D-LACTIC ACID FERMENTATION PLATFORM FROM OIL PALM TRUNK SAP BY *Sporolactobacillus terrae* SBT-1

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ต้นแบบการหมักกรดดี-แลคติกจากน้ำบีบต้นปาล์มโดย *Sporolactobacillus terrae* SBT-1

น.ส.นภัสวรรณ สุขเอี่ยม

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กรดแลคติกเป็นกรดอินทรีย์ซึ่งประกอบด้วยสองอีแนนทิโอเมอร์คือ กรดแอล-แลคติกและ ดี-แลคติก กรดแลคติกจัดเป็นมอนอเมอร์ที่สำคัญสำหรับผลิตภัณฑ์ในอุตสาหกรรมต่างๆ กรดแลคติกถูกนำไปใช้อย่างแพร่หลายในอุตสาหกรรมยา อาหาร สิ่งทอ เคมี และเครื่องหนัง นอกจากนี้ยังใช้ในกระบวนการสังเคราะห์พอลิแลคติกแอซิด ขณะที่กรดแอล-แลคติกเป็นที่รู้จักในผลิตภัณฑ์ทางการค้าและงานวิจัย มีเพียงงานวิจัยจำนวนน้อยเท่านั้นที่ศึกษากรดดี-แลคติก จากกระบวนการการผลิตกรดแลคติกโดยวิธีการหมักด้วยจุลินทรีย์และการสังเคราะห์ทางเคมี กว่าร้อยละ 90 ของการผลิตกรดแลคติกทั่วโลกใช้วิธีการผลิตด้วยเทคโนโลยีการหมัก ซึ่งจะให้ผลิตภัณฑ์กรดแอล- หรือ ดี-แลคติก ที่มีความบริสุทธิ์เชิงแสง เพื่อลดต้นทุนการผลิตกรดแลคติกได้มีการนำของเสียจากการเกษตรซึ่งเป็นชีวมวลรุ่นที่ 2 มาใช้ในกระบวนการหมัก น้ำบีบจากต้นปาล์มซึ่งเป็นของเสียจากกระบวนการรีไซเคิลต้นปาล์มมีคุณสมบัติสำหรับใช้เป็นอาหารเพื่อการเพาะเลี้ยงแบคทีเรีย เนื่องด้วยองค์ประกอบของน้ำบีบต้นปาล์มที่ประกอบด้วย ซูโครส กลูโคส ฟรัคโทสและกรดอะมิโน ในงานวิจัยนี้จึงนำน้ำบีบต้นปาล์มมาใช้เป็นแหล่งคาร์บอนร่วมกับน้ำตาลทางการค้า (กลูโคส, ซูโครส, น้ำตาลทรายดิบ และกากน้ำตาล) หมักโดยแบคทีเรียผลิตกรดดี-แลคติกสายพันธุ์ *Sporolactobacillus terrae* SBT-1 ผลการทดลองการเพิ่มประสิทธิภาพกระบวนการผลิตกรดดี- แลคติกในขวดทดลองได้ผลอัตราการผลิตกรดดี- แลคติกต่อปริมาณน้ำตาลที่สูง (0.87-0.91 กรัมต่อกรัม) และอัตราการผลิตที่สูง (2.42-2.36 กรัมต่อลิตรต่อชั่วโมง) รวมถึงให้ค่าความบริสุทธิ์เชิงแสงสูง (ร้อยละ 96-99) จึงได้ศึกษาต่อในถังหมักขนาด 5 ลิตร ผลการทดลองแสดงถึงประสิทธิภาพที่ดี โดยมีอัตราการผลิตกรดดี- แลคติกต่อปริมาณน้ำตาลสูง (0.84-1.04 กรัมต่อกรัม) รวมถึงอัตราการผลิตสูง (3.45-6.21 กรัมต่อลิตรต่อชั่วโมง) และค่าความบริสุทธิ์เชิงแสงที่ค่อนข้างสูงกว่าการทดลองในขวดทดลอง ผลการทดลองที่ใช้น้ำบีบต้นปาล์มร่วมกับน้ำตาลทรายดิบสามารถลดต้นทุนการผลิตได้ถึงร้อยละ 14.65 ดังนั้นจากผลการทดลองดังกล่าวข้างต้นจึงสามารถสรุปได้ว่าน้ำบีบต้นปาล์มเป็นชีวมวลรุ่นที่ 2 ที่มีประสิทธิภาพสูงสำหรับใช้ในการผลิตกรดแลคติกโดยแบคทีเรียสายพันธุ์ *S. terrae* SBT-1

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Naphatsawan Sukaiem : D-LACTIC ACID FERMENTATION PLATFORM FROM OIL PALM TRUNK SAP BY *Sporolactobacillus terrae* SBT-1. Advisor: Assoc. Prof. SUCHADA CHANPRATEEP NAPATHORN, Ph.D. Co-advisor: Prof. NUTTHA THONGCHUL, Ph.D.

