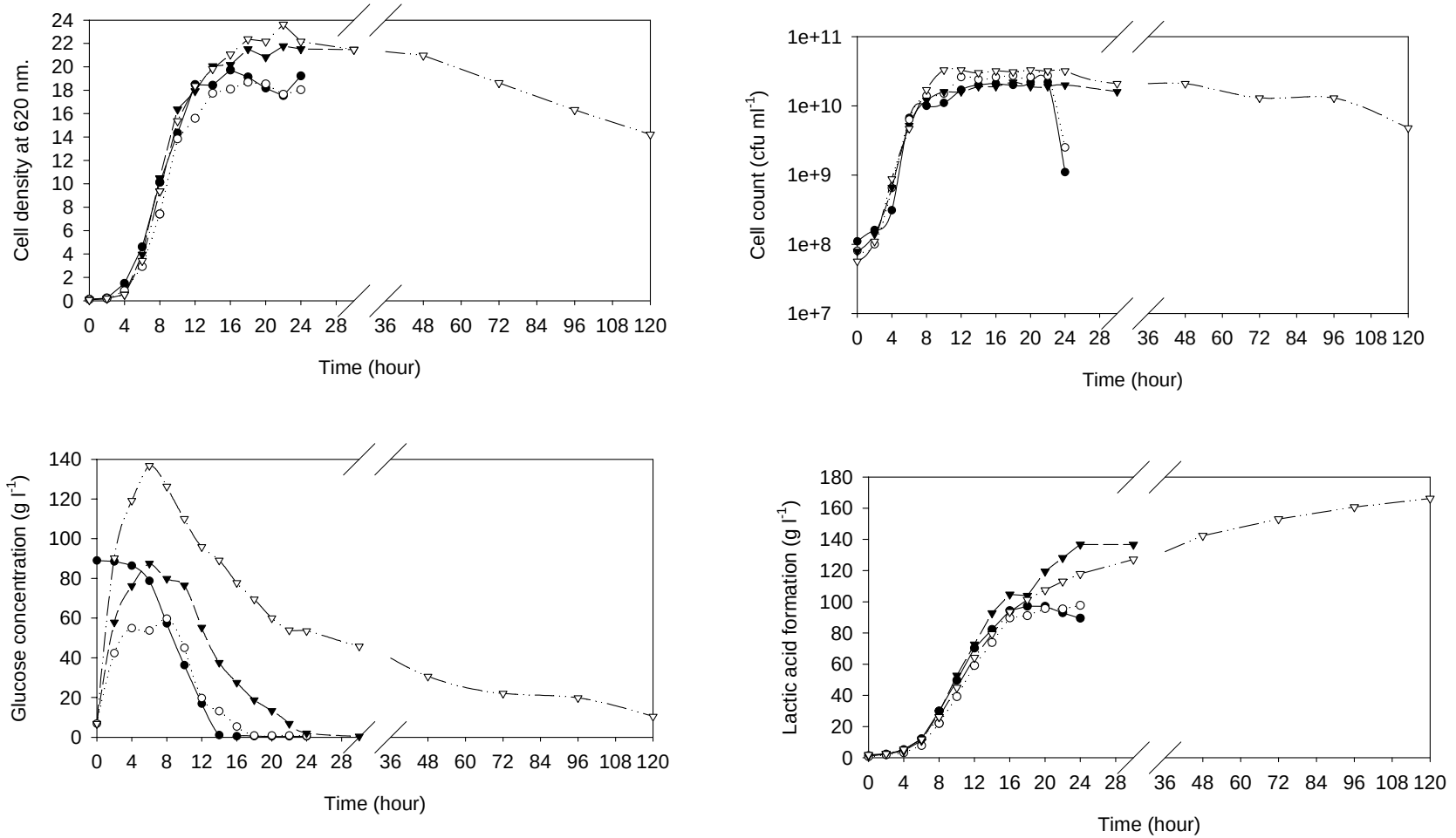


Figure 21 Changes in cell density (at 620 nm), cell counts (CFU/ml), glucose and lactic acid concentrations during lactic acid fermentation with DM3 strain by SS and SSF process at various substrate concentrations; SS 100 g l^{-1} (●), SSF 100 g l^{-1} (○), SSF 150 g l^{-1} (▼) and SSF 200 g l^{-1} (▽).

Part 3: Application of bipolar electrodialysis on recovery of free lactic acid after simultaneous saccharification and fermentation of cassava starch

1. Comparison of different BED systems

To evaluate the BED system of free lactic acid recovery from lactate salt, the model solution prepared from ammonium lactate (100 g l^{-1} adjusted to pH 6, similar to the actual fermentation broth) was used. During the experiment, voltage was fixed at 20 V and the process was terminated when the electrical current was reduced. Performance parameters for electrodialysis are summarized in Table 16.

When the BED-anion system was employed, the total operating time and the total current accumulated (coloumeter) were 2.83 h and 20 A.h, respectively. The concentration of lactic acid in the feed stream dropped from 97.7 to 7.8 g l^{-1} and, in product stream, rose from 0 to 138.3 g l^{-1}. The final lactic acid concentration of about 290.4 g, corresponding to 99% recovery ratio was achieved. The lactate flux was 466.4 g m^{-2} h^{-1}, the energy consumption for the recovery of 1 kg lactic acid was 1.58 kW.h and the current efficiency was 86.1%.

The total operating time and the total current accumulated of BED-cation system were 3.83 h and 21.3 A.h, respectively. The lactic acid concentration in feed stream did not change (about 103 g l^{-1}) since it could not move to the product stream. The final lactic acid concentration of 298.7 g was obtained with the recovery ratio of 2%. The lactate flux was 354.5 g m^{-2} h^{-1} and the energy consumption for the recovery of 1 kg lactic acid was 2.11 kW.h.

For BED-anion-cation system, the total operating time and the total current accumulated were 4.16 h and 13.4 A.h, respectively. In the product steam, 106.0 g l^{-1} was obtained and approximately 40.2 g l^{-1} remained in the feed steam, resulting in a low recovery ratio of 49%. The lactate flux was 116.2 g m^{-2} h^{-1} and the current efficiency was 65.5%. The energy consumption for lactic acid recovery was 5.87 $kW.h.kg^{-1}$.

Regardless of the BED systems, i.e. BED-anion, BED-cation and BED-anion-cation, when the direct current was applied to the electrodes, lactic acid ions always permeated through the anion exchange membranes whereas the ammonium ions (NH_4^+) permeated through the cation exchange membranes. The water was split by the bipolar membranes. As a result, lactic acid could be produced in the product stream in case of the BED-anion and BED-anion-cation, whereas be produced in the feed stream in case of the BED-cation. Different BED systems should be practical to recover chemicals present in different forms. The two-compartment cells with BED-cation only are expected to be useful to convert the salts of weak acids and strong bases, such as sodium acetate, lactate, formate, glycinate, etc. and of other organic and amino acid. The application of BED-cation as well as BED-anion-cation to recover free lactic acid from sodium lactate had been reported with promising results (Habova et al., 2004; Madzingaidzo et al., 2002). In contrast, the two-compartment cells with BED-anion are likely to be useful to convert the salts of weak bases (ammonia) and strong acids, such as ammonium chloride, ammonium sulfate and ammonium lactate. Among the three systems evaluated in this study, the BED-anion system gave the highest performance for all parameters reported, whereas the energy consumption was the lowest. In this case, no grate benefits could be achieved with the standard three-compartment configuration since the organic acids are usually weak acids that are not very conductive. Therefore, the following experiments were performed by using the BED-anion system.

2. Comparison of operating conditions of BED

A model solution was applied to BED-anion system at a fixed voltage or fixed current (Figure 22 and Table 17). At fixed current (5 A), after starting the operation, the voltage was decreased from 20 V until 14.6 V thereafter it started to increase. The total operating time and current accumulated were 3.66 h and 17.9 A.h, respectively. The lactic acid concentration in the feed stream was reduced from 92 to 25.6 g l^{-1} and increased from 0 to 110.5 g l^{-1} in the product stream. The lactate flux, the current efficiency, the energy consumption and the recovery ratio were 301.9 g $m^{-2}h^{-1}$, 80.4%, 2.46 kW.h.kg^{-1} and 88%, respectively.

In contrast to fixed current, at fixed voltage (20 V), the current was increased until 8.3 A thereafter it was reduced. The total operating time and current accumulated were 3 h and 20.4 A.h, respectively. The lactic acid concentration was dropped from 89.4 to 2.2 g l^{-1} in the feed stream and rose from 0 to 117.6 g l^{-1} in the product stream. The final lactic acid concentration of 278.7 g, corresponding to 100% recovery ratio, was accomplished. The lactate flux was 422.3 g m^{-2} h^{-1}, the energy consumption for the recovery of 1 kg lactic acid was 1.75 kW.h and the current efficiency was 81%.

It can be observed that the fixed current condition of BED-anion system used the total current lower than fixed voltage, however, operation at the fixed voltage demonstrated the better performance of all parameters, as compared to the fixed current. Thus, the BED-anion system being operated at fixed voltage (20 V) was further applied to purification of free lactic acid from the actual fermentation broth.

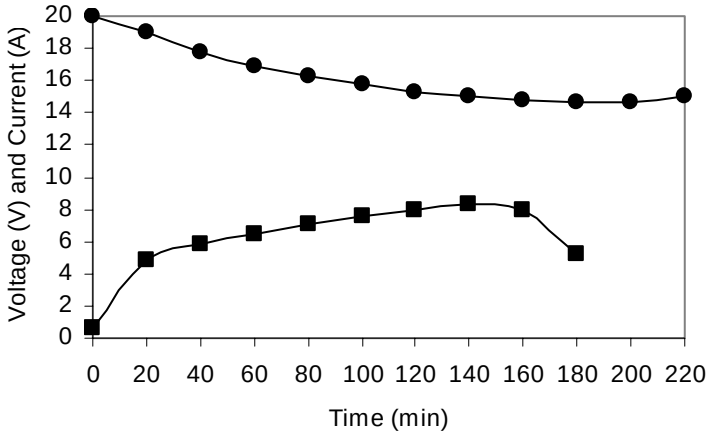


Figure 22 Changes in voltage and current values during lactic acid recovery by bipolar electrodialysis (BED-anion system) using a model solution of ammonium lactate at fixed current (●) and fixed voltage (■).

Table 16 Changes in some parameters during lactic acid recovery by different bipolar electrodialysis system (BED-anion, BED cation and BED-anion-cationsystem) using a model solution of ammonium lactate at fixed voltage.

Parameter	BED-anion	BED-cation	BED-anion-cation
Operating time (h)	2.83	3.83	4.16
Total current (A.h)	20	21.3	13.4
Initial volume in feed stream (l)	3	3	3
Final volume in feed stream (l)	2.3	2.9	2.7
Initial lactic acid concentration in feed stream (g l^{-1})	97.7	103	100.4
Final lactic acid concentration in feed stream (g l^{-1})	7.8	103	40.2
Initial volume in product stream (l)	1.5	1.5	1.5
Final volume in product stream (l)	2.1	1.4	1.4
Initial lactic acid concentration in product stream (g l^{-1})	0	0	0
Final lactic acid concentration in product stream (g l^{-1})	138.3	3.7	106.0
Final lactic acid in product stream (g)	290.4	NA	148.4
Initial lactic acid in feed stream (g)	293.1	309.0	301.2
Final lactic acid in feed stream (g)	NA	298.7	NA
Flux (g $m^{-2}.h^{-1}$)	466.4	354.5	111.6
Current efficiency (%)	86.1	NA	65.5
Energy consumption (kW.h. kg^{-1})	1.58	2.11	5.87
Recovery ratio (%)	99	2	49

NA: not available

Table 17 Changes in some parameters during lactic acid recovery by bipolar - electrodialysis (BED-anion system) using a model solution of ammonium lactate at different conditions (fixed current or fixed voltage).

Parameter	**At fixed current (5 A)**	**At fixed voltage (20 V)**
Operating time (h)	3.66	3.00
Total current (A.h)	17.9	20.4
Initial volume in feed steam (l)	3	3
Final volume in feed steam (l)	2.24	2.46
Initial lactic acid concentration in feed steam (g l^{-1})	92.0	89.4
Final lactic acid concentration in feed steam (g l^{-1})	25.6	2.2
Initial volume in product steam (l)	1.5	1.5
Final volume in product steam (l)	2.2	2.37
Initial lactic acid concentration in product steam (g l^{-1})	0	0
Final lactic acid concentration in product steam (g l^{-1})	110.5	117.6
Final LA in product steam (g)	243.1	278.7
Initial LA in feed steam (g)	276.0	268.2
Flux (g m^{-2} h^{-1})	301.9	422.3
Current efficiency (%)	80.4	81.0
Energy consumption(kW.h.kg^{-1})	2.46	1.75
Recovery ratio (%)	88	100

3. BED trial with actual fermented broth

Cassava starch was initially liquefied by α-amylase and further preceded to lactic acid production by SSF process. At the end of fermentation, the broth contained approximately 98 g l^{-1} lactic acid. Figure 23 shows the flow diagram of two-step electrodialysis recovery process of free lactic acid from fermentation broth.

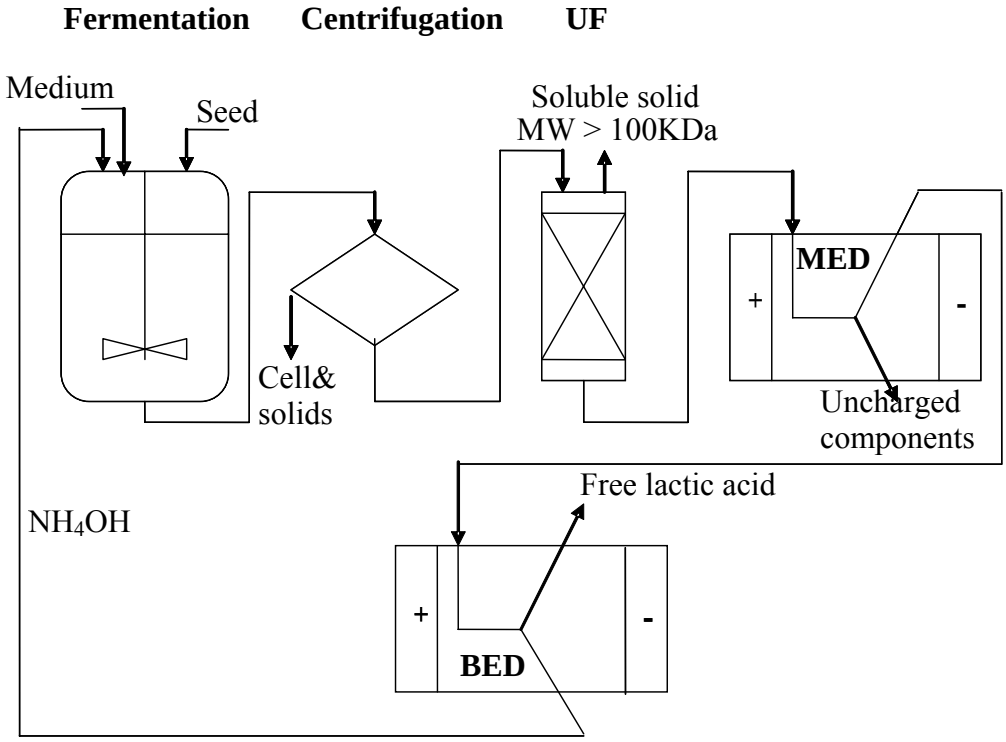


Figure 23 Flow diagram of two-step electrodialysis recovery process of free lactic acid from LAB fermentation broth.

In this work, the purification process was initially achieved by cell mass removal, using the centrifuge. Secondly, all suspended solids greater than 100 kDa were removed by the membrane filtration. Next, the sample was purified by mono-polar electrodialysis (MED) or simply called electrodialysis at fixed voltage (20 V), to separate ammonium lactate salts from an aqueous solution and other uncharged components. Figure 24 exhibit the time course of lactic acid concentration in the feed

stream and the product stream. The current efficiency was kept stable after 20 min and the highest current efficiency approximately 87.4 % was achieved at 120 min. The lactate concentration in the feed stream was decreased from 108.1 to 10.7 g l^{-1} and increased from 0 to 121.8 g l^{-1} in the product stream. The lactate flux was achieved at 810.3 g m^{-2} h^{-1}, while the energy consumption was 0.83 kW.h. kg^{-1} and the recovery ratio was 83 %. Moreover, the brown color of the fermentation broth was removed after the MED process, thus the use of activated carbon is no longer necessary.

The lactate solution, that being passed through MED, was continued to split free lactic acid and ammonium hydroxide. Based on the previous experiment, the acid recovery by BED-anion system was performed at the fixed voltage (20 V). The results, as shown in Table 18, exhibited the total current and the total operating time of 39.4 A at 240 min, respectively. Changes in lactic acid concentrations in feed and product streams (Figure 25) were observed to be from 117.2 to 10.4 g l^{-1} and 0 to 143.7 g l^{-1}, respectively. The final lactic acid concentration of 340.6 g, corresponding to 78% recovery ratio was obtained. The lactate flux was 387 g m^{-2} h^{-1}, the energy consumption for the recovery of 1 kg lactic acid was 1.93 kW.h and the current efficiency was 51.3%. It was noticeable that an unstable current during experiment, higher operating time and total current and lower performance parameters (flux, current efficiency, energy consumption and recovery ratio) were obtained when compare to a model solution. Presumably, it was due to impurities present in the fermentation broth and had adverse effects to ion membranes. Lee *et al.* (1998) had described the efficiency of lactic acid recovery from sodium lactate by BED-cation system when the feed solutions contained some metal ions, such as calcium and magnesium. In a presence of metal ion in broth water-splitting electrodialysis had the drawback of membrane fouling, resulting from the precipitation of metal ions on the membrane. Moreover, during operating, the volume of feed steam always decreased and of product steam increased. It introduced problems in electrodialysis as it limited the final concentration of lactate in the product steam. Habova *et al.* (2004) reported

that could be due to water passage through the membranes simultaneously with the lactate ions by electrodialysis.