Lactic acid is an organic acid that contains two enantiomers, L- and D-lactic acid, and is considered the most potential monomer for many industrial products. Lactic acid has versatile applications in the pharmaceutical, food, textile, chemical, and leather industries moreover in poly-lactic acid (PLA) manufacturing. While L-lactic acid is well known in many commercial products and studies, only a few studies on D-lactic acid were found. Lactic acid can be produced either by microbial fermentation or chemical synthesis. Approximately 90 percent of the total lactic acid produced worldwide is via microbial fermentation which provides an optically pure L- or D-lactic acid. To reduce the lactic acid production cost, agricultural waste which is second-generation biomass is applied for lactic acid fermentation. Oil palm trunk sap (OPT sap), the waste from the oil palm trunk recycle process, has the potential as a bacterial culture medium, due to the composition such as sucrose, glucose, fructose, and amino acid. In this study, by applying OPT sap as a carbon substrate together with other commercial sugars (glucose, sucrose, raw sugar, and molasses), fermented by *Sporolactobacillus terrae* SBT-1, the efficient D-lactic acid producer, the result from the enhancing D-lactic acid fermentation in shake flask provide a high D-lactic acid yield (0.87-0.91 g/g) and productivity (2.42-4.36 g/L·h) and also the high optical purity (96-99% ee). Thus, this platform was optimized in 5L fermenter, the results showed a good fermentation performance. The lactic acid production yield was high (0.84-.1.04 g/g), also the productivity (3.45-6.21 g/L·h), and the optical purity was almost higher than flask culture. In the fermentation using OPT sap supplemented with raw sugar as the carbon substrates, the most effective cost reduction by 14.65% was observed. From the result mentioned above, we can claim that OPT sap is a potential second-generation feedstock for lactic acid fermentation by *S. terrae* SBT-1.

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CHAPTER 1
INTRODUCTION

1.1 Background and Rationale

Lactic acid ($CH_3CHCOOH$) is an organic, hydroxycarboxylic acid that is naturally found. It is a simple chiral molecule containing two enantiomers, L- and D-lactic acid, and is considered the most potential monomer for chemical conversion due to its carboxylic and hydroxyl group. A worldwide demand for lactic acid is remarkable due to its versatile applications in pharmaceutical, food, textile, chemical, and leather industries (Lasprilla *et al.*, 2012). Since, lactic acid is an odorless and non-volatile organic acid, it is allowed to be used as a general-purpose food additive by Food and Drug Administration (FDA) in the USA (Reddy *et al*., 2008).

Polylactic acid (PLA) is a biodegradable and biocompatible polymer, derived from a lactic acid monomer building block. They are usually used in the packaging and fiber industry, moreover, in medical and clinical products (Ahmad *et al*., 2020). In 2017, the global market for PLA was estimated to be USD 2.23 billion and was expected to be greater than 20.6% from 2018 to 2026 by a stable compound annual growth rate (CAGR) (Mehrpouya *et al*., 2021). The optical purity of L- or D-lactic acid is very important for the monomer feedstock in polymer manufacturing (Petrova *et al*., 2013). In PLA manufacturing, the optical purity of lactic acid can lead to the physical properties of the polymer backbone. The modified structure of PLA by mixing L- and D- isomers for crystalline provides the polymer properties that can be used for food contact (Garlotta, 2001). Since it is required that PLA must possess the thermal stability for preventing degradation at high temperature, to promote the heat resistant property, the injection of 1% D-isomer is suitable for crystallization in a

short cycle of PLA processing (Lim *et al*., 2008). While L-lactic acid is well known in many commercial products and studies, only a few studies on D-lactic acid was found although it naturally exists in humans (Pohanka, 2020). Although D-lactic acid plays an important role in improving polylactic acid (PLA) thermal property, the study on D-lactic acid production is still limited (Zhang *et al*., 2018).

Lactic acid can be produced either by microbial fermentation or chemical synthesis. In chemical synthesis, a racemic mixture of the two isomers, L- and D-lactic acid, is produced by the hydrolysis of lactonitrile using a strong acid. While the microbial fermentation can produce an optically pure L- or D-lactic acid (Petrova *et al*., 2013)). Approximately 90 percent of the total lactic acid produced worldwide is via microbial fermentation. This is because of the low production cost, the high process performance, and the environmentally benign process (Lasprilla *et al*., 2012).

According to the importance of D-lactic acid in PLA manufacturing, a high fermentation process performance is mandatory. In terms of industrial application, the production of D-lactic acid requires the essential components including D-lactic acid producing strain, the inexpensive raw material, and the productive fermentation strategies that supply the high yield and high enantiomeric purity (Klotz *et al*., 2016). The production of D-lactic acid has been studied by using a wide range of lactic acid producing microorganisms for example bacteria, fungi, and algae as well as cyanobacteria (Alexandri *et al*., 2019). The wild-type strain or even the genetically engineered strain, ferment various raw materials such as commercial sugar, starch, whey, pulp residue, and molasses *(Bai et al., 2016; Fukushima et al., 2004; Tanaka et al*., 2006; Zhang & Vadlani, 2015).

To achieve high lactic acid concentration, the ability to ferment high sugar content using the promising strains that overcome the substrate inhibition is required. This can provide the high product yield and productivity (Abdel-Rahman & Sonomoto, 2016). For example, Yu et al. (2008) used the genetically engineered *Lb. rhamnosus* ATCC 1144 strain for enhancing lactic acid production. They obtain the increased lactic acid concentration from 160 g/L and 200 g/L glucose media (Yu *et al*., 2008).

D-lactic acid can be produced by many bacterial strains including *Escherichia coli*, *Lactobacillus bulgaricus*, *Lactobacillus delbrueckii*, *Lactobacillus coryniformis*, and *Sporolactobacillus* sp. (Benthin & Villadsen, 1995; Bustos *et al*., 2004; Calabia & Tokiwa, 2007; Shukla *et al*., 2004; Zhao *et al*., 2010). In the group of *Sporolactobacillus* sp., they have several productive D-lactic acid producing strains, such as *Sporolactobacillus inulinus* which can produce a high concentration of D-lactic acid up to 107.5 g/L by fermenting the low nutrient resource. *Sporolactobacillus nakayamae* when cultured in the optimal medium condition yielded high D-lactic acid concentration at 112.93 g/L. Moreover, *Sporolactobacillus laevolacticus* also produced high D-lactic acid concentration at 144 g/L from inexpensive cottonseed (Bai *et al*., 2016; Beitel *et al*., 2016; Li *et al*., 2013). As mentioned above, *Sporolactobacillus* sp. show a high potential as the production strain for efficient D-lactic acid production. It was reported that *Sporolactobacillus terrae* SBT-1 which was isolated from the previous study produced a high lactic acid from a high sugar concentration up to 360 g/L during batch fermentation (Sitanan Thitiprasert et al., 2021).

To reduce PLA production costs, agricultural waste is applied for lactic acid fermentation (Bustamante *et al*., 2019). According to the cultivation of palm trees, oil palm trees have been planted in all regions of Thailand for food and fuel consumption (Jaroenkietkajorn & Gheewala, 2021). After plantation between 12 to 15 years, palm trunks are felled and chopped into pieces then spreaded out on the plantation area to allow decomposition for nutrition purposes. Due to the high residual sugars and moisture content of the felled palm tree, microbial contamination during wood decomposition at the plantation area can lead to infection by plant pathogens of the new oil palm tree. Previous study reported that the palm sap consisted of mainly 80% water and 10-15% sugars, i.e., glucose, fructose, and sucrose, with some vitamins, minerals, and amino acids at trace amounts (Sarma *et al*., 2021). For this reason, the extracted oil palm trunk sap can be a potential microbial culture medium (Dirkes *et al*., 2021). By extracting the oil palm trunk sap from the felled palm tree, the remaining pressed trunks can be also a good source of lignocelluloses for converting into high-valued chemicals (Bukhari *et al*., 2021).