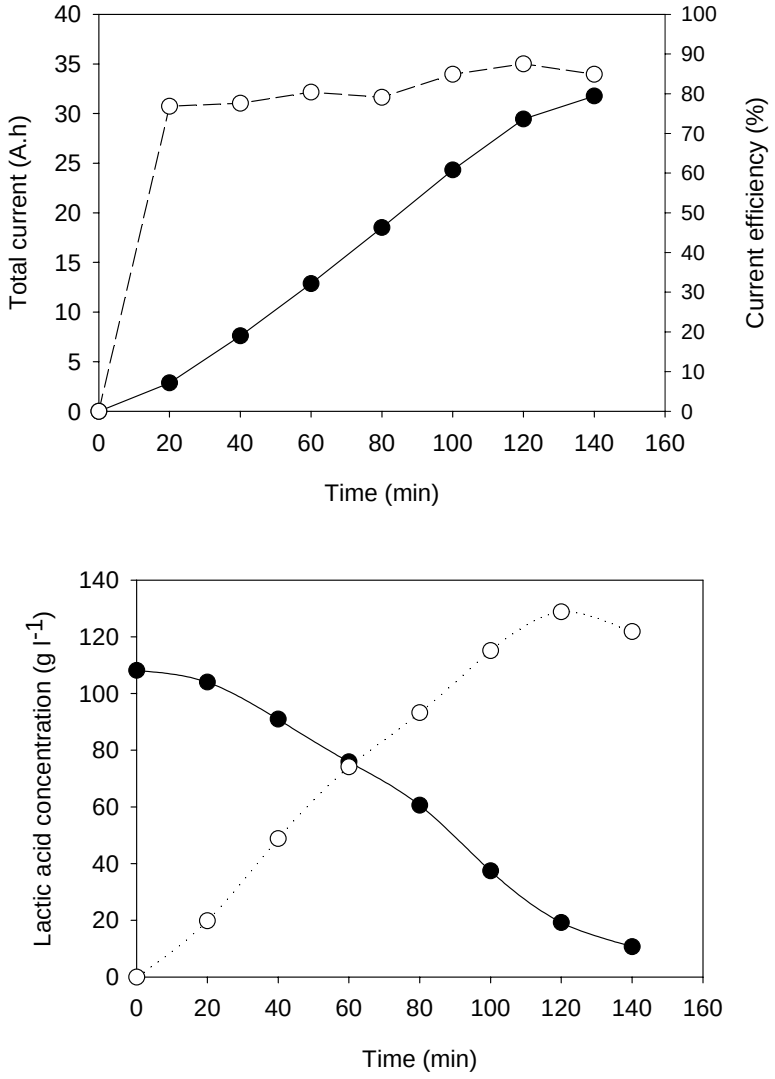


Figure 24 Purification of ammonium lactate from the actual fermentation broth by mono-polar electrodialysis; total current (●), current efficiency (○), lactic acid concentration in feed stream (■) and lactic acid concentration in product stream (□)

Table 18 Two-step purification of ammonium lactate from fermentation broth using mono-polar electrodialysis and bipolar electrodialysis.

Parameter	MED	BED*
Operating time (h)	2.33	4.00
Total current (A.h)	31.8	39.4
Initial volume in feed steam (l)	5	3.72
Final volume in feed steam (l)	3.22	2.36
Initial lactic acid concentration in feed steam (g l^{-1})	108.1	117.2
Final lactic acid concentration in feed steam (g l^{-1})	10.7	10.4
Initial volume in product steam (l)	2.0	1.5
Final volume in product steam (l)	3.72	2.37
Initial lactic acid concentration in product steam (g l^{-1})	0	0
Final lactic acid concentration in product steam (g l^{-1})	121.8	143.7
Final lactic acid in product steam (g)	453.1	340.6
Initial lactic acid in feed steam (g)	540.5	436.0
Flux (g m^{-2} h^{-1})	810.3	387.0
Current efficiency (%)	84.6	51.3
Energy consumption(kW.h.kg^{-1})	0.83	1.93
Recovery ratio (%)	83	78

*BED-anion system at fixed voltage (20 V).

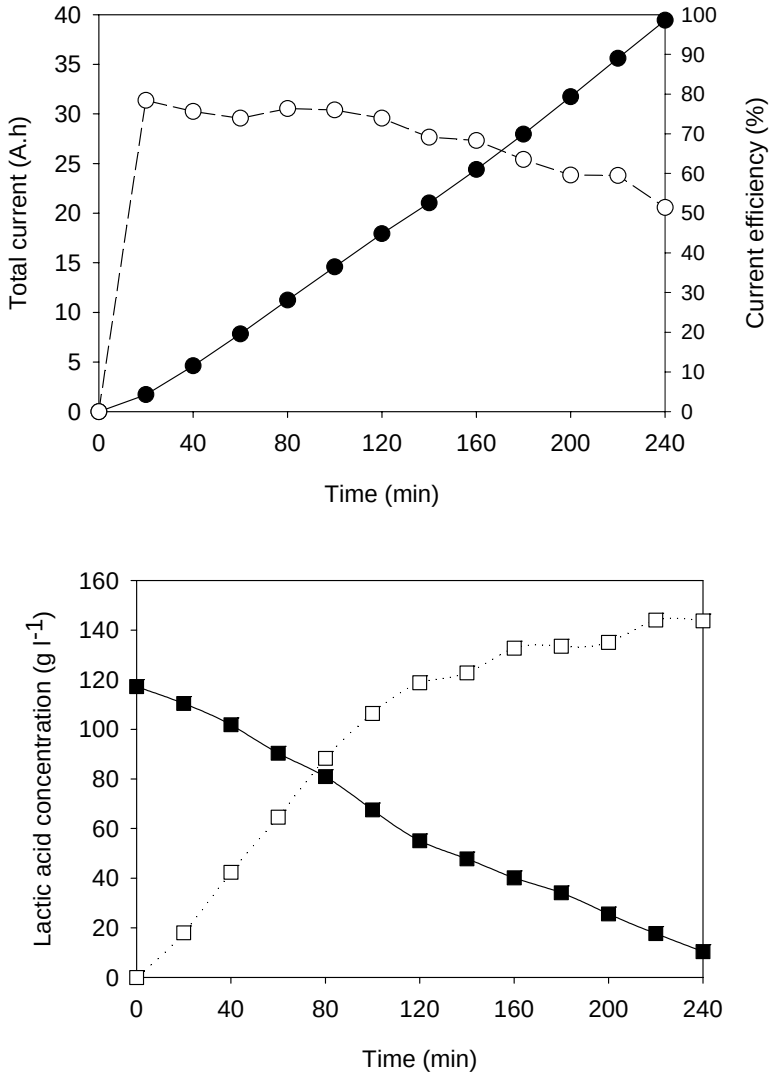


Figure 25 Recovery of free lactic acid from the actual fermentation broth after treated with mono-polar electrodialysis as operated by bipolar electrodialysis (BED-anion system) at fixed voltage (20 V); total current (●), current efficiency (○), lactic acid concentration in feed stream (■) and lactic acid concentration in product stream (□)

Part 4: Use of nitrogen-containing Thai agro-industrial wastes to minimize lactic acid production cost

Some previous reports delineated the advantages of the fungal process over the bacterial process such as the use of a chemically defined medium (including inorganic nitrogen sources), which simplifies product purification and produce steriospecific pure L-lactic acid (Rojan *et al.,* 2007), but it also requires vigorous aeration because *Rizopus oryzae* is an obligate aerobe. In fungal fermentation, the low production rate, below 3 g $l^{-1}h^{-1}$, is probably due to the low reaction rate caused by mass transfer limitation. The lower product yield from fungal fermentation is attributed partially to the formation of by-products, such as fumaric acid and ethanol (Tay and Yang, 2002; Wee *et al*., 2006). LAB have received wide interest because of their growth rate and product yield. Moreover, due to the oxygen tolerance of LAB, most fermentations were performed without aeration control. However, LAB have complex nutrient requirements because of their limited ability to synthesize B-vitamins and amino acids. Using agro-industrial wastes with low costs as nitrogen supplement to media is necessary to develop lactic acid production at industrial scale. In previous reports, corn steep liquor (CSL) is an agro-industrial waste known to support growth and lactic acid production (Wee *et al.,* 2006b; Lee, 2005; Oh *et al.,* 2005). However, in Thailand, CSL has low potential to be used due to its availability (in term of tons generated per year) and its high cost if imported. Thus, to develop commercial scale of lactic acid production, alternative nutritional sources shall be screened from local agro-industrial-based.

1. Preparation and characterization of agro-industrial wastes

Lactic acid can be produced by the fermentation of simple sugars using the LAB under an anaerobic condition. Even sugar-containing materials are recognized as the major substance being used for cell growth, other substances are also important for LAB growths including nitrogen, vitamin and minerals (Kwon *et al.,* 2000). Earlier works have demonstrated an improved cell growth as well as lactic acid

productivity, when nitrogen is supplemented along with the carbon feedstock during fermentation (Kurbanoglu and Kurbanoglu, 2003; Kwon *et al*., 2000; Rivas *et al*., 2004; Wee *et al.,* 2006b; Gao *et al.,* 2006a; Gao *et al.,* 2006b; Timbuntam *et al*., 2006).

In this study, several processing wastes obtained from major Thai agro-industries including sodium monoglutamate production, canned fish and frozen shrimp production likely contain nitrogen compounds that may be beneficial to lactic acid fermentation by LAB. The liquid waste can be received from a production of monosodium glutamate, as a salt form of glutamic acid. Fish waste is a by-product generated from tuna canning industry; the total production capacity of the whole country is approximately 350,000 tons/year of which about 100,000 tons are head, bone and viscera. Previously, some studies have reported the utilization of this waste as fish meal, fish silage or fish protein concentrate as a nitrogen-supplementary source for production of microbial-fermented products such as ethanol, etc. (Ruanglek *et al.,* 2006; Goddard and Perret, 2005). Another large-productivity sector of protein-rich agro-industry is the production of shrimp products. Around 300,000 tons/year of shrimp are produced to frozen products and other export products. Approximately 170,000 tons of wastes are head and shell which contain around 30-35% tissue protein and chitin being the other major component. The wastes are used as a source for production of chitin, chitosan and protein hydrolysates.

When the wastes from fish and shrimp processing are used, the hydrolysis should be applied firstly so that the high molecular weight proteins are broken down to lower ones, i.e. peptides and amino acids, which are more readily consumed by LAB. Protein hydrolysis can be implemented either by enzyme, acid or both. For enzymatic hydrolysis, although, it brings out products of high functionality and high nutritive value but it has a slow reaction rate and a high production cost. Alternatively, a relatively low-cost acid hydrolysis process is introduced for this purpose. Yet the neutralization of hydrolysates can generate some salts which should be closely evaluated for their any possible effect on LAB performance.

The qualities of agro-industrial wastes that were used as a nitrogen supplement instead of yeast extract (YE) were characterized (Table 19). YE contained highest contents of nitrogen, free amino acid and protein. Most of the proteins present in YE were in forms of amino acids as indicated by a high ratio of free amino acid to protein (≈ 107%). Definitely, the highest ratio of free amino acid to protein was evident in the liquid wastes from glutamic acid processing. The hydrolysates derived from fish and shrimp wastes were quite low in the nitrogen, protein and free amino acid contents since these products were received from acid hydrolysis and then used directly without dewatering or concentrating to minimize the cost. The intention was to reveal the potential use of these wastes and their on-site preparation that could be adopted by the industry.

Table 19 Characterization of agro-industrial wastes including glutamic acid processing waste water (GM), fish extract (FE), fish waste hydrolysates (FWH) and shrimp waste hydrolysates (SWH) as compared to yeast extract (YE), a commonly used nitrogen supplement in lactic acid fermentation.

Agro-industrial wastes / Sources	**Content (% wet basis)**				**Ratio of free amino acid to protein (%)**
	Total nitrogen by Kjeldahl method	**Protein***	**Free amino acid**	**Ash**	
Glutamic acid processing waste water (GM)	2.84±0.05	17.75	21.00±0.00	6.31±0.05	118.3
Fish extract (FE)	8.57±0.05	53.56	14.50±0.71	15.99±0.13	27.1
Fish waste hydrolysate (FWH)	1.23±0.02	7.69	4.00±0.00	3.00±0.02	52.0
Shrimp waste hydrolysate (SWH)	0.66±0.01	4.13	2.00±0.00	2.55±0.01	48.4
Yeast extract (YE)	10.88±0.23	68.00	72.67±2.89	12.62±0.25	106.9

*Calculated by multiplying the nitrogen content with a factor of 6.25.

2. Lactic acid fermentation of cassava starch by SSF process with the substitution of yeast extract (YE) by agro-industrial wastes

LAB require a high level of nutrient supplementation like peptides, amino acids, vitamins, etc. YE has been reported as the best nutrient source for this purpose, but it is relatively expensive for large-scale fermentation. Therefore, in this experiment, the application of various nitrogen-containing agro-industrial waste sources for lactic acid production, i.e. GM, FE, FWH and SWH were evaluated in comparison with a control medium containing YE (10 g l^{-1}). The concentration of these nitrogen supplements were adjusted to an equivalence of total nitrogen content, i.e. 1 g l^{-1}. For this reason, different concentrations of the nitrogen supplements were reported; 38, 13, 80 and 100 g l^{-1} for GM, FE, FWH and SWH, respectively. As shown in Figure 26 and Table 20, with a supplement of 10 g YE l^{-1}, the starch (150 g dry solid l^{-1}) was completely consumed within 36 h. The lactic acid production reached a maximum concentration of 141.4 g l^{-1} (productivity 3.93 g $l^{-1}h^{-1}$ and yield 94.3 %). When other inferior nitrogen-containing wastes were applied, the lactic acids were potentially produced, but dry cell weight of LAB were fairly low and longer fermentation times were need, making the acid productivity comparatively low. In particular, GM and SWH demonstrated the lowest fermentation efficiencies (substrate utilization ≈ 59% and productivity range of 1.12-1.22 g $l^{-1}h^{-1}$) with a high level of unfermented substrate leftover at the end of process. Substitution of YE with FE or FWH provided improved acid fermentation comparatively to GM and SWH. The total lactic acid formation by supplementing with FE and FWH was 141 and 146 g l^{-1}, respectively, which were comparable to fermenting with YE supplementation. However, the total fermentation periods with FE and FWH addition were prolonged to be twice of fermentation with YE and, consequently, the productivities were only a half of YE. LAB fermentation in the presence of FE showed a few glucose residuals after 72 h, suggesting incomplete glucose consumption. FWH had a slight advantage over FE as glucose residual was negligible at the end of fermentation.

A previous study reported by Ruanglek *et al.* (2006) has demonstrated the possibility of replacing the complex nutritional source for *Z. mobilis* by many Thai

agro-industrial wastes such as the ami-ami solution from glutamate-synthesizing process, autolysates of brewer's yeast and hydrolysates of fish soluble waste. Especially, the fish soluble waste was the best applicable nitrogen source of nutritional replacement in ethanol production since it could significantly promote both specific growth rate and ethanol productivity as compared with the YE. Gao *et al.* (2007) has discussed a possible problem associated with the application of low-cost nutrient sources in fermentation that they had to be used in much larger amounts than YE, resulting in much larger amounts of impurities in fermentation broths. For this reason, they defined the ratio of ash to nitrogen concentration in fermentation broths as an indicator of impurities. Similarly, in this work, the total ash contents in fermentation broths with the addition of agro-industrial wastes were comparable as in the range of 0.46-0.76 % w/w, suggesting similar ratios of ash to nitrogen concentrations.

Even the addition of FWH and FE in LAB fermentation could give good yields of acid, but the productivities were much lower relatively to YE. This might be due to a limitation of other growth factors in agro-industrial wastes that were different from YE. Hujanen and Linko (1996) suggested that costly YE could partially be replaced with more economical malt sprout extract without any significant change in lactic acid production. Kurbanoglu and Kurbanoglu (2003) reported the content of lactic acid in the culture broth containing 6% ram horn hydrolysates with 0.5% YE (44 g l^{-1}) for 26 h was 22% higher than that of control culture broth (36 g l^{-1}). Rivas *et al.* (2004) concluded the suitability of supplementing glucose solutions with CSL and hydrolysates-grown yeast cells for high yield and high productivity lactic acid fermentation with *L. rhamnosus* CECT-288. Oh *et al.* (2005) reported the medium contained 200 g l^{-1} of whole wheat flour hydrolyzate, 15 g l^{-1} of CSL and 1.5 g l^{-1} of YE resulted in significant improvement of lactic acid productivity and cell growth. Also, Gao *et al*. (2006) presented the combination of 5 g l^{-1} YE and the spent cell hydrolysates has high performance in lactic acid production relative to 15 g YE l^{-1}. It was interesting if the potential low-priced nitrogen supplement, which was FWH, could be used as a combination with YE (50% substitution), to reduce the cost at some extent.