Thus, to investigate D-lactic acid production using the low-cost substrate, *S. terrae* SBT-1, a potent D-lactic acid producing bacterium, was selected for utilizing oil palm sap as a carbon substrate together with other commercial sugars. This led to the main purposes of this research that included medium formulation for enhanced D-lactic acid production in a shake flask culture and fermentation process development in a 5 L stirred bioreactor using the commercial sugars mixed with the actual oil palm trunk sap obtained from the local factory in Thailand.

1.2 Research objective

1.2.1 To formulate the medium compositions that contained the oil palm trunk sap as the carbon substrate and to validate the D-lactic acid fermentation performance of *S. terrae* SBT-1 in a flask culture

1.2.2 To develop the fermentation platform for D-lactic acid production using the low-cost medium containing oil palm trunk sap in a 5 L stirred bioreactor

1.3 Expected outcome

D-lactic acid fermentation process conditions that gave a sufficiently high production performance and cost saving.

CHAPTER 2

LITERATURE REVIEW

2.1 Lactic acid

Lactic acid ($CH_3CH(OH)COOH$) is the hydroxycarboxylic acid that was most commonly found. Lactic acid is an organic acid that contains two enantiomers of L (+) lactic acid and D (-) lactic acid (Figure 1) (Datta & Henry, 2006). Due to the important role of lactic acid in metabolic pathways, the global lactic acid market was estimated to be worth USD 1.1 billion in 2020, and is expected to reach USD 2.1 billion by 2025 (Chahal & Starr, 2000; Elkhateeb *et al*., 2022). It is considered GRAS (Generally Recognized As Safe) by the United States Food and Drug Administration (FDA). Lactic acid manufacturers comprise many enterprises in many countries, such as NatureWorks LLC (United States), Purac and Hycail (Netherlands), Futerro and Galactic (Belgium), Hisun Industries Co. Ltd. (China), and Mitsui Chemicals (Japan) (Rodrigues *et al*., 2017).

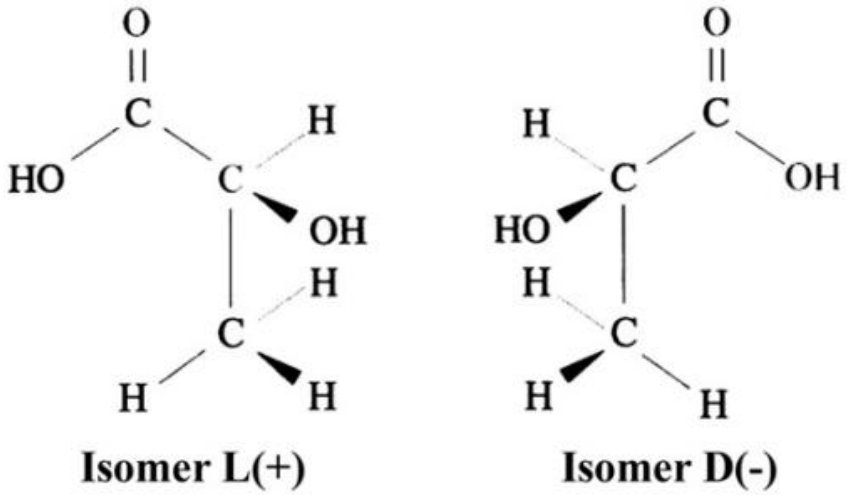


Figure 1 The two enantiomers of lactic acid, D(-) lactic acid and L(+) lactic acid (Martinez *et al*., 2013)

The general physical and chemical properties of L (+) lactic acid and D (-) lactic acid are shown in Table 1 (Komesu *et al*., 2017; Ren, 2010). Lactic acid can be produced by chemical synthesis or by microbial fermentation (Martinez *et al*., 2013).

Table 1 Lactic acid general properties (Komesu *et al*., 2017; Ren, 2010)

Properties	**Characteristics**
Formula	$CH_3CH(OH)COOH$
Color	Yellow or colorless
Density at 20°C (g/L)	1.249
Melting point (°C)	52.8 (D), 53.0 (L), 16.8 (DL)
Boiling point (°C)	122°C (12 mmHg)
Specific gravity	1.2 g/mL
Molar mass	90.08 g/mol

2.1.1 The differentiation between L- and D-lactic acid

As the exit of two enantiomers, lactic acid in chemical terms is entitled by its isomer**.** L-lactic acid is identified as L (+) or S (+) lactic acid while D-lactic acid is identified as D (-) or R (-) lactic acid**.** L-lactic acid is the predominant form in humans and the higher life forms. However, a high level of lactate in blood can cause hyperlactatemia. In most organisms, the metabolism of L-lactate is moderated by L-lactate dehydrogenase (EC 1.1.1.27) with NAD^+ as a cofactor. The same reaction is present in the oxidation of D-lactate by D-lactate dehydrogenase (EC 1.1.1.28). The oxidation of L-lactate and D-lactate is described in Figure 2. L-lactate

dehydrogenase not only is involved in the basic metabolism in humans and higher life forms but also in the fermentation process. L-lactate dehydrogenase is only specific to produce L-isomer. In contrast with L-lactate, D-lactate is not involved in basic metabolism (Pohanka, 2020). D-lactate is a lactate form which can be produced by some bacteria species such as *Lactobacillus delbrueckii*, *Leuconostoc* sp., and *Sporolactobacillus* sp (Prasirtsak *et al*., 2017).

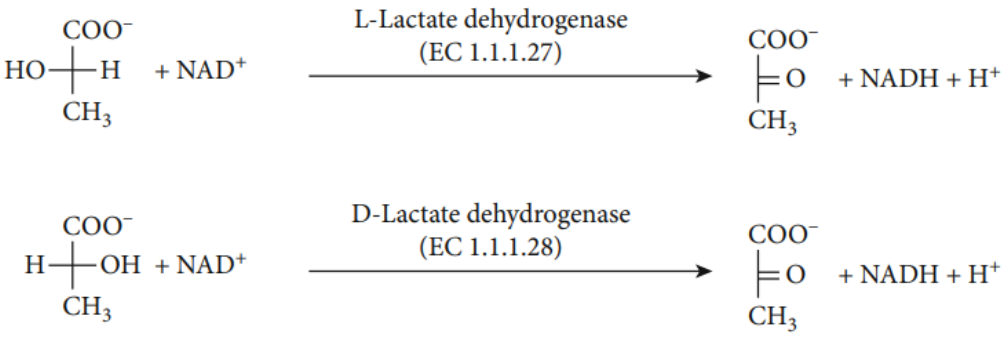


Figure 2 Oxidation of L-lactate by L-lactate dehydrogenase and D-lactate by D-lactate dehydrogenase to pyruvate.

Lactide, or the cycling polymer of lactic acid, has three forms, including (i) L-lactide (LLA), (ii) D-lactide (DLA), and (iii) meso-lactide (MLA), which is the racemic mixture of L- and D-lactic acid. The polymer from all of these isomeric forms, poly L-lactic acid (PLLA), is the one that is most often commercially produced because it is easily adapted and assimilated to the human body (Rodrigues *et al*., 2017); it is used as surgical implant material, and for drug delivery, packaging, and other consumer products. However, to increase the resistance of hydrolytic and thermal degradation,

stereo complexation between enantiomer PLLA and PDLA is required. Racemic lactide, or DL-lactide (DLLA), is a racemic stereo complex compound, which has a melting temperature of 230 °C, which is higher than LLA or DLA, which have melting temperatures in the range of 95-99°C (Tsuji, 2013*;* Zhao *et al.*, 2010).

2.2 Lactic acid production

2.2.1 *Chemical synthesis*

The process is based on lactonitrile, which was obtained by adding hydrogen cyanide to acetaldehyde. Then, the crude lactronitrile was purified and recovered by distillation. The concentrated HCl, or H_2SO_4, was added for a hydrolysis reaction to produce ammonium salt and lactic acid. The lactic acid was then esterified by methanol to produce methyl lactate. Both the lactic acid and methanol were recycled for purification by distillation and hydrolyzed by water under acid catalyzation. The steps of lactic acid production by chemical synthesis are detailed in the following information (Ren, 2010).