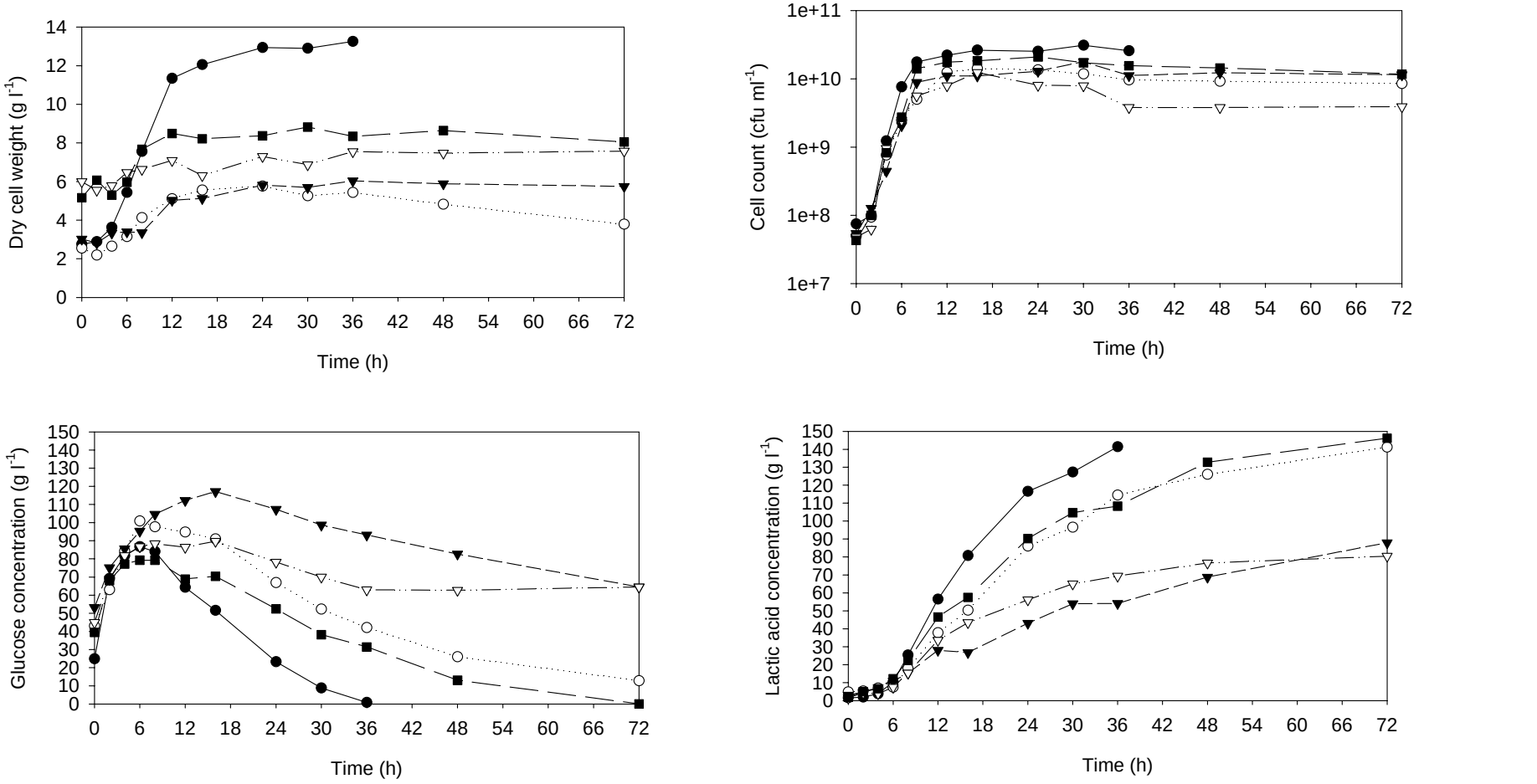


Figure 26 Comparison of various nitrogen sources during fermentation of lactic acid by DM3, at cassava starch hydrolysates (150 g dry solid l^{-1}), pH 6 and temperature of 40^{O}C by SSF process; (●) YE, (○) FE, (▼) GM1, (▽) SWH and (■) FWH.

Table 20 Parameters in lactic acid production of cassava starch hydrolysates* by the strain DM3 with the addition of various agro-industrial wastes (Glutamic acid processing waste-GM; Fish extract-FE; Fish waste hydrolysates-FWH and Shrimp waste hydrolysates-SWH) for complete substitution of yeast extract (YE) at an equivalent nitrogen content of 1g l^{-1}**

Parameter	YE (10 g l^{-1})	GM (38 g l^{-1})	FE (13 g l^{-1})	FWH (80 g l^{-1})	SWH (100 g l^{-1})
Cell density at 620 nm.	19.32	5.43	6.76	11.92	6.75
Glucose residual (g l^{-1})	0.85	64.4	12.8	0	64.4
Starch consumption (g l^{-1})	150	85.6	137.2	150	85.6
Starch consumption (g)	375	220.4	343	375	220.4
Substrate utilization (%)	100	58.8	91.5	100	58.8
Total lactic acid concentration (g l^{-1})	141.4	87.9	141.1	146.1	80.5
Total lactic acid concentration (g)	353.5	211.0	352.8	365.3	193.2
$\%Yield_{P/S}$	94.3	95.7	102.9	97.4	87.7
Fermentation time (h)	36	72	72	72	72
Productivity (g $l^{-1}h^{-1}$)	3.93	1.22	1.96	2.03	1.12
Ash content (%w/w)	0.46	0.65	0.52	0.67	0.76

*Cassava starch hydrolysates (150 g dry solid l^{-1}) was fermented at 40°C, pH 6 by SSF process with the strain DM3

**The number in parenthesis was the amount of nitrogen-rich agro-industrial wastes added at an equivalent nitrogen content of 1 g l^{-1}, based on the nitrogen content as reported in Table 19.

A mixture of YE (5 g l^{-1}) and FWH (40 g l^{-1}), with a total nitrogen content of 1 g l^{-1}, was applied to lactic acid fermentation of cassava starch hydrolysates (Figure 27 and Table 21). Lactic acid production with the addition of 5 g l^{-1} YE was also performed as a control experiment. When YE was reduced from 10 g l^{-1} to 5 g l^{-1}, not only the cell density, substrate consumption (from 100 to 96%), yield (from 94.3 to

85.9%) and lactic acid content (from 353.5 to 309.5 g) was lower, but also the productivity (from 3.93 to 2.58 g $l^{-1}h^{-1}$) was inferior since the system required longer periods (from 36 to 48 h) of time to complete fermentation. Moreover, some glucose residuals were also found when fermentation process was terminated.

Nevertheless, an extra addition of FWH (40 g l^{-1}) with YE (5 g l^{-1}) could improve the acid production performance which was similar to the use of YE (10 g l^{-1}). In addition, a slightly lower fermentation time could achieved (30 h), making the acid productivity greater by 19%; the productivity was 4.69 and 3.93 g $l^{-1}h^{-1}$ for supplementation with a FWH/YE mixture and YE, respectively. As evidenced by experimental results, a nitrogen source of YE complemented with FWH could be an effective substitute for the application of YE only. By this way, the nutrient cost could be minimized whereas the acid productivity and yield were still maintained or even improved. Gao *et al.* (2006b) discussed that some trace nutrients such as vitamins were liable and be destroyed when subjected to the acidic condition, the supplementation in a combination with YE, at a small amount, could overcome that problem readily and still allow a high performance in lactic acid production. As a result, this promising low-cost nitrogen supplement, i.e. a mixture of 5 g l^{-1} YE and 40 g l^{-1} FWH was further applied to a real material for industrial uses, i.e. cassava feedstock.

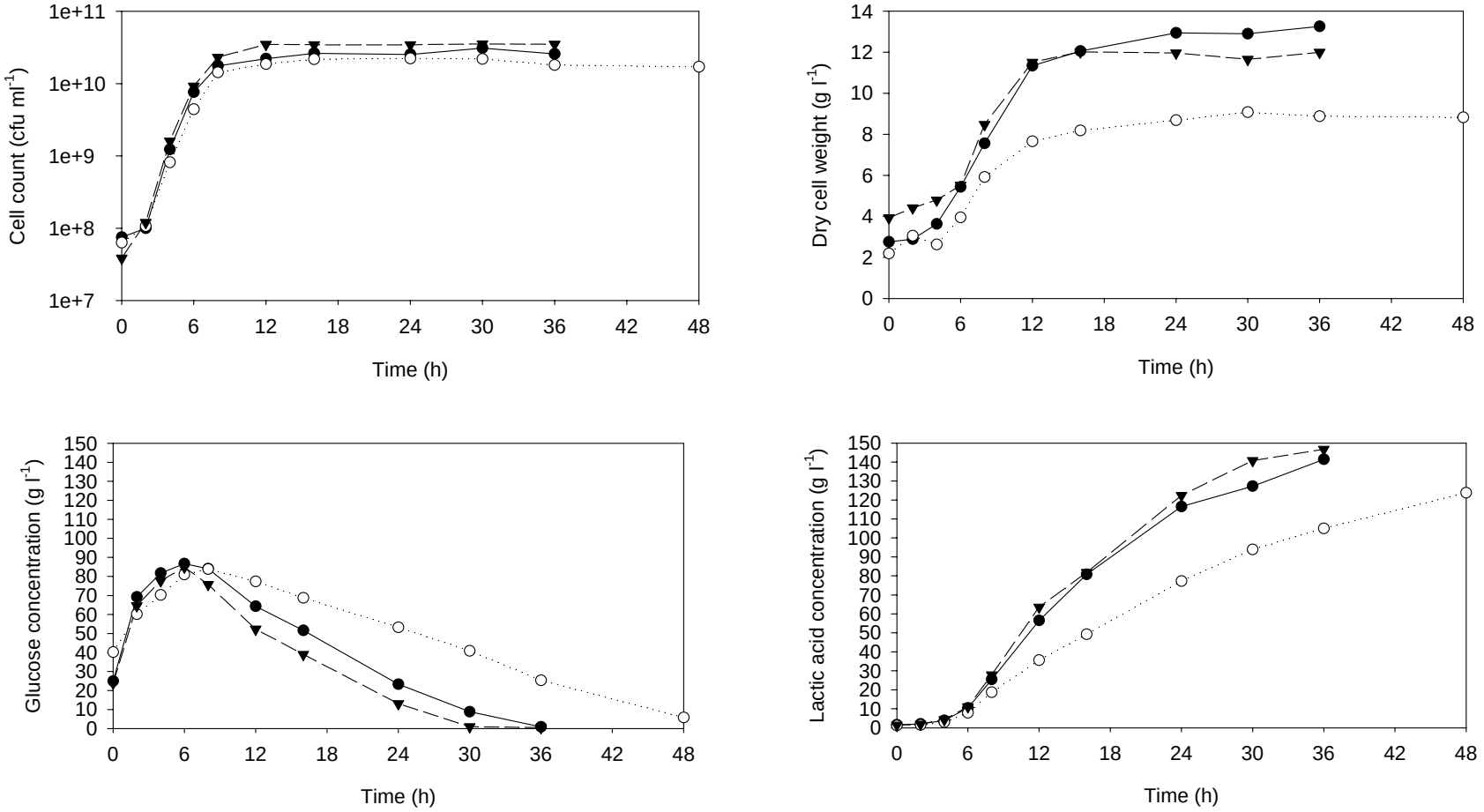


Figure 27 Comparison of using YE and a mixture of YE and FWH during lactic acid fermentation by DM3 strain, at cassava starch hydrolysates (150 g dry solid l^{-1}), pH 6 and temperature of $40^{O}C$ by SSF process; (●) YE 1%, (○) YE 0.5% and (▼) YE 0.5% + FWH 4%.

Table 21 Parameters in lactic acid production of cassava starch hydrolysate* by the strain DM3 with the addition of yeast extract (YE), fish waste hydrolysate (FWH) and a mixture of both to partially substitute yeast extract at an equivalent nitrogen content of 1 g l^{-1}**.

Parameter	**YE (10 g l^{-1})**	**FWH (80 g l^{-1})**	**YE (5 g l^{-1})**	**YE (5 g l^{-1}) +FWH (40 g l^{-1})**
Cell density at 620 nm.	19.32	11.92	13.16	17.83
Glucose residual (g l^{-1})	0.85	0	5.8	0.94
Starch consumption (g l^{-1})	150	150	144.2	150
Starch consumption (g)	375	375	360.5	375
Substrate utilization (%)	100	100	96.1	100
Total lactic acid concentration (g l^{-1})	141.4	146.1	123.8	140.8
Total lactic acid concentration (g)	353.5	365.3	309.5	359.0
$\%Yield_{P/S}$	94.3	97.4	85.9	95.7
Fermentation time (h)	36	72	48	30
Productivity (g $l^{-1}h^{-1}$)	3.93	2.03	2.58	4.69
Ash content (%w/w)	0.46	0.67	0.4	0.57

*Cassava starch hydrolysate (150 g dry solid l^{-1}) was fermented at 40°C, pH 6 by SSF process with the strain DM3

**The number in parenthesis was the amount of nitrogen supplements added at an equivalent nitrogen content of 1g l^{-1}, based on the nitrogen content as reported in Table 19.

3. Lactic acid fermentation of cassava materials by Simultaneous Saccharification and Fermentation (SSF) process with the substitution of yeast extract (YE) by agro-industrial wastes

A raw material cost is one of the major constrains in the economic production of lactic acid. Various starch materials have been studied for lactic acid production, including wheat, corn, potato, sorghum and cassava. When cassava is used, different forms of cassava are available-starch, dried chips and fresh roots; all are used industrially. Although, starch can be stored for a long period but the price is higher than chips and roots. Chips and roots are of great interest from an industrial and economic point of views. In this work, the lactic acid production from chips and roots were performed with the substrate concentration of 150 g dry solid l^{-1}; the starch content in chips and roots were about 72.5 % (w/w) and 26.3 % (w/w), respectively. Figure 28 exhibits changes in glucose and lactic acid concentrations during lactic acid production from cassava starch, chips and roots. The glucose consumption and lactic acid production profiles were similar for all tested feedstock. The glucose concentration was increased to the maximum at 6 h and afterward started to decline. However, the glucose consumption of each material was completed at different time, resulting in different fermentation periods and productivity (Table 22). Among all types of feedstock, fermentation of cassava starch gave the highest productivity (4.69 g $l^{-1}h^{-1}$) whereas the productivity of fresh root fermentation was lowest (3.30 g $l^{-1}h^{-1}$). Nevertheless, all significant parameters of fermentation performance did indicate the applicable uses of all types of cassava feedstock with the partial YE supplementation with nitrogen-containing agro-industrial waste in fermentation which was agreeable with a previous study of Ghofar *et al.* (2005). Ghofar *et al.* (2005) reported the production of L-lactic acid from fresh cassava roots, being slurred with tofu liquid wastes by *Streptococcus bovis* in order to reduce an overall production cost for biodegradable plastic uses. The result showed that the maximum L-lactic acid concentration, productivity and yield were 67 g l^{-1}, 0.77 (g $l^{-1}h^{-1}$) and 58.6%, respectively. Different fermentation performance might be accounted by different fermentation conditions, i.e. microbial strain, the nitrogen content and sources as well as other impurities that probably associated with the liquid waste used.

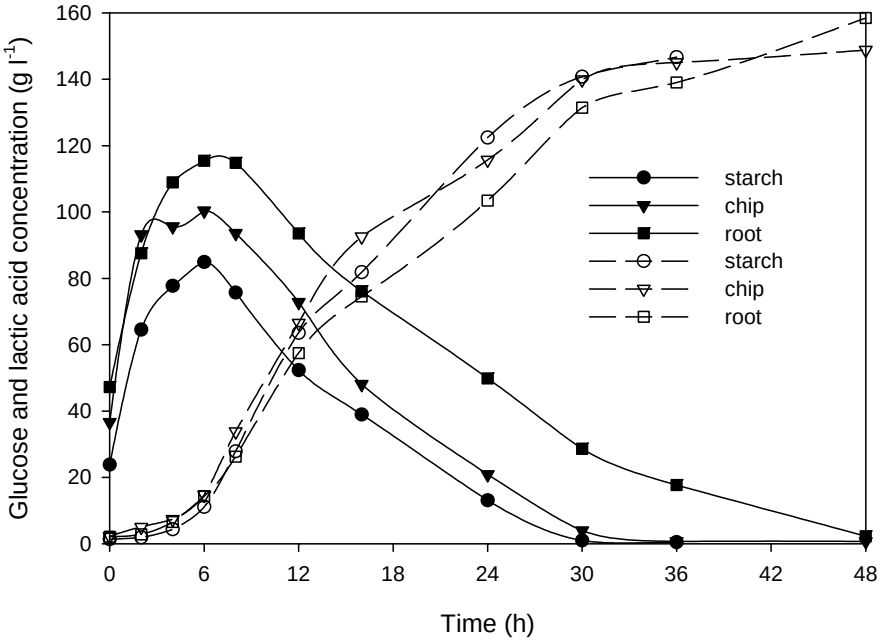


Figure 28 Profiles of glucose and lactic acid concentration (g l^{-1}) changes during lactic acid fermentation of various cassava materials using a mixture of yeast extract (5 g l^{-1}) and fish waste hydrolysate (FWH; 40 g l^{-1}) as a nitrogen supplement; close symbols and open symbols were glucose and lactic acid concentrations, respectively. (Fermentation condition: substrate concentration = 150 g l^{-1}, pH = 6, temperature = 40°C, agitation speed = 150 rpm).