(a) Addition of Hydrogen Cyanide

$$CH_3CHO + HCN \rightarrow CH_3CHOHCN$$

(b) Hydrolysis by H_2SO_4

$$CH_3CHOHCN + H_2O + \frac{1}{2} H_2SO_4 \rightarrow CH_3CHOHCOOH + \frac{1}{2} (NH_4)_2SO_4$$

(c) Esterification

$$CH_3CHOHCOOH + CH_3OH \rightarrow CH_3CHOHCOOCH3 + H_2O$$

(d) Hydrolysis by H_2O

$$CH_3CHOHCOOCH_3 + H_2O \rightarrow CH_3CHOHCOOH + CH_3OH$$

Although the chemical synthesis of lactic acid production has more routes -- for example, 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 -- all of these routes are technically and economically non-viable processes (Datta et al., 1995; John et al., 2007).

2.2.2 *Fermentation*

The biological process of lactic acid fermentation uses microorganisms such as bacteria, mold, or yeast to ferment substrate, which has some sugar content. The homofermentative microorganism converts one molecule of glucose into two molecules of lactic acid, while the heterofermentative microorganism converts one molecule of glucose into carbon dioxide, ethanol, and lactic acid (Ren, 2010).

Two molecules of ATP are produced, two molecules of NAD^+ are reduced to NADH, were form by glycolysis. Two of the three carbon molecules are produced by pyruvate; 95% of the chemical energy from glucose is also trapped in the pyruvate. The thorough oxidation of pyruvate is performed in the Kreb's cycle and electron transport system (ETS) to completely break down the glucose into carbon dioxide. As a result, the

pyruvate is converted to lactic acid. This step also creates the NAD^+, which goes on into the glycolysis process (Ren, 2010).

The optically pure L (+) or D (-) lactic acids are produced by carbohydrate fermentation. The fermentation product depends on the selection of lactic acid producer strain. The steps in lactic acid fermentation by microorganism are described in details below (Ren, 2010).

(a) Fermentation and neutralization

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

(Carbohydrate) (Calcium hydroxide) (Calcium lactate)

(b) Hydrolysis by H_2SO_4

$$2(CH_3CHOHCOO^-)\,Ca^{2+} + H_2SO_4 \rightarrow 2\,CH_3CHOHCOOH + 2H_2O + CaSO_4$$

(Calcium lactate) (Sulfuric acid) (Calcium lactate) (Calcium sulfate)

(c) Esterification

$$CH_3CHOHCOOH + CH_3OH \rightarrow CH_3CHOHCOOCH_3 + 2H_2O$$

(Lactic acid) (Methanol) (Methyl lactate)

(d) Hydrolysis by H_2O

$$CH_3CHOHCOOCH_3 + H_2O \rightarrow CH_3CHOHCOOH + CH_3OH$$

(Methyl lactate) (Lactic acid) (Methanol)

To get lactic acid and calcium sulfate, the fermentation broth is filtered to remove cells, carbon treated, evaporated, and acidified by using sulfuric acid. Lactic acid is obtained by hydrolysis and esterification (Narayanan *et al*., 2004; Ren, 2010). For comparison of chemical synthesis and biological fermentation for the synthesis of lactic acid, the steps are described in Figure 3.

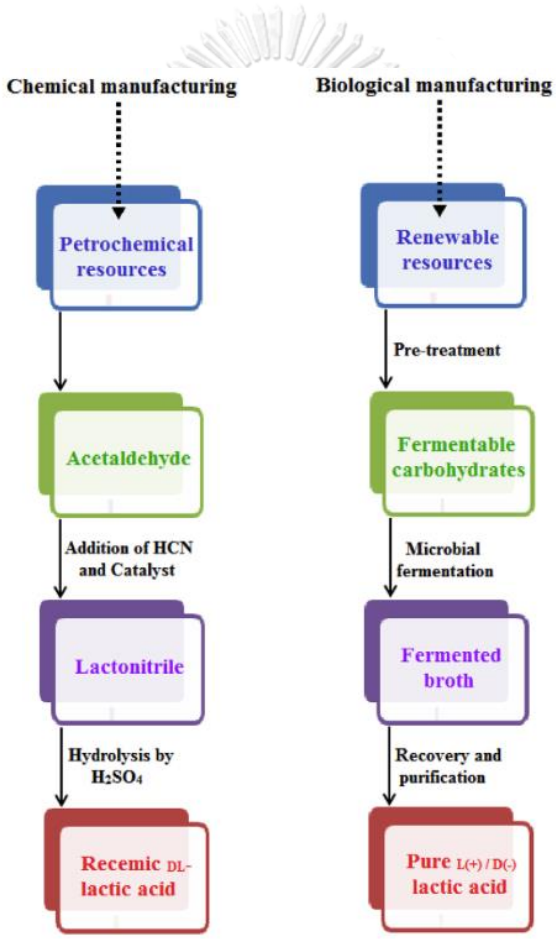


Figure 3 The two lactic acid manufacturing processes (Ghaffar *et al*., 2014)