Table 22 Parameters in lactic acid production of cassava hydrolysate from dried chips and fresh roots* by the strain DM3 with the addition of a mixture of yeast extract (YE) and fish waste hydrolysate (FWH) as a nitrogen supplement**.

Parameter	Cassava dried chip	Cassava fresh root
Cell density at 620 nm.	24.46	24.35
Glucose residual (g l^{-1})	0.7	2.24
Starch consumption (g l^{-1})	150	148
Starch consumption (g)	375	369
Substrate utilization (%)	100	98.4
Total lactic acid concentration (g l^{-1})	145.1	158.5
Total lactic acid concentration (g)	370	404.2
$\%Yield_{P/S}$	98.7	109.5
Fermentation time (h)	36	48
Productivity (g $l^{-1}h^{-1}$)	4.03	3.30
Ash content (%w/w)	0.84	0.95

**Cassava hydrolysate (150 g dry solid/l) was fermented at 40°C, pH by Simultaneous Saccharification and Fermentation (SSF) process with the strain DM3
**An equivalent nitrogen content of 1 g l^{-1}.

Table 23 shows the data of lactic acid production by *L. rhamnosus* from this work and from some previous reports. When the results of batch lactic acid fermentation obtained in this work are compared with the literature cited in Table 23, it could be seen that final lactic acid concentration with *L. rhamnosus* DM3 are higher than any of the reported so far.

Table 23 Comparison of different strains of *L. rhamnosus* for lactic acid production

L. rhamnosus	**Substrate**	**Temp. (^{O}C)**	**pH**	**pH control**	**nutrient**	**LA (g l^{-1})**	**Yield (%)**	**Productivity (g $l^{-1}h^{-1}$)**	**Ref.**
DM3	Cassava, SSF	40	6.0	NH_4OH	0.5%YE +				This work
	Starch				4%FWH	140.8	95.7	4.69	
	Chip					145.1	98.7	4.03	
	Roots					158.5	109.5	3.3	
ATCC10863	Glucose	30				67	74	3.3	Hujanen and Linko, 1996
		37				70	78	3.3	
		41				68	76	3.5	
		45				75	83	3.3	
	Whey permeate	40				30	71	1.5	Mulligan and Gibbs.1991
	Glucose				0.25%YE+0.5%trp	57	81		Olmos–Dichara *et al.,* 1997
					0.5%YE +1%trp	58	95		
	hydr molasses, SSF				YE + pep	14	70		Aksu Z, Kutsal, 1986
	Glucose		5.0	NaOH		65	65	2.3	Yoo *et al*., 1996
			5.5			78	78	3.9	
			6.0			79	79	4.9	
			6.5			78	78	4.9	
			6.0	NH_4OH		71		1.3	Kaufman *et al*., 1996
ATCC7469	Glucose	45				28	93	8.0	Siebold *et al*., 1995
		36				24	80		
					0.2%YE	34	110	0.5	Guoqiang *et al.,* 1991
					1%YE	26	81	2.6	
	Whey permeate	37				30	71	1.9	Mulligan and Gibbs.1991
	Glucose		6.2	$CaCO_3$		26	81	2.6	Guoqiang *et al.,* 1991
ATCC11443	Glucose				0.4%YE	53	66	2.8	Ho *et al.,* 1997
					0.8%YE	53	66	3.7	

CONCLUSION

Lactic acid bacterial (LAB) were isolated from various sources (waste from starch factories, dairy products, fermented vegetable and meat products). A total of 156 purified cultures were obtained from 57 samples of which 94 strains were homofermentative, the rest were heterofermentative. The characterization of the LAB that being suitable for industrial lactic acid fermentation were studied under nourished conditions with various concentrations of sodium lactate (3-20% w/v), glucose (3-30% w/v), pH 2-8 and evaluated for the product stereospecificity test. The strain code as DM3 was selected the best strain. It showed the highest lactic acid formation (16.6 g l^{-1}) and L-specific (92.8%). A ribosomal DNA-based identification of bacteria by 16S rDNA gene sequencing demonstrated the strain DM3 was closely related to *Lactobacillus rhamnosus* with similarities of 99.94%.

DM3 could function maximally under the optimal condition, i.e. pH 6.0, temperature of 40^{O}C, an agitation speed of 150 rpm and the initial glucose concentration of 100 g l^{-1}. The fed-batch mode did not seem to improve the fermentation efficiency. The selected strain DM3 could be applied effectively to produce lactic acid from cassava starch by SSF process. Cassava starch was initially liquefied by α-amylase (0.1% v/w) and subsequently saccharified and fermented, simultaneously, by glucoamylase (0.5% v/w) and DM3 (5% v/v). The yield of lactic acid by DM3 was 136.8 g l^{-1} and the productivity was 5.70 g $l^{-1}h^{-1}$, when the initial starch concentration was 150 g l^{-1}.

The efficiency of biopolar electrodialysis (BED) on recovery of free lactic acid from ammonium lactate salts in fermentation broths had been demonstrated by first a model solution of ammonium lactate and further applied to fermentation broth. Purification and concentration of ammonium lactate to free lactic acid after fermentation were demonstrated by four steps, firstly; cell mass removal by centrifugation, secondly; soluble solid removal by ultrafiltration membrane, thirdly; uncharged components and brown colour removal by MED, finally; free lactic acid recovery from ammonium hydroxide and cationic components by BED-anion. The

final lactic acid concentration increased by 32% and total required energy in both electrodialysis processes consisting of energy consumption for lactate transfer and for its electroconversion to lactic acid was about 2.76 $kW.h.kg^{-1}$. Furthermore, the remained ammonium hydroxide in feed stream could be recycled to use for next fermentation process. This work had demonstrated the possibility of purifying ammonium lactate to free lactic acid with the use of MED and BED-anion, which represents one of the most important membrane processes for environmentally clean technology in biochemical industries.

Yeast extract (YE) is required to improve the efficiency of lactic acid production by LAB. However, it is not likely preferred from an economical point of view. An effort to completely or partially substitute YE by agro-industrial wastes has been reported. Among several agro-industrial wastes used in this work, fish wastes obtained from canning process being acid-hydrolyzed prior to use exhibited the best promising performance especially when applied with a reduced amount of YE by 50%. With a partial supplementation of high-priced YE with the fish waste hydrolysates, a nutrient cost in lactic acid production can be reduced to some extent without a significant decrease in a performance of lactic acid production. This lower-priced nitrogen supplementation can be applied to lactic acid production from cassava chips and roots, which are less expensive and more preferred in the actual industry.

LITERATURE CITED

Abe, S. and M. Takagi. 1991. Simultaneous saccharification and fermentation of cellulose to lactic acid. **Biotechnol. Bioeng.** 37: 93–96.

Akerberk, C., K. Hofvendahl, G. Zacchi and B.H. Hagerdal. 1998. Modeling the influence of pH, temperature, glucose, and lactic acid concentrations on the kinetics of lactic acid production by *Lactococcus lactis* ssp. *lactis* ATCC 19435 in whole-wheat flour. **Appl. Microbiol. Biotechnol.** 49: 682-690.

Akerberg, C. and G. Zacchi. 2000. An economic evaluation of the fermentative production of lactic acid from wheat flour. **Biores. Technol**. 75: 119-126.

Amass, W., A. Amass and B. Tighe. 1998. A review of biodegradable polymers: uses, current developments in the synthesis and characterization of biodegradable polymers, blends of biodegradable polymers and recent advances in biodegradation studies. **Polym. Int.** 47: 89-114.

Amrane, A. 2000. Evaluation of lactic acid bacteria autohydrolyzate for the supplementation of lactic acid bacteria fermentation. **World J. Microbiol. Biotechnol.** 16: 207–209.

Anuradha, R., A.K. Suresh and K.V. Venkatesh. 1999. Simultaneous saccharification and fermentation of starch to lactic acid. **Proc. Biochem**. 35: 367-375.

A.O.A.C. 1980. **Official Methods of Analysis**, 13th Edn. Association of Official Agricultural Chemist, Washington, DC.

Arasaratnam, V., A. Senthuran and K. Balasubramaniam. 1996. Supplementation of whey with glucose and different nitrogen sources for lactic acid production by *Lactobacillus delbrueckii.* **Enzyme Microbiol. Technol.** 19: 482-486.

ASTOM Corporation. 2007. **NEOSEPTA BIPOLAR.** Available Source: http://www.astom-corp.jp/en/en-main2-bipolar.html

Axelsson, L.T. 1993. Lactic Acid Bacteria: Classification and Physiology, pp. 1-63. *In*: Salminen, S. and A. Wright eds. **Lactic Acid Bacteria**. Marcel Dekker, New York, USA.

Axelsson, L. 2004. Lactic Acid Bacteria: Classification and Physiology, pp. 1-66. *In:* Salminen, S., A. von Wright, and A. Ouwehand eds. **Lactic acid bacteria: microbiological and functional aspects.** 3rd rev. and exp. ed. Marcel Dekker, Inc., New York.

Bai, D.M., Q. Wei, Z.H. Yan, X.M. Zhao, X.G. Li and S.M. Xu. 2003. Fed-batch fermentation of *Lactobacillus lactis* for hyper-production of L-lactic acid. **Biotechnol. Lett.** 25: 1833-1835.

Bailly, M. 2002. Production of organic acids by bipolar electrodialysis: realizations and perspectives. **Desalination.** 144: 157-162.

Beatriz, R., A. B. Moldes and J.M. Domınguez, J.C. Parajo. 2004. Lactic acid production from corn cobs by simultaneous saccharification and fermentation: a mathematical interpretation. **Enzyme Microbiol. Technol.** 34: 627–634

Berry, A.R., C.M.M. Franco, W. Zhang and A.P.J. Middelberg. 1999. Growth and lactic acid production in batch culture of *Lactobacillus rhamnosus* in a defined medium, **Biotechnol. Lett.** 21: 163–167.

Bibal, B., G. Goma, Y. Vassier and A. Pareilleux. 1988. Influence of pH, lactose and lactic acid on the growth of *Streptococcus cremoris*: a kinetic study. **Appl. Microbial. Biotechnol.** 28: 340-344.

Boniardi, N., R. Rota, G. Nano and B. Mazza. 1997. Lactic acid production by electrodialysis Part I: Experimental tests. **J. Appl. Electrochem.** 27: 125-133.

Borzani, W., P.A. Sanchez-Podlech, M.F. Luna, R. Jerkep, M.A.C.F. Stein. Kinetics of semicontinuous microbial transformation of whey by *Lactobacillus bulgaricus* varying the initial concentration of yeast autolysate. **J. Biotechnol**. 31: 61-66.

Boyaval, P., C. Corre and S. Terre. 1987. Continuous lactic acid fermentation with concentrate product recovery by ultrafiltration and electrodialysis. **Biotechnol. Lett.** 9: 207-212.

Burgos-Rubio, C.N., M.R. Okos and P.C. Wankat. 2000. Kinetic study of the conversion of different substrates to lactic acid using *Lactobacillus bulgaricus*. **Biotechnol. Progr**. 16: 305– 314.

Bustos, G., A.B. Moldes, J.M. Cruz and J.M. Domnguez. 2004. Production of fermentable media from vine-trimming wastes and bioconversion into lactic acid by *Lactobacillus pentosus*. **J. Sci. Food Agric.** 84: 2105–2112.

Chen, R. and Y.Y. Lee. 1997. Membrane-mediated extractive fermentation for lactic acid production from cellulosic biomass. **Appl. Biochem. Biotechnol.** 63-65: 435-438.

Choi, J.H., S.H. Kim and S.H. Moon. 2002. Recovery of lactic acid from sodium lactate by ion substitution using ion-exchange membrane. **Sep. Purif. Technol.** 28: 69-79.

Christine, P.M., H.H. Huss and L Gram. 1999. Characterization of lactic acid bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as substrate for fermentation. **Int. J. of Food Microbiol.** 46: 219-229.

Chukwu, U.N. and M. Cheryan. 1999. Electrodialysis of acetate fermentation broths. **Appl. Biochem. Biotechnol.** 77-79: 485-499.

Czytko, M., K. Ishii and K. Kawai. 1987. Continuous glucose fermentation for lactic acid production: recovery of acid by electrodialysis. **Chem. Ing. Tech.** 59: 952-954.

Danner, H., L. Madzingaidzo, M. Holzer, L. Mayrhuber and R. Braun. 2000. Extraction and purification of lactic acid from silages. **Biores. Technol.** 75: 181-187.

Datta, R., S.P. Tsai., P. Bonsignore and S.H. Moon. 1995. Technological and economic potential of poly(lactic acid) and lactic acid derivatives. **FEMS Microbial. Rev.** 16: 221-231.

Datta, R. and M. Henry. 2006. Lactic acid: recent advances in products, processes and technologies-a review. **J. Chem. Technol. Biotechnol.** 81:1119–1129.

Davidson, B.E., R.M. Llanos, M.R. Cancilla, N.C. Redman and A.J. Hillier. 1995. Current research on the genetics of lactic acid production in lactic acid bacteria. **Int. Dairy J.** 5: 763-784.

Dimerci, A., A.L. Pometto and K.E. Johnson. 1993. Lactic acid production in a mixed culture biofilm reactor. **Appl. Environ. Microbiol.** 59:203–207.

Ercolini, D., G. Moschetti, G. Blaiotta and S. 2001. Coppola. Behavior of variable V3 region from 16S rDNA of lactic acid bacteria in denaturing gradient gel electrophoresis. **Curr. Microbiol.** 42: 199-202.

Fadda, S., G. Oliver and G. Vignolo. 2002. Protein degradation by *Lactobacillus plantarum* and *Lactobacillus casei* in a sausage model system. **J. of Food Sci.** 67(3): 1179-1183.

Fitzpatrick, J.J. and U.O. Keeffe. 2001. Influence on whey protein hydrolyste addition to whey permeate batch fermentations for producing lactic acid. **Proc. Biochem.** 37: 183–186.

Franken, T. 2000. Bipolar membrane technology and its applications. **Membrane Technol.** 125: 8-11.

Fu, W. and A.P. Mathews. 1999. Lactic acid production from lactose by *Lactobacillus plantarum*: Kinetic model and effects of pH, substrate, and oxygen, **Biochem. Engineer. J.** 3: 163–170.

Gao, M-t, M. Hirata, M. Koide, H. Takanashi and T. Hano. 2004. Production of L-lactic acid by electrodialysis fermentation (EDF). **Proc. Biochem.** 39: 1903-1907.

Gao, M-t., M. Koide, R. Gotou, H. Takanashi, M. Hirata and T. Hano. 2005. Development of a continuous electrodialysis fermentation system for production of lactic acid by *Lactobacillus rhamnosus*. **Proc. Biochem.** 40: 1033-1036.

Gao, M-t, M. Hirata, E. Toorisaka and T. Hano. 2006a. Study on acid-hydrolysis of spent cells for lactic acid fermentation. **Biochem. Engineer. J.** 28: 87-91.

Gao, M-t, M. Hirata, E. Toorisaka and T. Hano. 2006b. Acid-hydrolysis of fish wastes for lactic acid fermentation. **Biores. Technol.** 97: 2414-2420.

Gao, M-t, M. Hirata, E. Toorisaka and T. Hano. 2007. Lactic acid production with the supplementation of spent cells and fish wastes for the purpose of reducing impurities in fermentation broth. **Biochem. Engineer. J.** 36(3): 276-280.

Gauss, W.F., S. Suzuki and M. Takagi. 1976. **Manufacture of alcohol from cellulosic material.** US Patent 3, 990, Nov.

Ghofar, A., S. Ogawa and T. Kokugan. 2005. Production of L-lactic acid from fresh cassava roots slurried with tofu liquid waste by *Streptococcus bovis*. **J. of Bioscie. Bioeng.** 100(6): 606-612.

Ghose, T.K., P.K. Roychoudhury and P. Ghosh. 1984. Simultaneous saccharification and fermentation (SSF) of lignocellulosics to ethanol under vacuum cycling and step feeding. **Biotechnol. Bioeng.** 26: 377–381.

Gibson, V.C. 2008. **Poly (lactic acid) PLA**. Available Source: http://www.ch.ic.ac.uk/vgibson/research5.htm

Giraud, E., B. Lelong and M. Raimbault. 1991. Influence of pH and initial lactate concentration on the growth of *Lactobacillus plantarum*. **Appl. Microbiol. Biotechnol.** 36: 96-99.

Glassner, D.A. and R. Datta. 1990. **Process for Production and Purification of Lactic Acid.** EP 0 393 818 A1.

Goddard, J.S. and J.S.M. Perret. 2005. Co-drying fish silage for use in aquafeeds. **Animal Feed Sci. Technol**. 118: 337-342.

Goksungur, Y. and U. Guvenc. 1999. Batch and continuous production of lactic acid from beet molasses by immobilized *Lactobacillus delbrueckii* IFO 3202. **J. Chem. Technol. Biotechnol.** 74: 131–136.

Goncalves, L.M.D., A.M.R.B. Xavier, J.S. Almeida and M.J.T. Carronda. 1991. Concomitant substrate and product inhibition kinetics in lactic acid production. **Enzyme Microbiol. Technol**. 13: 314-319.

Goncalves, L.M.D., A. Ramos, J.S. Almeida, A.M.R.B. Xavier and M.J.T. Carrondo. 1997. Elucidation of the mechanism of lactic acid growth inhibition and production in batch cultures of *Lactobacillus rhamnosus*. **Appl. Microbiol. Biotechnol.** 48: 346-350.

Guoqiang, D., R. Kaul and B. Mattiasson. 1991. Evaluation of alginate-immobilized *Lactobacillus casei* for lactate production. **Appl. Microbiol. Biotechnol.** 36: 309 –314.

Habova, V., K. Melzoch, M. Rychtera and B. Sekavova. 2004. Electrodialysis as a useful technique for lactic acid separation from a model solution and a fermentation broth. **Desalination.** 163: 361-372.

Hammes, W.P., A.Bantleon and S. Min. 1990. Lactic acid bacteria in meat fermentation. **FEMS Microbiol. Rev.** 87: 165– 173.

Hassan, K.S., B. Moldes, G.K. Richard and J.S. Richard. 2001. Lactic acid production by simultaneous saccharification and fermentation of alfalfa fiber. **J. Biosc. Bioeng.** 92: 518-523.

Heriban, V., J. Gkgra, E. Gturdik and J. Ilavskj. 1993. Isolation of free lactic acid using electrodialysis. **Biotechnol. Technol.** 7: 63-68.

Hilde, M.O., J. Treimo and J.A. Narvhus. 2005. Effect of temperature on growth and metabolism of probiotic bacteria in milk. **Int. Dairy J.** 15: 989-997.

Ho, K.L.G., A.L. Pometto and P.N. Hinz. 1997. Optimization of L-(1)-lactic acid production by ring and disc plastic composite supports through repeated-batch biofilm fermentation. **Appl. Environ. Microbiol.** 63: 2533– 2542.

Hofvendahl, K., C. Akerberg, G. Zacchi and B. Hahn-Hägerdal. 1999. Simultaneous enzymatic wheat starch saccharifiaction and fermentation to lactic acid by *Lactococcus lactis.* **Appl. Microbiol. Biotechnol.** 52: 163–169.

Hofvendahl, K. and B. Hahn-Hagerdal. 1997. L-lactic acid production from whole wheat flour hydrolysates using strains of Lactobacilli and Lactococci. **Enzyme Microbiol. Technol.** 20: 301-307.

Hofvendahl, K. and B. Hahn-Hagerdal. 2000. Factors affecting the fermentative lactic acid production from renewable resources. **Enzyme Microbiol. Technol.** 26: 87-107.

Holzapfel, W.H., P. Haberer, R. Geisen, J. Björkroth and U. Schillinger. 2001. Taxonomy and important features of probiotic microorganisms in food nutrition. **Am. J. Clin. Nutr**. 73: 365S-373S.

Hongo, M., Y. Nomura and M. Iwahara. 1986. Novel method of lactic acid production by electrodialysis fermentation. **J. Appl. Environ. Microbiol**. 52(2): 314-319.

Huang, L.P., B. Jin, P. Lant and J. Zhou. 2003. Biotechnological production of lactic acid integrated with potato wastewater treatment by *Rhizopus arrhizus*. **J. Chem. Technol. Biotechnol.** 78: 899-906.

Hujanen, M. and Y.Y. Linko. 1996. Effect of temperature and various nitrogen sources on L (+) lactic acid production by *Lactobacillus casei.* **Appl. Microbiol Biotechnol**. 45: 307-313.

Hujanen, M., S. Linko, Y.Y. Linko and M. Leisola. 2001. Optimisation of media and cultivation conditions for L(+) lactic acid production by *Lactobacillus casei* NRRL B-441. **Appl. Microbiol. Biotechnol.** 56: 126-130.

Jay, J.M. 2000. Fermentation and fermented dairy products, pp. 113-130. *In*: **Modern Food Microbiology**, 6th edition. An Aspen Publication, Aspen Publishers, Inc. Gaithersburg, USA.

Jin, B., L.P. Huang and P. Lant. 2003. *Rhizopus arrhizus*-a producer for simultaneous saccharification and fermentation of starch waste materials to L(+)-lactic acid, **Biotechnol. Lett.** 25: 1983–1987.

John, R.P., K.M. Nampoothiri and A. Pandey. 2006a. Solid-state fermentation for L-lactic acid production from agro wastes using *Lactobacillus delbrueckii*. **Proc. Biochem.** 41: 759–763.

John, R.P., K.M. Nampoothiri and A. Pandey. 2006b. Simultaneous saccharification and L-(+)-lactic acid fermentation of proteasetreated wheat bran using mixed culture of lactobacilli. **Biotechnol. Lett.** 28: 1823–1826.

John, R.P., K.M. Nampoothiri and A. Pandey. 2007. Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. **Appl. Microbiol. Biotechnol.** 74: 524-534.

Jong, I.L.C. and W.H. Hong. 1999. Recovery of lactic acid by batch distillation with chemical reactions using ion exchange resin. **Japan J. Chem. Eng.** 32: 56-59.

Kashket, E.R. 1987. Bioenergetics of lactic acid bacteria: cytoplasmic pH and osmotolerance. **FEMS Microbiol. Rev.** 46: 233– 244.

Kaufman, E.N., S.P. Cooper, M.K. Budner and G.R. Richardson. 1996. Continuous and simultaneous fermentation and recovery of lactic acid in a biparticle fluidized-bed bioreactor**. Appl. Biochem. Biotechnol**. 57–58: 503–515.

Kim, Y.H. and S.H. Moon. 2001. Lactic acid recovery from fermentation broth using one-stage electrodialysis. **J. Chem. Technol. Biotechnol**. 76: 169-178.

Konings, W.N., J. Kok, O.P. Kuiters and V. Poolman. 2000. Lactic acid bacteria: The bugs of new millennium. **Current Opinions in Microbiol.** 3: 276-282.

Kotzamanidis, C., T. Roukas and G. Skaracis. 2002. Optimization of lactic acid production from beet molasses by *Lactobacillus delbrueckii* NCIMB 8130. **World J. Microbiol. Biotechnol.** 18: 441–448.

Krishnan, S., S. Bhattacharya and N.G. Karanth. 1998. Media optimization for production of lactic acid by *Lactobacillus plantarum* NCIM 2084 using response surface methodology. **Food Biotechnol.** 12: 105–121.

Kumar, Dutta, S., A. Mukherjee and P. Chakraborty. 1996. Effect of product inhibition on lactic acid fermentation: simulation and modeling. **Appl. Microbiol. Biotechnol.** 46: 410-413.

Kurbanoglu, E.B. and N.I. Kurbanoglu. 2003. Utilization for lactic acid production with a new acid hydrolysis of ram horn waste. **FEMS Microbiol. Lett.** 225: 29-34.

Kwon, S., P.C. Lee, E.G. Lee, Y.K. Chang and N. Chang. 2000. Production of lactic acid of *Lactobacillus rhamnosus* with vitamin-supplemented soybean hydrolysate. **Enzyme Microbiol. Technol.** 26: 209-215.

Lee, K. 2005. A media design program for lactic acid production coupled with extraction by electrodialysis. **Biores. Technol.** 96: 1505-1510.

Lee, E.G, S.H. Moon, Y.C Chang, I.K. Yoo and H.N. Chang. 1998. Lactic acid recovery using two-stage electrodialysis and its modelling. **J. Membr. Sci.** 145: 53-66.

Li, H., M. Roberta, J.K. Christopher, S. Wolfgang, S. Garry, D. Ian and A.J. Simon. 2004. An electrokinetic bioreactor: using direct electric current for enhanced lactic acid fermentation and product recovery. **Tetrahedron.** 60: 655-661.

Liew, S.L., A.B. Ariff, A.R. Raha and Y.W. Ho. 2005. Optimization of medium composition for the production of a probiotic microorganism, *Lactobacillus rhamnosus*, using response surface methodology. **Int. J. of Food Microbiol.** 102: 137-142.

Linko, Y.Y. and P. Javanainen. 1996. Simultaneous liquefaction, saccharification, and lactic acid fermentation on barley starch. **Enzyme Microbiol. Technol.** 19: 118–123.

Liu, T., S. Miura, M. Yaguchi, T. Arimura, E.Y. Park and M. Okabe. 2006. Scale-up of l-lactic acid production by mutant strain *Rhizopus* sp. MK-96-1196 from 0.003m3 to 5m3 in airlift bioreactors. **J. Biosci. Bioeng.** 101: 9–12.

Luo, J., L. Xia, J. Lin and P. Cen. 1997. Kinetics of simultaneous saccharification and lactic acid fermentation processes. **Biotechnol. Prog.** 13: 762-767.

Macedo, M.G., C. Lacroix, N.J. Gardner and C.P. Champagne. 2002. Effect of medium supplementation on exopolysaccharide production by *Lactobacillus rhamnosus* RW-9595M in whey permeate. **Int. Dairy J.** 12: 419-426.

Madzingaidzo, L. H. Danner and R. Braun. 2002. Process development and optimization of lactic acid purification using electrodialysis. **J. Biotechnol**. 96: 223-239.

Marinova, M., G. Kyuchoukov, J. Albet, J. Molinier and G. Malmary. 2004. Separation of tartaric and lactic acid by means of solvent extraction. **Sep. Purif. Technol.** 37: 199-207.

Martak, J., S. Schlosser, E. Sabolova, L. Kristofikova and M. Rosenberg. 2003. Fermentation for lactic acid with *Rhizopus arrhizus* in a stirred tank reactor with a periodical bleed and feed operation. **Proc. Biochem.** 38: 1573–1583.

Mattey, M. 1992. The production of organic acids. **CRC. Critic. Rev. Biotechnol.** 12: 87–132.

Mauro, M. and F. Sappino. 2000. Electrodialytic recovery of some fermentation products from model solutions: techno-economic feasibility study. **J. Membr. Sci.** 164: 129-140.

Mehaia, M. and M. Cheryan. 1987. Production of lactic acid from sweet whey permeate concentrates. **Proc. Biochem.** 22: 185-188.

Miura, S., T. Arimura, M. Hoshino, M. Kojima, L. Dwiarti and M. Okabe. 2003. Optimizaiton and scale-up of l-lactic acid fermentation by mutant strain *Rhizopus* sp. MK-96-1196 in airlift bioreactors. **J. Biosci. Bioeng.** 96: 65–69.

Mohd, Adnan, A.F. and I.K.P. Tan. 2007. Isolation of lactic acid bacteria from Malaysian foods and assessment of the isolates for industrial potential. **Biores. Technol.** 98: 1380-1385.

Moldes, A.N., J.L. Alonso and J.C. Parajo. 2001. Strategies to improve the bioconversion of processed wood into lactic acid by simultaneous saccharification and fermentation. **J. Chem. Technol. Biotechnol.** 76: 279–284.

Monteagudo, J.M., L. Rodriguez, J. Rincon and J. Fuertes. 1994. Optimization of the conditions of the fermenatation of beet molasses to lactic acid by *Lactobacillus delbrueckii*. **Acta. Biotechnol.** 14: 251-260.

Mulligan, C.N. and B.F. Gibbs. 1991. Batch conversion of whey permeate to ammonium lactate by *Streptococcus cremoris*. **Biotechnol. Appl. Biochem.** 14: 41–53.

Nancib, A., N. Nancib, D. Meziane-Cherif, A. Boudenbir, M. Fick and J. Boudrant. 2005. Joint effect of nitrogen sources and B vitamin supplementation of date juice on lactic acid production by *Lactobacillus casei* subsp. *rhamnosus*. **Biores. Technol.** 96: 63–67.

Narayanan, N., P.K. Roychoudhury, A. Srivastava. 2004. **L (+) lactic acid fermentation and its product polymerization.** Available Source: http://www.ejbiotechnology.info/content/vol7/issue2/full/7/index.html

Naveena, B.J. 2004. **Amylolytic bacterial L(+) lactic acid production in solid state fermentation and molecular identification of the strain.** Ph.D. thesis, Osmania University, Hyderabad, India.

Nexant. 2008. **Biotech Route to Lactic Acid/ Polylactic Acid.** Available Source: http://nexant.ecnext.com/coms2/summary_0255-130_ITM

Niel, E.W.J. and B. Hahn-Hagerdal. 1999. Nutrients requirements of lactococci in defined growth media. **Appl. Microbiol. Biotechnol.** 52: 617–627.

Nolasco-Hipolito, C., T. Matsunaka, G. Kobayashi, K. Sonomoto and A. Ishizaki. 2002. Synchronised fresh cell bioreactor system for continuous L(+) lactic acid production using *Lactococcus lactis* IO-1 in hydrolysed sago starch. **J. Biosci Bioeng.** 93: 281-287.

Nomura, Y., M. Iwahara and M. Hongo. 1987. Lactic acid production by electrodialysis fermentation using immobilized growing cells. **Biotechnol. Bioeng.** 30: 788-793.

Noonpakdee, W., C. Santivarangkna, P. Jumriangrit, K. Sonomoto and S. Panyim. 2003. Isolation of nisin-producing *Lactococcus lactis* WNC 20 strain from nham, a traditional Thai fermented sausage. **Int. J. of Food Microbiol.** 81: 137-145.

Oh, H., Y.J. Wee, J.S. Yun, S.H. Han, S. Jung and H.W. Ryu. 2005. Lactic acid production from agricultural resources as cheap raw materials. **Biores. Technol.** 96: 1492-1498.

Oh, S., S. Rheem, J. Sim, S. Kim and Y. Baek. 1995. Optimizing conditions for the growth of *Lactobacillus casei* YIT 9018 in tryptone-yeast extract-glucose medium by using response surface methodology. **Appl. Microbiol. Biotechnol.** 61: 3809-3814.

Olmos-Dichara, A., F. Ampe, J.L. Uribelarrea, A. Pereilleux and G. Coma. 1997. Growth and lactic acid production by *Lactobacillus casei* spp. *rhamnosus* in bath and membrane bioreactor: influence of yeast extract and tryptone enrichment. **Biotechnol. Lett.** 19: 709-714.

Pauli, T. and J.J. Fitzpatrick. 2002. Malt combing nuts as a nutrient supplement to whey permeate for producing lactic by fermentation with *Lactobacillus casei*. **Proc. Biochem.** 38: 1–6.

Payot, T., Z. Chemaly and M. Fick. 1999. Lactic acid production by *Bacillus coagulans*-Kinetic studies and optimization of culture medium for batch and continuous fermentations. **Enzyme Microbiol. Technol.** 24: 191-199.

Philippidis, G.P., T.K. Smith and C.E. Wyman. 1993. Study of the enzymatic hydrolysis of cellulose for production of fuel ethanol by the simultaneous saccharification and fermentation process. **Biotechnol. Bioeng.** 41: 846–853.

Pourcelly, G. 2002. Electrodialysis with bipolar membranes: principles, optimization and applications. **Russian J. of Electrochem.** 38(8): 919-926.

Rincon, J., J. Fuertes, A. Moya, J.M. Aontegudo and L. Rodriguez. 1993. Optimization of the fermentation of whey by *Lactobacillus casei.* **Acta Biotechnol**. 13: 323-331.

Rivas, B., A.B. Moldes, J.M. Dominguez and J.C. Parajo. 2004. Development of culture media containing spent yeast cells of *Debaryomyces hansenii* and corn steep liquor for lactic acid production with *Lactobacillus rhamnosus*. **Inter. J. of Food Microbiol.** 97: 93-98.

Rojan, P.J., K.M. Nampoothiri, A.S. Nair and A. Pandey. 2005. L(+)-Lactic acid production using *Lactobacillus casei* in solid-state fermentation. **Biotechnol. Lett.** 27: 1685–1688.

Rojan, P.J., K.M. Nampoothiri, A.S. Nair and A. Pandey. 2007. Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. **Appl. Microbiol. Biotechnol.** 74: 524-534.

Rosen, H. 1957. A modified ninhydrin colorimetric analysis of amino acids. **Arch. Biochem. Biophys.** 67: 10–15.

Rosenberg, M. and L. Kristofikova. 1995. Physiological restriction of the l-lactic acid production by *Rhizopus arrhizus*. **Acta Biotechnol.** 15: 367–374.

Roukas, T. and P. Kotzekidou. 1998. Lactic acid production from de-proteinized whey by mixed cultures of free and coimmobilized *Lactobacillus casei* and *Lactococcus lactic* cells using fedbatch culture. **Enzyme Microbiol. Technol.** 22: 199-204.

Roy, S., R.D. Gudi, K.V. Venkatesh and S.S. Shah. 2001. Opimal control strategies for simultaneous saccharification and fermentation of starch. **Proc. Biochem.** 36: 713-722.

Ruanglek, V., D. Maneewatthana and S. Tripetchkul. 2006. Evaluation of Thai agro-industrial wastes for bio-ethanol production by *Zymomonas mobilis*. **Proc. Biochem.** 41: 1432-1437.

Schepers, A.W., J. Thibault and C. Lacrox. 2002. *Lactobacillus helveticus* growth and lactic acid production during pH controlled bath cultures in whey permeate/yeast extract medium. Part I. multiple factor kinetic analysis. **Enzyme Microbiol. Technol.** 30: 176-186.

Siebold, M., P. Frieling, R. Joppien, D. Rindfleisch, K. Schugerl, H. Roper. 1995. Comparison of the production of lactic acid by three different *Lactobacilli* and its recovery by extraction and electrodialysis. **Proc. Biochem.** 30: 81–95.