2.3 Lactic acid producer

2.3.1 Bacteria

Bacteria used in the production of lactic acid can be divided in to 4 main producers: lactic acid bacteria (LAB), *Bacillus strains*, *Escherichia coli*, and *Corynebacterium glutamicum* (Abdel-Rahman *et al*., 2013). LAB are well known for their use as a major lactic acid product producer. They are Gram-positive, non-spore forming, non-catalase, micro aerobes in rod and cocci shapes. Lactic acid bacteria can be classified by their carbo hydrate metabolism as either homofermentative or heterofermentative bacteria. The homofermentative bacteria use the Embden-Meyerhof-Parnas (EMP) pathway for glycolysis. They can produce only lactic acids in form of D (-) or L (+) lactic acid, or a racemic mixture of these two isomers, while the heterofermentative bacteria uses the pentose phosphoketolase pathway, or 6-phosphogluconate pathway, to produce lactic acid, carbon dioxide, ethanol, and acetate, as shown in Figure 4 (Von Wright & Axelsson, 2019).

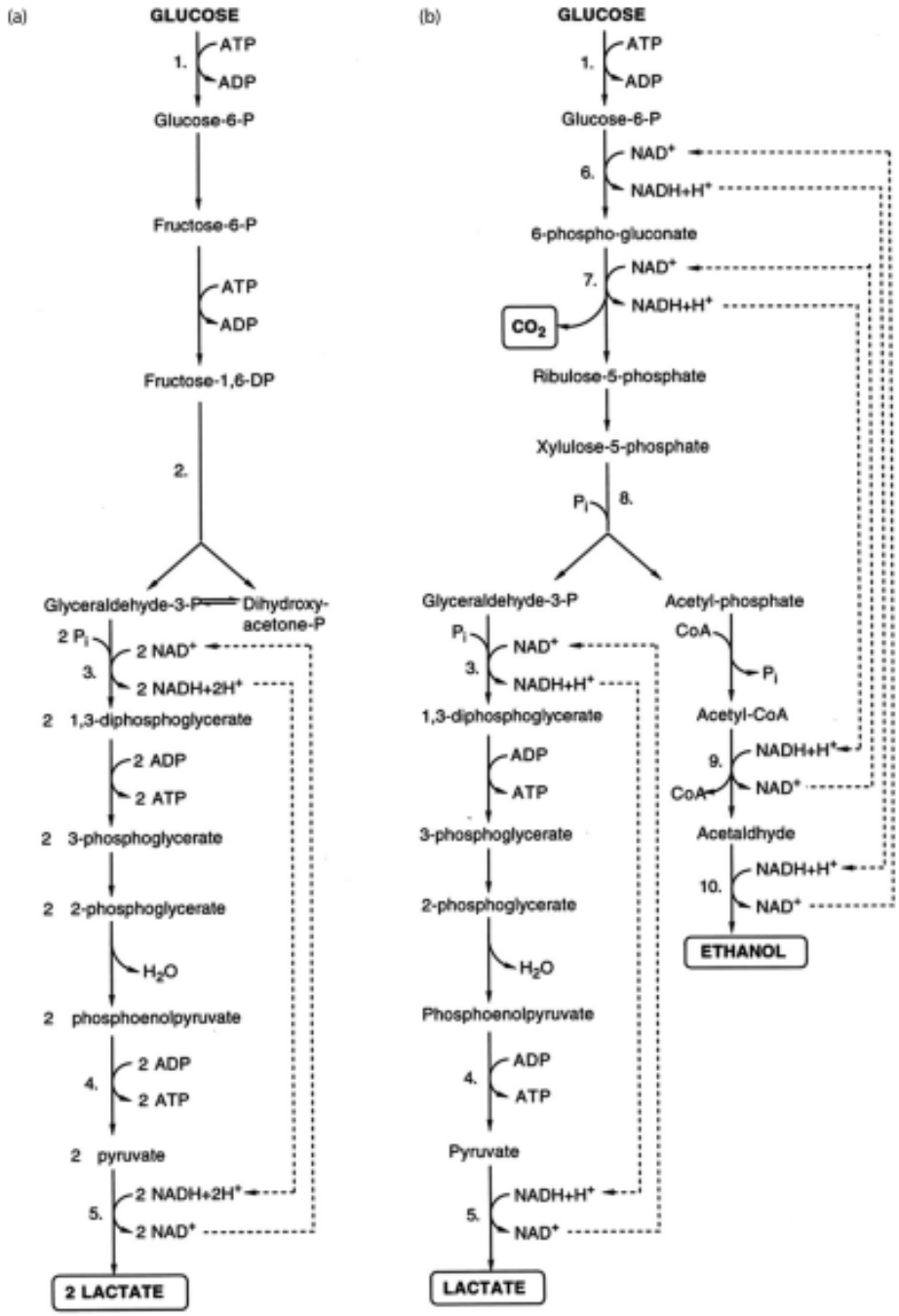


Figure 4 Major glucose fermentation pathways of lactic acid bacteria (Von Wright & Axelsson, 2019)

(a) homolactic acid fermentation

(b) heterotactic acid fermentation

The traditional lactic acid producing bacterium is a non-spore former. However, spore-forming lactic acid producers have been described. In 1923, the genera *Bacillus* was identified as spore-forming lactic acid producing bacteria. This group was limited accepted as the only aerobic spore-forming rod shape bacteria. Nowadays, five additional genera have been added: *Sporosarcina*, *Thermoactinomyces*, *Sporolactobacillus*, *Amphibacillus*, and *Alicyclobacillus* (Fritze & Claus, 1995). The spore-forming lactic acid producers are able to survive in many different conditions because of resistant endospores. Like most spore-forming bacteria, they are mesophilic to thermophilic. They can be found in soil, which is their natural habitat, even though soil is contaminated by almost everything.

2.3.1.1 The genus *Sporolactobacillus*