Senthuran, A., V. Senthuran, B. Mattiasson and R. Kaul. 1997. Lactic acid fermentation in a recycle batch reactor using immobilized *Lactobacillus casei*. **Biotechnol Bioeng.** 55: 843–853.

Senthuran, A., V. Senthuran, R. Hatti-Kaul and B. Mattiasson. 1999. Lactic acid production by immobilized *Lactobacillus casei* in recycle batch reactor: a step towards optimization. **J. of Biotechnol.** 73: 61-70.

Sethi, V. and S.B. Maini. 1999. **Biotechnology: food fermentation**. Asiatech Publishers, New Delhi.

Shirai, K., I. Guerrero, S. Huerta, G. Saucedo, A. Gastillo, R.O. Gonzalez and G.M. Hall. 2001. Effect of initial glucose concentration and inoculation level of lactic acid bacteria in shrimp waste ensilation. **Enzyme Microbiol. Technol.** 28: 446-452.

Somogyi, M. 1952. Notes on sugar determination. **J. Biol. Chem.** 195: 19-23.

Sreenath, H.K., A.B. Moldes, R.G. Koegel and R.J. 2001. Lactic acid production by simultaneous saccharification and fermentation of alfalfa fiber. **J. Biosci. Bioeng.** 92: 518-523.

Stenberg, K., M. Galbe and G. Zacchi. 2000. The influence of lactic acid formation on the simultaneous saccharification and fermentation of softwood to ethanol. **Enzyme Microbiol. Technol.** 26: 71–79.

Sriroth, K., S. Wanlapatit, K. Piyachomkwan and C.G. Oates. 1998. Improved cassava starch granule stability in the presence of sulphur dioxide. **Starch/Starke.** 50**:** 466–473.

Sunhoon, K., P.C. Lee, E.G. Lee, Y.K. Chang and N. Chang. 2000. Production of lactic acid by *Lactobacillus rhamnosus* with vitamin-supplemented soybean hydrolysate. **Enzyme Microbiol. Technol.** 26: 209-215.

Stanier, R.Y., J.L. Ingraham, J.L. Wheelis and P.R. Painter. 1986. **General microbiology**. Macmillan, London.

Stiles, M.E. and W.H. Holzapfel. 1997. Review article: Lactic acid bacteria of foods and their current taxonomy. **Int. J. Food Microbiol.** 36: 1-29.

Tay, A. and S.T. Yang. 2002. Production of l(+)-lactic acid from glucose and starch by immobilized cells of *Rhizopus oryzae* in a rotating fibrous bed bioreactor. **Biotechnol. Bioeng.** 80: 1–12.

The Commission of the European communities. 1999. Commission Directive 1999/79/EC. **Official J. of the European Communities.** 209: 23-27.

Timbuntam, W., K. Sriroth and Y. Tokiwa. 2006. Lactic acid production from sugar-cane juice by a newly isolated *Lactobacillus* sp. **Biotechnol. Lett.** 28: 811–814.

Tom, R. 2007. **Index of /tomsplace/scientific/phd/Introduction.** Available Source: http://www.brighton73.freeserve.co.uk/tomsplace/scientific/phd/Introduction/

Tomas, J.R. 2006. **The development of a novel antifungal silage inoculant.** Available Source: www.brighton73.freeserve.co.uk/tomsplace/scientific/phd/Introduction/

Tomasz, L., C. Jones, X Lemercinier, A. Korzeniowska-Kowal, M. Strus, J. Rybka, A. Gamian and P.B. Heczko. 2003. Structural analysis of the *Lactobacillus rhamnosus* strain KL37C exopolysaccharide. **Carbohydrate Research.** 338: 605-609.

Venkatesh, K.V. 1997. Simultaneous saccharification and fermentation of cellulose to lactic acid. **Biores. Technol.** 62: 91–8.

Vernon, C.G. 2006. **Poly (lactic acid) (PLA).** Available Source: http://www.ch.ic.ac.uk/vgibson/research5.htm

VickRoy, T.B. 1985. Lactic acid, pp. 761-776. *In*: Blanch, H.W., S. Drew and D.I.C. Wang eds. **Comprehensive Biotechnology.** Vol. 3. Pergamon Press, Oxford.

Von Eysmondt, J. and C. Wandrey. 1990. Integrated product work-up by electrodialysis in the microbial production of organic acids. **J. Chem. Eng. Technol.** 62: 134-135.

Wang, Q., G. Cheng, X. Sun and B. Jin. 2006. Recovery of lactic acid from kitchen garbage fermentation broth by four-compartment configuration electrodialyzer. **Proc. Biochem.** 41: 152-158.

Wasewar, K.L., A.A. Yawalkar, J.A. Moulijn and V.G. Pangarkar. 2004. Fermentation of glucose to lactic acid coupled with reactive extraction: a review. **Ind. Eng. Chem. Res.** 43: 5969–5982

Wee, Y.J., J.N. Kim and H.W. Ryu. 2006a. Biotechnological production of lactic acid and its recent applications. **Food Technol. Biotechnol**. 44(2): 163-172.

Wee, Y.J., H.O. Kim, J.S. Yun and H.W. Ryu. 2006b. Pilot-scale lactic acid production via batch culturing of *Lactobacillus* sp. RKY2 using corn steep liquor as a nitrogen source. **Food Technol. Biotechnol.** 44(2): 293-298.

Wouters, J.A., H.H. Kamphuis, J. Hugenholtz, P. Kuipers, W.M. De Vos and T. Abee. 200. Changs in glycolytic activity of *Lactococcus lactis* induced by low temperature. **Appl. Environ. Microbiol.** 66: 3686-3691.

Yao, P.X. and K. Toda. 1990. Lactic acid production in electrodialysis culture. **J. Gen**. **Appl. Microbiol.** 36: 111-125.

Ye, K., S. Jin and K. Shimizu. 1996. Performance improvement of lactic acid fermentation by multistage extractive fermentation. **J. Ferment. Bioeng.** 81: 240–246.

Yoo, I.K., H.N. Chang, E.G. Lee, Y.K. Chang and S.H. Moon. 1996. Effect of pH on the production of lactic acid and secondary products in batch cultures of *Lactobacillus casei.* **J. Microbiol. Biotechnol.** 6: 482–486.

Yoo, I.K., H.N. Chang, E.G. Lee, Y.K. Chang and S.H. Moon. 1997. Effect of B vitamin supplementation of lactic acid production by *Lactobacillus casei*. **J. of Ferment. Bioeng.** 84(2): 172-175.

Yu, R.C. and Y.D. Hang. 1989. Kinetics of direct fermentation of agricultural commodities to L(+)-lactic acid by *Rhizopus oryzae*. **Biotechnol. Lett.** 11: 597–600.

Yun, J.S., Y.J. Wee, J.N. Kim and H.W. Ryu. 2004. Fermentative production of DL lactic acid from amylase treated rice and wheat brans hydrolysate by a novel lactic acid bacterium, *Lactobacillus* sp. **Biotechnol Lett.** 18:1613–1616.

Zhang, Z.N., B. Jin and J.M. Kelly. 2007. Production of lactic acid from renewable materials by *Rhizopus* fungi. **Biochem. Engineer. J.** 35(3): 251-263.

Zhou, S.D., T.A. McCaskey and J. Broder. 1995. Evaluation of nitrogen supplement for bioconversion of municipal solid waste for lactic acid. **Appl. Biochem. Biotechnol.** 57–58: 517–527.

APPENDIX

Appendix Table 1 Results of total LAB plate count from various fermented samples.

Sample	Code	Source	Total plate count on MRS agar (CFU/g)
Fermented cabbage	CH3	Chaiyaphum	1.54×10^7
Fermented bamboo	CH4	Chaiyaphum	1.28×10^4
Fermented fish	CH5	Chaiyaphum	1.66×10^7
Fermented pork	CH6	Chaiyaphum	1.67×10^6
Fermented cabbage	SS	Samutsongkhram	1.30×10^5
Fermented mango	CS	Chachurngsual	1.22×10^7
Fermented pork	DM	Donmerng	1.83×10^5
Yoghurt (Dutchie)	YD		1.7×10^6
Yoghurt (Richesse)	YR1		1.85×10^6
Yoghurt (Richesse)	YR2		7.30×10^7
Yoghurt (Foremost)	YF		3.90×10^6
Drinking yoghurt (Dutch Mill)	DD		2.00×10^3
Drinking yoghurt (Foremost)	DF		3.50×10^5
Drinking yoghurt (Yakult)	DY		2.10×10^7
Drinking yoghurt (Richesse)	DR		1.62×10^5
Yoghurt (Nestle, LC1)	YN		1.32×10^6
Drinking yoghurt (Betagen)	DB		9.60×10^6
Fermented mollusk	KU2	Kasetsart University	8.50×10^6
Cassava pulp	CB	Chonburi	4.10×10^6
Fermented cassava pulp	CB(R2)	Chonburi	5.40×10^4

Appendix Table 1 (Continued)

Sample	Code	Source	Total plate count on MRS agar (CFU/g)
Fermented bamboo	BK1	Bangkla	7.90×10^3
Fermented cabbage1	BK2	Bangkla	5.25×10^6
Fermented cabbage 2	BK3	Bangkla	6.00×10^2
Fermented crab	BK5	Bangkla	$> 300 \times 10^4$
Fermented vegetable	BK6	Bangkla	2.00×10^6
Fermented cassava pulp	CP	Nakornratchasrima	3.10×10^6
Waste water	WW	Nakornratchasrima	1.13×10^7
Fermented pork	NM	Bangkhen, BKK	1.57×10^8
Fermented garlic	TB1	Tharadbangbaou, BKK	3.60×10^7
Fermented pork	TB2	Tharadbangbaou, BKK	1.31×10^8
Fermented fish	TB3	Tharadbangbaou, BKK	2.60×10^6
Fermented pork	TB4	Tharadbangbaou, BKK.	3.20×10^8
Fermented bamboo	KU5	Kasetsart university, BKK.	5.00×10^2
Fermented cassava pulp	FCP	Chonburi	1.85×10^7
Water from line	WL	Chonburi	7.60×10^5
Fermented pork 1	CM1	Changmai	5.50×10^8
Fermented pork 2	CM2	Changmai	1.45×10^8
Fermented pork 3	CM3	Changmai	3.63×10^8
Fermented pork 4	CM4	Changmai	1.55×10^9
Fermented pork 5	CM5	Changmai	1.63×10^8
Waste water	AT	Aungtong	4.10×10^6
Fermented pork	BA	Bangkok	5.30×10^8
Fermented fish	SP1	Saphanmai, BKK	1.00×10^3
Fermented crab water	SP2	Saphanmai, BKK	3.90×10^3

Appendix Table 1 (Continued)

Sample	Code	Source	Total plate count on MRS agar (CFU/g)
Fermented pork 1	CHK1	Charunsanitwong, BKK	4.40×10^8
Fermented pork 2	CHK2	Charunsanitwong, BKK	1.06×10^9
Fermented pork 1	CHR1	Changrai	3.36×10^8
Nham Changrai 2	CHR2	Changrai	1.79×10^9
Waste water	KP1	Kumpangpet	1.27×10^7
Fermented cassava pulp	KP2	Kumpangpet	3.23×10^7
Fermented olive	SMS	Samutsongkram	4.50×10^6
Fermented cabbage	YT1	Yasothorn	5.10×10^6
Sausage	YT2	Yasothorn	3.00×10^6
Fermented pork	YT3	Yasothorn	3.70×10^6
Waste water1	ND1	Nakornphrathom	4.10×10^6
Waste water2	ND2	Nakornphrathom	1.10×10^6
Noodle	ND3	Nakornphrathom	3.00×10^7

Appendix Table 2 Lactic acid bacteria from culture collection.

Strain	TISTR No.		Medium	Condition
Lactobacillus plantarum	926	NRIC 1838	MRS	37^{O}C
Lactobacillus delbrueckii	326	NRRL B-763	MRS	37^{O}C
Lactobacillus bulgaricus	451	TUA 093L	MRS	37^{O}C
Lactobacillus delbrueckii ssp. bulgaricus	895	ATCC 33409	MRS	37^{O}C
Lactabacillus bulgaricus	1339	DMCU	MRS	37^{O}C
Lactobacillus delbrueckii ssp. lactis	785	ATCC 7830	MRS	37^{O}C
Lactobacillus amylovorus	1110	ATCC 33620	MRS	37^{O}C
Lactobacillus salivarius ssp. salivarius	1112	ATCC 11741	MRS	37^{O}C
Lactobacillus lactis	452	TUA 026L	MRS	37^{O}C
Lactobacillus casei	1340	DMCU	MRS	37^{O}C
Lactobacillus casei	1341	DMCU	MRS	37^{O}C
Lactobacillus casei ssp. rhamnosus	047	ATCC 7469	MRS	37^{O}C
Lactobacillus casei ssp. rhamnosus	372	TUA 333	MRS	37^{O}C
Lactobacillus casei	1463		MRS	37^{O}C
Lactobacillus lactis	1464		MRS	37^{O}C
Lactobacillus plantarum	1465		MRS	37^{O}C
Lactobacillus brevis	-		MRS	37^{O}C

ATCC: American Type Culture Collection, Rockville, Maryland, U.S.A

DMCU: Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok, Thailand

NRIC: NODAI Research Institute Culture Collection, Tokyo University of Agriculture, Tokyo Japan.

NRRL: Northern Regional Research Center, Agricultural Research Service, USDA, 1815 N. University St., Peoria, lllinois, U.S.A

Appendix Table 3 Results of evaluating result of lactic acid production and homofermentative test of isolated culture from fermented samples.

Code	pH	Cell density ($OD_{620\ nm}$)	Lactic acid formation* ($g\ l^{-1}$)	Types of fermentation	
				Homo	Hetero
CH3-1(white)	3.81	2.56	12.60	+	-
CH3-1(green)	4.41	1.46	6.30	-	+
CH3-2	3.85	2.47	10.35	+	-
CH3-3	3.55	2.11	11.7	+	-
CH3-4	3.80	2.37	12.15	+	-
CH3-5	3.84	2.62	11.25	+	-
CH3-6	3.83	2.59	12.60	+	-
CH3-7	3.92	1.74	9.00	-	+
CH5-1	4.25	1.63	6.75	-	+
CH5-2	4.14	1.80	8.10	-	+
CH5-3	3.88	1.72	8.55	-	+
CH6-1	3.70	2.37	9.45	+	-
CH6-2	4.12	0.74	12.60	+	-
CH6-3	3.58	2.80	12.60	+	-
CH6-4	3.70	2.52	9.90	+	-
SS1	3.83	2.48	9.90	+	-
SS2	3.75	3.17	12.15	+	-
SS3	3.84	2.57	11.25	+	-
SS4	3.67	2.26	11.25	+	-
CS1	3.94	2.61	14.85	+	-
CS2	3.65	9.98	15.30	+	-
CS3	3.87	4.58	16.2	+	-
DM1	3.77	2.15	12.15	+	-
DM2	3.93	4.48	14.85	+	-

Appendix Table 3 (Continued)

Code	pH	Cell density ($OD_{620\ nm}$)	Lactic acid formation* (g l^{-1})	Types of fermentation	
				Homo	Hetero
DM3	3.84	3.86	15.75	+	-
DM4	3.85	3.77	16.2	+	-
YD	4.23	0.80	5.85	+	-
YR1-1	4.82	0.71	5.4	+	-
YR1-2	4.65	0.59	4.5	+	-
YF	5.09	0.44	4.95	+	-
DD	4.68	0.44	4.05	+	-
DF1	4.94	0.46	3.6	+	-
DF2	4.44	0.76	5.85	+	-
DY	3.49	3.36	13.50	+	-
DY(R)	3.32	3.63	14.40	+	-
DR2	4.27	1.06	6.30	+	-
DR3	4.34	1.02	5.40	+	-
YN1	4.65	0.58	4.50	+	-
YN2	5.00	0.47	4.50	+	-
DB1	3.54	3.65	13.05	+	-
KU2-1	3.46	2.10	13.05	+	-
KU2-2	3.74	2.74	9.90	+	-
KU2-3	3.73	2.77	9.90	+	-
TB1-1	3.72	2.11	10.35	+	-
TB1-2	3.56	2.93	12.60	+	-
CB1	3.59	2.24	11.70	+	-
CB2	3.59	2.20	12.60	+	-
YR2-1	4.25	1.00	6.30	+	-
YR2-2	4.09	0.92	6.30	+	-
CB3	3.78	3.73	11.70	-	+

Appendix Table 3 (Continued)

Code	pH	Cell density ($OD_{620\ nm}$)	Lactic acid formation* ($g\ l^{-1}$)	Types of fermentation	
				Homo	Hetero
CB4	3.51	3.16	11.70	+	-
CB(R2)-1	3.29	2.78	15.30	+	-
CB(R2)-2	3.43	3.55	14.40	+	-
CB(R2)-Beef	3.44	3.50	14.40	+	-
BK1-1	3.77	2.51	11.70	-	+
BK2-1	3.61	3.12	11.70	+	-
BK2-2	3.68	2.90	11.70	+	-
BK3-1	4.33	0.48	6.30	+	-
BK5-1	4.13	0.95	7.20	+	-
BK6-1	3.48	4.57	13.50	+	-
BK6-2	3.51	3.39	14.40	+	-
$CP2_{cream}$	3.24	3.79	14.40	+	-
$CP2_{White}$	3.22	4.01	15.30	+	-
CP3	3.23	4.11	15.30	+	-
CP4	3.22	4.22	15.30	+	-
WW1	3.55	3.12	10.80	-	+
WW2	3.55	4.17	10.80	-	+
WW3	3.57	3.67	10.80	-	+
TB2-1	3.19	4.51	16.20	+	-
TB2-2	3.26	4.56	16.20	-	+
TB2-3	3.24	3.86	14.40	+	-
TB2-4	3.23	3.62	15.30	+	-
KU5	3.85	1.91	7.20	-	+
FCP2	3.19	3.60	15.30	+	-
AT1	3.71	1.18	7.20	+	-
AT2	3.78	1.18	7.20	-	+

Appendix Table 3 (Continued)

Code	pH	Cell density ($OD_{620\ nm}$)	Lactic acid formation* (g l^{-1})	Types of fermentation	
				Homo	Hetero
AT3	3.72	1.11	6.30	+	-
TB3-1	3.13	3.69	15.30	+	-
TB3-2	3.78	0.95	6.30	+	-
TB3-3	3.56	1.79	7.20	+	-
TB3-4	3.41	3.32	10.08	-	+
TB4-1	3.20	4.40	13.50	+	-
TB4-2	3.35	1.96	10.80	+	-
TB4-3	3.43	2.48	9.90	+	-
BA1	3.16	4.18	14.40	-	+
BA2	3.14	3.82	15.30	+	-
BA3	3.16	4.06	14.40	+	-
SP1-1	3.41	2.96	11.70	+	-
SP2-1	3.83	1.70	7.65	-	+
SP2-2	3.28	4.35	14.85	+	-
SP2-5	3.30	2.88	13.50	+	-
CHK1-1	3.79	2.18	8.10	-	+
CHK1-2	3.75	2.17	9.90	-	+
CHK1-4	3.76	2.24	9.0	-	+
CHK2-1	3.40	3.31	10.80	+	-
CHK2-2	3.21	4.73	15.30	+	-
CHK2-3	3.77	2.05	8.10	+	-
CHK2-4	3.19	4.61	14.40	+	-
CHK2-5	3.18	2.30	9.0	-	+
CHR1-1	3.28	4.53	13.50	+	-
CHR1-2	3.17	4.84	15.30	+	-
CHR1-3	3.41	3.32	13.50	+	-

Appendix Table 3 (Continued)

Code	pH	Cell density ($OD_{620\ nm}$)	Lactic acid formation* (g l^{-1})	Types of fermentation	
				Homo	Hetero
CHR1-4	3.35	2.95	11.70	+	-
CHR1-5	3.36	4.23	17.10	+	-
CHR2-1	3.38	4.28	15.30	+	-
CHR2-2	3.23	4.69	15.30	+	-
CHR2-3	3.44	2.18	10.80	+	-
CHR2-4	3.40	2.98	12.60	+	-
CHR2-5	3.22	4.20	14.40	+	-
KP1-2	3.21	4.41	16.20	+	-
KP2-2	3.22	4.62	15.30	+	-
SMS	5.11	0.55	3.60	+	-
YT1-1	3.75	1.36	10.8	+	-
YT1-2	4.04	0.79	8.10	-	+
YT1-3	3.77	1.41	10.8	+	-
YT1-4	3.91	1.08	9.0	+	-
YT2-1	4.10	1.58	7.20	-	+
YT2-2	4.13	1.57	7.20	+	-
YT3-2	3.51	4.49	15.30	+	-
YT3-3	4.02	2.06	9.90	-	+
ND1-1	3.71	4.06	12.60	-	+
ND1-2	3.93	1.24	6.30	+	-
ND1-3	3.72	4.33	6.30	-	+
ND2-1	3.73	3.47	11.70	+	-
ND2-2	3.72	3.48	12.6	+	-
ND2-3	3.76	3.65	7.20	+	-
ND2-4	3.76	2.70	15.30	-	+
ND3-1	3.54	4.63	13.50	+	-

Appendix Table 3 (Continued)

Code	pH	Cell density ($OD_{620\ nm}$)	Lactic acid formation* (g l^{-1})	Types of fermentation	
				Homo	Hetero
ND3-2	4.01	2.12	8.10	-	+
926	3.53	4.24	14.40	+	-
326	4.07	0.94	7.20	+	-
451	3.49	1.90	9.0	+	-
895	3.42	1.86	11.70	+	-
1339	3.25	1.90	10.80	+	-
785	4.05	0.73	6.30	+	-
1110	4.59	0.243	3.90	+	-
1112	3.17	3.97	15.30	+	-
452	3.45	3.08	11.70	+	-
1340	3.19	3.75	16.20	+	-
1341	3.26	3.45	15.90	+	-
047	3.21	4.03	15.30	+	-
372	3.24	3.30	15.90	+	-
1463	3.39	3.13	14.85	+	-
1464	3.55	2.75	13.50	+	-
1465	3.55	2.72	12.15	+	-
L. brevis	3.52	2.96	13.50	+	-
Kob-1	4.05	1.08	8.10	+	-
Kob-2	4.05	1.05	5.40	+	-
Kob-3	3.98	1.18	6.30	+	-

* The lactic acid production was performed at glucose concentration 20 g l^{-1}, pH 6 and temperature of 37^{O}C.

- no cell growth

Appendix Table 4 Results of lactic acid formation (g l^{-1}) at various concentrations of sodium lactate*.

Code	Lactate concentration (w/v)				
	3%	**5%**	**10%**	**15%**	**20%**
CH3-1 (white)	9.5	-	-	-	-
CH3-2	13.5	-	-	-	-
CH3-3	8.1	-	-	-	-
CH3-4	8.1	-	-	-	-
CH3-5	7.2	-	-	-	-
CH3-6	3.5	-	-	-	-
CH6-2	-	-	-	-	-
CH6-3	12.2	8.1	-	-	-
SS2	13.1	10.4	-	-	-
SS3	12.6	9.9	-	-	-
SS4	13.1	9.9	-	-	-
CS1	13.5	-	-	-	-
CS2	13.1	-	-	-	-
CS3	13.5	-	-	-	-
DM1	13.1	10.4	-	-	-
DM3	13.5	-	-	-	-

Appendix Table 4 (Continued)

Code	Lactate concentration (w/v)				
	3%	**5%**	**10%**	**15%**	**20%**
DY	10.4	7.7	-	-	-
DY (R)	11.3	-	-	-	-
DB	15.3	10.8	-	-	-
KU2-1	14.0	-	-	-	-
TB1-1	8.6	-	-	-	-
TB1-2	13.1	10.8	-	-	-
CB1	14.0	-	-	-	-
CB2	11.7	9.0	-	-	-
CB(R2)-1	14.4	10.8	-	-	-
CB(R2)-2	12.6	-	-	-	-
CB(R2)-bf	13.1	-	-	-	-
BK2-1	11.7	-	-	-	-
BK2-2	10.8	-	-	-	-
BK6-1	12.6	-	-	-	-
BK6-2	11.3	-	-	-	-
$CP2_{cream}$	14.4	11.7	-	-	-

Appendix Table 4 (Continued)

Code	Lactate concentration (w/v)				
	3%	**5%**	**10%**	**15%**	**20%**
$CP2_{white}$	13.5	-	-	-	-
CP3	14.4	11.3	-	-	-
CP4	13.1	-	-	-	-
TB2-1	13.1	-	-	-	-
TB2-3	13.5	11.7	-	-	-
TB2-4	12.2	-	-	-	-
FCP2	12.6	-	-	-	-
WL2	12.2	11.25	-	-	-
NM1	12.6	11.7	-	-	-
NM2	12.6	10.8	-	-	-
NM4	13.1	10.8	-	-	-
CM1-1	10.8	-	-	-	-
CM1-2	12.6	10.8	-	-	-
CM1-3	-	-	-	-	-
CM2-1	12.2	8.6	-	-	-

Appendix Table 4 (Continued)

Code	Lactate concentration (w/v)				
	3%	**5%**	**10%**	**15%**	**20%**
CM2-2	12.6	10.4	-	-	-
CM2-5	10.8	-	-	-	-
CM2-7	11.3	9.0	-	-	-
CM3-1	10.4	-	-	-	-
CM3-2	11.7	-	-	-	-
CM3-3	11.3	-	-	-	-
CM4-1	12.6	-	-	-	-
CM4-2	11.7	8.1	-	-	-
CM4-3	12.6	-	-	-	-
CM4-4	12.2	-	-	-	-
CM5-1	12.6	11.3	-	-	-
CM5-2	13.1	9.9	-	-	-
CM5-3	12.6	10.4	-	-	-
CM5-4	12.2	9.5	-	-	-
CM5-5	14.0	11.7	-	-	-

Appendix Table 4 (Continued)

Code	Lactate concentration (w/v)				
	3%	**5%**	**10%**	**15%**	**20%**
TB3-1	13.5	10.8	-	-	-
TB4-1	14.0	-	-	-	-
TB4-2	13.5	11.3	-	-	-
BA2	14.4	-	-	-	-
BA3	12.6	-	-	-	-
SP1-1	11.7	-	-	-	-
SP2-2	13.5	10.8	-	-	-
SP2-5	13.5	12.2	-	-	-
CHK2-1	12.6	-	-	-	-
CHK2-2	14.9	11.3	-	-	-
CHK2-4	12.6	11.3	-	-	-
CHR1-1	11.7	-	-	-	-
CHR1-2	12.6	9.9	-	-	-
CHR1-3	9.5	-	-	-	-
CHR1-4	13.1	10.4	-	-	-

Appendix Table 4 (Continued)

Code	Lactate concentration (w/v)				
	3%	**5%**	**10%**	**15%**	**20%**
CHR1-5	11.7	-	-	-	-
CHR2-1	12.2	-	-	-	-
CHR2-2	13.5	10.8	-	-	-
CHR2-3	8.6	-	-	-	-
CHR2-4	10.8	-	-	-	-
CHR2-5	13.5	10.8	-	-	-
KP1-2	13.5	-	-	-	-
KP2-2	13.1	-	-	-	-
YT1-1	13.5	-	-	-	-
YT1-3	14.9	10.8	-	-	-
YT3-2	-	-	-	-	-
ND2-1	14.0	10.8	-	-	-
ND2-2	14.0	10.4	-	-	-
ND3-1	12.6	10.8	-	-	-
926	13.1	9.0	-	-	-

Appendix Table 4 (Continued)

Code	Lactate concentration (w/v)				
	3%	**5%**	**10%**	**15%**	**20%**
326	14.4	11.7	-	-	-
895	14.4	-	-	-	-
1339	10.4	9.5	-	-	-
1112	14.0	11.3	-	-	-
452	12.6	-	-	-	-
1340	13.5	-	-	-	-
1341	12.6	10.8	-	-	-
047	13.1	11.3	-	-	-
372	14.9	11.3	-	-	-
1463	13.1	-	-	-	-
1464	13.5	10.4	-	-	-
1465	13.5	-	-	-	-
L. brevis	13.5	10.4	-	-	-

***** The lactic acid production was performed at glucose concentration 20 g l^{-1}, pH 6 and temperature of 37^{O}C.

- no cell growth

Appendix Table 5 Results of lactic acid formation (g l^{-1}) at various pH*.

Code	pH						
	2	**3**	**4**	**5**	**6**	**7**	**8**
CH3-1 (white)	-	-	10.4	11.3	12.6	12.6	13.5
CH3-2	-	11.7	16.7	18.5	18.0	18.5	18.9
CH3-3	-	-	9.9	10.8	11.7	10.8	11.3
CH3-4	-	-	13.1	14.0	13.5	13.1	13.5
CH3-5	-	-	5.9	9.9	11.7	12.6	13.5
CH3-6	-	-	8.6	11.3	11.7	11.7	12.6
CH6-2	-	-	-	8.1	8.1	7.7	7.7
CH6-3	-	-	14.0	17.1	18	17.1	18.5
SS2	-	10.4	11.3	13.5	14.0	14.0	14.0
SS3	-	-	10.4	13.1	14.4	14.4	13.1
SS4	-	9.9	16.7	17.6	17.1	17.1	17.6
CS1	-	-	12.2	17.1	17.1	17.1	17.6
CS2	-	11.3	14.9	15.8	17.1	17.1	17.1
CS3	-	-	16.2	17.6	18.9	19.4	20.3

Appendix Table 5 (Continued)

Code	pH						
	2	**3**	**4**	**5**	**6**	**7**	**8**
DM1	-	11.7	16.7	18.0	17.1	18.9	18.5
DM3	-	-	12.6	15.8	17.1	17.1	18.5
DY	-	-	13.1	15.8	17.1	16.7	18.5
DY (R)	-	-	13.5	15.3	17.1	18.0	19.4
DB	-	15.8	15.3	13.5	14.0	12.2	11.7
KU2-1	-	12.6	15.3	17.6	18.5	18.0	18.9
TB1-1	-	-	9.0	13.5	13.1	14.0	12.6
TB1-2	-	13.1	16.7	16.7	16.7	17.1	16.7
CB1	-	-	15.3	18.0	17.6	17.6	18.0
CB2	-	-	11.7	17.6	18.0	18.0	17.1
CB(R2)-1	-	-	13.5	14.9	14.0	17.1	17.6
CB(R2)-2	-	-	11.7	16.2	17.6	18.5	19.8
CB(R2)-bf	-	-	15.3	16.7	18.0	19.4	18.9
BK2-1	-	-	10.8	13.1	13.5	14.0	15.3
BK2-2	-	-	11.7	11.7	11.7	12.6	12.6

Appendix Table 5 (Continued)

Code	pH						
	2	**3**	**4**	**5**	**6**	**7**	**8**
BK6-1	-	-	13.5	15.3	15.3	15.8	16.2
BK6-2	-	-	14.0	15.3	15.3	14.4	14.4
$CP2_{cream}$	-	9.9	13.1	14.9	14.4	14.4	12.2
$CP2_{white}$	-	11.7	13.1	14.9	16.2	16.2	17.6
CP3	-	8.6	12.6	13.1	13.1	11.7	11.7
CP4	-	10.8	9.9	14.0	14.8	14.9	15.8
TB2-1	-	11.3	16.7	16.7	18.0	17.1	18.0
TB2-3	-	12.6	16.2	17.1	17.1	16.2	16.7
TB2-4	-	-	13.5	15.3	17.1	17.1	16.7
FCP2	-	-	15.3	18.5	19.4	19.4	18.0
WL2	-	-	16.2	16.2	17.1	18.9	18.0
NM1	-	-	14.9	17.1	17.1	18.5	17.6
NM2	-	9.0	14.9	16.7	17.1	18.0	17.1
NM4	-	9.45	16.2	17.1	17.6	18.5	17.1
CM1-1	-	-	14.9	16.7	17.1	16.7	15.8

Appendix Table 5 (Continued)

Code	pH						
	2	**3**	**4**	**5**	**6**	**7**	**8**
CM1-2	-	10.8	16.2	17.1	16.7	18.0	18.5
CM1-3	-	-	9.9	11.7	11.7	12.2	12.2
CM2-1	-	-	14.9	16.2	17.1	17.1	18.0
CM2-2	-	12.6	15.3	17.1	16.7	17.1	16.7
CM2-5	-	-	9.5	12.6	12.6	15.8	15.3
CM2-7	-	-	14.9	17.6	16.2	17.1	17.1
CM3-1	-	-	11.3	14.4	15.8	14.9	15.3
CM3-2	-	-	14.4	16.2	17.1	16.7	17.2
CM3-3	-	-	9.5	14.9	14.9	15.3	15.8
CM4-1	-	-	14.9	18.0	18.0	18.0	17.1
CM4-2	-	-	15.8	18.0	18.0	17.6	18.0
CM4-3	-	10.8	16.2	17.6	17.6	17.6	18.0
CM4-4	-	10.8	16.2	17.1	17.1	17.1	18.0
CM5-1	-	11.3	16.7	18.5	18.5	18.5	16.7
CM5-2	-	11.3	15.8	16.7	16.7	17.1	16.2

Appendix Table 5 (Continued)

Code	pH						
	2	**3**	**4**	**5**	**6**	**7**	**8**
CM5-3	-	10.8	16.7	17.1	18.0	18.0	17.6
CM5-4	-	10.8	15.8	16.2	16.2	17.6	17.1
CM5-5	-	9.9	12.2	14.9	14.9	17.1	16.7
TB3-1	-	11.7	16.2	16.2	18.0	18.0	18.0
TB4-1	-	-	14.9	15.8	18.5	17.6	18.0
TB4-2	-	11.7	14.9	16.2	17.1	17.1	17.6
BA2	-	12.2	16.2	17.1	16.7	18.0	18.0
BA3	-	11.7	16.7	17.1	17.1	16.7	17.6
SP1-1	-	-	12.6	12.6	12.6	12.2	13.5
SP2-2	-	13.1	15.3	16.2	17.6	17.1	18.5
SP2-5	-	10.8	17.1	17.1	17.1	18.0	18.0
CHK2-1	-	-	13.5	13.5	13.1	12.6	13.5
CHK2-2	-	12.6	16.7	16.2	16.7	17.1	18.0
CHK2-4	-	10.4	15.3	16.2	17.1	17.6	18.0
CHR1-1	-	-	14.4	16.2	17.1	16.2	16.2

Appendix Table 5 (Continued)

Code	pH						
	2	**3**	**4**	**5**	**6**	**7**	**8**
CHR1-2	-	-	16.7	16.7	17.1	17.1	18.5
CHR1-3	-	-	9.0	15.3	15.8	16.7	17.1
CHR1-4	-	-	14.0	14.9	16.2	16.7	16.7
CHR1-5	-	-	17.6	17.1	18.9	18.0	19.8
CHR2-1	-	11.7	15.3	18.9	17.6	18.0	17.6
CHR2-2	-	10.8	13.5	15.8	16.7	16.2	17.6
CHR2-3	-	-	11.7	12.2	12.6	12.6	14.0
CHR2-4	-	-	10.4	12.2	12.6	12.6	13.5
CHR2-5	-	10.8	16.2	16.2	17.6	17.1	17.6
KP1-2	-	-	16.7	17.6	18.0	18.0	18.0
KP2-2	-	11.7	16.7	17.6	17.1	17.1	17.1
YT1-1	-	14.9	14.4	14.4	13.5	11.7	10.8
YT1-3	-	15.8	15.3	14.9	14.9	15.3	14.0
YT3-2	-	-	7.7	8.1	8.6	7.7	9.6
ND2-1	-	15.3	14.0	14.0	13.1	12.6	10.4

Appendix Table 5 (Continued)

Code	pH						
	2	**3**	**4**	**5**	**6**	**7**	**8**
ND2-2	-	15.8	15.3	13.1	13.1	12.6	12.6
ND3-1	-	10.4	12.2	14.9	16.2	16.7	16.2
926	-	9.9	14.0	15.8	16.2	14.9	16.2
326	-	15.3	14.9	15.3	14.4	13.5	10.8
895	-	15.8	15.3	14.9	13.1	11.9	10.4
1339	-	-	14.4	16.7	16.7	16.7	17.6
1112	-	11.7	15.3	17.6	17.6	17.1	18.0
452	-	-	15.3	14.9	14.9	15.3	14.9
1340	-	-	15.3	17.1	18.0	18.0	16.2
1341	-	-	13.5	16.7	16.7	16.7	16.2
047	-	11.7	16.7	16.7	18.0	17.1	18.5
372	-	-	6.3	13.1	14.0	16.2	17.1
1463	-	-	11.25	16.65	18.0	18.5	19.4
1464	-	15.8	15.8	15.8	14.4	13.5	12.6
1465	-	-	15.75	17.1	17.1	17.1	18.5

Appendix Table 5 (Continued)

Code	pH						
	2	**3**	**4**	**5**	**6**	**7**	**8**
L. brevis	-	14.9	15.3	14.9	14.4	14.4	13.1

***** The lactic acid production was performed at glucose concentration 20 g l^{-1} and temperature of 37^{O}C.

- no cell growth

Appendix Table 6 Results of lactic acid formation (g l^{-1}) at various concentrations of glucose*.

Code	Glucose concentration (w/v)						
	3%	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
CH3-1 (white)	13.5	13.1	13.1	12.6	12.6	11.3	10.4
CH3-2	18.9	18.5	18.0	18.9	16.2	16.2	16.2
CH3-3	11.7	12.6	12.6	12.6	12.6	11.7	11.7
CH3-4	13.5	13.5	13.5	11.7	12.2	11.3	10.4
CH3-5	13.5	14.0	13.5	13.5	12.2	10.8	9.9
CH3-6	11.7	12.6	14.4	12.6	10.8	10.8	9.9
CH6-2	8.1	7.7	8.1	8.1	-	-	-
CH6-3	17.1	17.6	18.5	18.0	14.9	14.4	12.6
SS2	13.1	15.8	15.8	16.7	16.7	14.4	15.8
SS3	12.6	14.4	14.4	14.0	14.0	15.8	11.7
SS4	17.1	16.7	17.6	17.6	17.1	14.4	14.9
CS1	18.5	18.5	18.9	18.5	18.5	15.3	15.3
CS2	18.0	18.0	18.0	18.5	15.8	15.8	15.8
CS3	19.8	18.9	19.8	18.9	16.2	15.8	15.8
DM1	18.0	18.9	18.9	18.9	16.7	18.0	15.8

Appendix Table 6 (Continued)

Code	Glucose concentration (w/v)						
	3%	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
DM3	17.1	19.4	19.4	18.0	15.8	13.5	13.5
DY	18.9	18.9	18.0	18.0	14.4	14.0	11.7
DY (R)	16.7	15.3	15.8	16.2	15.8	11.3	6.8
DB	13.1	13.5	12.6	12.6	11.7	12.2	12.6
KU2-1	18.0	18.0	18.0	18.9	18.0	15.8	15.3
TB1-1	12.2	13.1	11.7	10.8	18.8	11.3	9.5
TB1-2	15.3	16.7	16.2	16.7	17.6	17.6	15.8
CB1	18.0	18.9	18.9	18.5	17.1	17.6	15.3
CB2	16.2	17.6	17.6	17.1	15.8	14.4	13.1
CB(R2)-1	17.6	16.7	17.1	17.1	16.2	13.5	13.5
CB(R2)-2	19.4	19.4	18.9	18.5	13.5	13.1	12.6
CB(R2)-bf	17.1	19.4	18.9	19.4	18.0	17.6	16.7
BK2-1	14.9	14.4	15.3	16.2	16.2	14.4	14.4
BK2-2	12.6	12.6	13.1	13.5	12.6	13.5	12.6
BK6-1	16.7	17.1	17.1	16.7	16.2	16.2	16.2

Appendix Table 6 (Continued)

Code	Glucose concentration (w/v)						
	3%	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
BK6-2	15.3	15.3	15.8	15.3	13.5	13.5	13.1
$CP2_{cream}$	17.6	19.4	19.4	18.0	17.6	17.1	16.2
$CP2_{white}$	17.1	18.9	17.6	18.0	17.1	16.2	16.2
CP3	18.0	17.1	18.9	17.6	17.6	16.2	15.3
CP4	16.7	18.0	18.9	17.1	18.0	17.1	17.1
TB2-1	18.0	18.5	18.0	17.1	16.7	17.6	17.6
TB2-3	17.1	17.1	16.7	17.6	15.8	16.2	15.8
TB2-4	17.6	18.5	18.9	18.9	17.6	16.7	15.8
FCP2	17.6	18.9	18.0	17.1	14.9	14.4	12.6
WL2	17.1	18.0	18.9	18.0	14.9	16.7	14.9
NM1	16.7	16.7	18.45	18.0	17.1	17.1	12.6
NM2	17.6	17.6	18.9	18.0	17.1	16.7	17.1
NM4	16.2	17.6	18.0	16.2	16.7	15.8	15.8
CM1-1	16.7	16.7	17.6	17.6	17.6	17.1	16.7
CM1-2	17.1	17.1	18.0	17.1	16.2	14.4	16.2

Appendix Table 6 (Continued)

Code	Glucose concentration (w/v)						
	3%	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
CM1-3	12.6	13.1	12.6	11.7	11.7	11.7	11.3
CM2-1	17.6	17.6	18.0	17.6	15.8	15.8	14.4
CM2-2	17.1	17.6	17.1	17.1	15.3	12.6	15.3
CM2-5	15.3	14.9	16.7	17.1	16.2	16.2	13.1
CM2-7	16.7	17.1	16.7	16.7	14.9	15.3	14.0
CM3-1	13.1	12.6	12.2	12.6	11.7	10.8	10.8
CM3-2	18.0	18.0	18.0	18.5	17.1	15.3	15.3
CM3-3	16.2	17.1	16.7	16.2	14.9	14.0	13.5
CM4-1	17.6	18.0	18.0	18.0	17.1	17.6	14.9
CM4-2	18.5	18.9	18.9	18.5	14.4	14.9	14.4
CM4-3	18.9	18.5	18.0	18.0	15.8	14.9	16.2
CM4-4	18.5	18.0	18.0	18.0	16.7	15.8	16.2
CM5-1	18.0	18.5	18.0	17.6	17.1	15.8	14.9
CM5-2	15.3	16.2	17.6	17.1	18.0	17.1	17.1
CM5-3	15.3	16.7	18.0	18.0	17.6	17.1	16.7

Appendix Table 6 (Continued)

Code	Glucose concentration (w/v)						
	3%	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
CM5-4	15.3	16.7	17.1	18.0	15.8	16.2	16.2
CM5-5	15.3	17.6	17.6	18.0	18.0	17.6	17.6
TB3-1	18.0	18.5	17.6	16.7	17.6	16.2	15.3
TB4-1	17.6	18.0	18.0	17.6	17.6	18.0	17.6
TB4-2	17.6	18.0	18.0	18.0	18.0	17.1	18.5
BA2	16.7	18.5	18.5	18.5	17.6	16.7	16.2
BA3	18.0	18.0	17.1	18.0	15.3	17.1	16.2
SP1-1	14.0	14.9	13.1	11.7	10.8	9.0	9.5
SP2-2	17.1	17.6	18.9	17.6	18.5	16.7	16.2
SP2-5	18.0	18.0	18.9	18.5	17.1	17.1	16.2
CHK2-1	13.5	14.0	13.1	11.7	9.5	8.6	9.0
CHK2-2	15.3	17.1	16.2	17.6	17.6	17.6	15.8
CHK2-4	17.1	15.3	15.8	18.0	15.3	17.6	16.7
CHR1-1	16.2	16.2	17.1	17.1	17.1	16.7	15.8
CHR1-2	18.5	18.0	19.8	17.1	16.7	15.8	16.7

Appendix Table 6 (Continued)

Code	Glucose concentration (w/v)						
	3%	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
CHR1-3	16.7	17.1	17.1	17.6	17.1	17.1	17.1
CHR1-4	16.2	17.6	17.1	18.0	17.1	17.1	17.1
CHR1-5	18.9	18.9	18.9	19.8	18.0	18.0	18.0
CHR2-1	18.0	18.9	18.9	18.9	18.0	18.0	15.3
CHR2-2	15.8	18.5	18.0	18.9	18.0	18.9	16.2
CHR2-3	13.5	13.5	11.7	9.9	9.5	8.6	-
CHR2-4	13.5	12.6	13.5	13.1	12.6	11.3	11.3
CHR2-5	17.1	19.4	18.0	18.5	17.6	17.1	17.6
KP1-2	18.5	19.4	18.9	18.9	16.2	17.1	17.6
KP2-2	17.1	18.0	18.5	18.9	18.0	17.6	18.5
YT1-1	15.8	20.3	18.9	18.5	19.4	16.2	13.1
YT1-3	17.1	19.8	20.3	18.9	19.4	18.9	17.6
YT3-2	-	9.5	9.5	8.6	10.8	-	-
ND2-1	15.3	20.3	19.8	19.4	18.5	17.1	14.4
ND2-2	15.8	19.8	19.8	17.6	19.4	17.1	11.3

Appendix Table 6 (Continued)

Code	Glucose concentration (w/v)						
	3%	**5%**	**10%**	**15%**	**20%**	**25%**	**30%**
ND3-1	14.9	17.6	18.0	17.6	17.6	18.5	17.6
926	16.7	16.2	17.1	17.1	16.7	14.4	14.0
326	16.7	20.7	20.3	19.4	18.9	18.0	15.8
895	13.1	18.0	19.4	18.9	17.9	17.1	13.5
1339	17.1	16.7	16.7	17.1	16.7	16.2	14.0
1112	17.6	18.9	18.5	18.0	18.5	17.6	17.1
452	15.8	15.8	15.3	15.3	14.0	14.9	15.2
1340	15.3	15.3	17.1	17.1	17.6	17.1	14.4
1341	17.1	16.2	15.3	15.8	15.8	13.1	9.9
047	18.5	18.9	19.4	18.0	18.9	18.0	17.1
372	16.2	16.7	16.7	16.7	13.1	11.3	10.3
1463	18.9	18.9	18.0	17.1	15.3	14.0	12.6
1464	15.8	19.8	19.4	18.9	17.1	17.1	14.9
1465	18.5	18.0	17.6	18.5	15.3	15.3	14.4
L.brevis	17.6	19.4	18.5	18.5	17.6	17.1	16.2

***** The lactic acid production was performed at pH 6 and temperature of 37^{O}C.

Appendix Table 7 Results of lactic acid concentration and D, L- isomer test of isolated cultures from fermented samples*.

Code	D-lactic acid		L-lactic acid		Total
	Concentration ($g\ l^{-1}$)	% D-isomer	Concentration ($g\ l^{-1}$)	% L-isomer	lactic acid ($g\ l^{-1}$)
CH3-2	7.1	54.6	5.9	45.4	13
CH6-3	6.9	55.2	5.6	44.8	12.5
CS1	6.2	45.3	7.5	54.7	13.7
CS2	9.7	59.9	6.5	40.1	16.2
CS3	7.6	46.1	8.9	53.9	16.5
DM1	5.5	51.4	5.2	48.6	10.7
DM3	1.2	7.2	15.4	92.8	16.6
DY	1.7	10.1	15.2	89.9	16.9
DY(R)	5.6	47.5	6.2	52.5	11.8
DB	4.0	45.5	4.8	54.5	8.8
KU2-1	6.8	51.9	6.3	48.1	13.1
CB1	6.9	52.3	6.3	47.7	13.2
CB(R2)-2	5.0	35	9.3	65	14.3
CB(R2) - bf	1.6	9.8	14.8	90.2	16.4
CP2 cr.	5.3	48.2	5.7	51.8	11.0
CP2 wh.	8.7	56.1	6.8	43.9	15.5
CP3	5.0	48.5	5.3	51.5	10.3
CP4	7.6	53.1	6.7	46.9	14.3
TB2-1	6.2	55.9	4.9	44.1	11.1
TB2-4	6.2	54.9	5.1	45.1	11.3
FCP2	1.2	8.8	12.5	91.2	13.7
WL2	7.5	53.2	6.6	46.8	14.1
NM1	6.8	54.0	5.8	46.0	12.6
NM2	6.4	46.0	7.5	54.0	13.9

Appendix Table 7 (Continued)

Code	D-lactic acid		L-lactic acid		Total
	Concentration ($g\ l^{-1}$)	% D-isomer	Concentration ($g\ l^{-1}$)	% L-isomer	lactic acid ($g\ l^{-1}$)
CM1-2	6.5	54.2	5.5	45.8	12.0
CM2-1	7.1	57.3	5.3	42.7	12.4
CM3-2	5.3	57	4.0	43.0	9.3
CM4-1	7.4	58.7	5.2	41.3	12.6
CM4-2	5.9	56.2	4.6	43.8	10.5
CM4-3	5.9	56.7	4.5	43.3	10.4
CM4-4	6.0	54.5	5.0	45.5	11.0
CM5-1	6.3	56.8	4.8	43.2	11.1
CM5-2	6.2	56.9	4.7	43.1	10.9
CM5-3	5.7	54.8	4.7	45.2	10.4
CM5-4	5.4	53.5	4.7	46.5	10.1
CM5-5	7.8	61.9	4.8	38.1	12.6
TB3-1	7.6	60.3	5.0	39.7	12.6
TB4-1	5.5	56.7	4.2	43.3	9.7
BA2	5.5	42.3	7.5	57.7	13.0
BA3	5.9	54.1	5.0	45.9	10.9
SP2-2	6.5	50.4	6.4	49.6	12.9
SP2-5	6.3	54.8	5.2	45.2	11.5
CHR1-2	5.2	43.0	6.9	57.0	12.1
CHR1-5	6.1	57.5	4.5	42.5	10.6
CHR2-1	6.5	57.5	4.8	42.5	11.3
CHR2-2	6.7	67.8	3.2	32.2	9.9
CHR2-5	5.9	58.4	4.2	41.6	10.1
KP1-2	6.5	64.4	3.6	35.6	10.1
KP2-2	5.8	49.6	5.9	50.4	11.7
YT1-1	3.7	46.3	4.3	53.7	8.0

Appendix Table 7 (Continued)

Code	D-lactic acid		L-lactic acid		Total
	Concentration ($g\ l^{-1}$)	% D-isomer	Concentration ($g\ l^{-1}$)	% L-isomer	lactic acid ($g\ l^{-1}$)
YT1-3	4.4	45.4	5.3	54.6	9.7
ND2-1	3.9	48.1	4.2	51.9	8.1
ND2-2	3.8	50.7	3.7	49.3	7.5
326	4.7	49.0	4.9	51.0	9.6
895	4.1	42.3	5.6	57.7	9.7
1112	6.0	60.6	3.9	39.4	9.9
047	7.3	51.0	7.0	49.0	14.3
1463	7.0	39.3	10.8	60.7	17.8
1464	4.2	47.7	4.6	52.3	8.8
1465	6.9	47.6	7.6	52.4	14.5
L. brevis	4.0	49.4	4.1	50.6	8.1

* The isolated cultures were cultivated at $37^{O}C$ for 48 h in 10 ml of MRS medium.

CIRRICULUM VITAE

NAME : Ms. Walaiporn Timbuntam

BIRTH DATE : October 6, 1975

BIRTH PLACE : Bangkok, Thailand

EDUCATION :

YEAR	INSTITUTE	DEGREE/DIPLOMA
1998	Kasetsart Univ.	B.Sc.(Biotechnology)
2001	Kasetsart Univ.	M.S. (Biotechnology)

POSITION/TITLE : Assistant Researcher

WORK PLACE : Kasetsart Agricultural and Agro-Industrail Product Improvement Institute, Kasetsart University

SCHOLARSHIP/AWARDS : Graduate Research Scholarship from the Graduate School, Kasetsart University (2007)