Sporolactobacillus is an endospore-forming, catalase negative strain that prefers growth under microaerophilic conditions. *Sporolactobacillus* is in the middle between *Bacillaceae* and *Lactobacillaceae* (McClure, 2006). Initially, they were classified in *Lactobacillaceae*. Later, they were classified in *Bacillaceae* due to sharing their chemotaxonomic characteristics (Fritze & Claus, 1995; Suzuki & Yamasato, 1994). They are rod-shaped bacteria, like *Bacillus*, even though that they are microaerophilic and lack catalase.

Their habitat comprises soil and root crops located around Japan and Southeast Asia. The subgenus *Sporolactobacillus,* in particular, is D-lactic acid homofermentative under the microaerobic condition (Fritze & Claus, 1995). For cultivation, they are well grown in the medium for *Lactobacilli*.

To investigate phylogenetic relationships, G-C content was determined, and DNA-DNA hybridization was performed. The oligonucleotide pattern of ribosomal 16s RNA was also studied; the results indicated that *Sporolactobacillus* is included in the genus *Bacillus*. DNA homology groups from glucose, sucrose, fructose, maltose, mannose, and trehalose which was the different between strain (Claus *et al*., 2006).

- *Sporolactobacillus terrae*

This strain can grow at temperatures between 15-40°C. They can produce lactic acid from trehalose and inulin, but not from ribose, xylose, rhamnose, lactose, or sorbitol, and prefer lactic acid produced from galactose and sucrose. They can be isolated from soil (Yanagida *et al*., 1997). In lactic acid production, *Sporolactobacillus* has the potential to produce D-lactic acid via a homolactic fermentation pathway; the key enzyme is shown in Figure 6. A comparison between *Sporolactobacillus* from lactic acid production studies is summarized in Table 2. The phylogenetic relationships, according to this study using *Sporolactobacillus terrae* SBT-1 as a D-lactic acid producer, are shown in Figure 5.

Table 2 The comparison of D-lactic acid production from *Sporolactobacillus* sp.

Strain	Substrate	D-Lactic acid conc. (g/L)	Yield (g/g)	Time (h)
Sporolactobacillus sp. CASD (Wang *et al*., 2011)	Glucose	207	0.93	60
S. laevolacticus DSM 44 (Li *et al*., 2013)	Glucose	144.4	0.96	35
S. inulinus YBS1-5 (Bai *et al*., 2016)	Corn cob hydrolysates	107.2	0.85	90

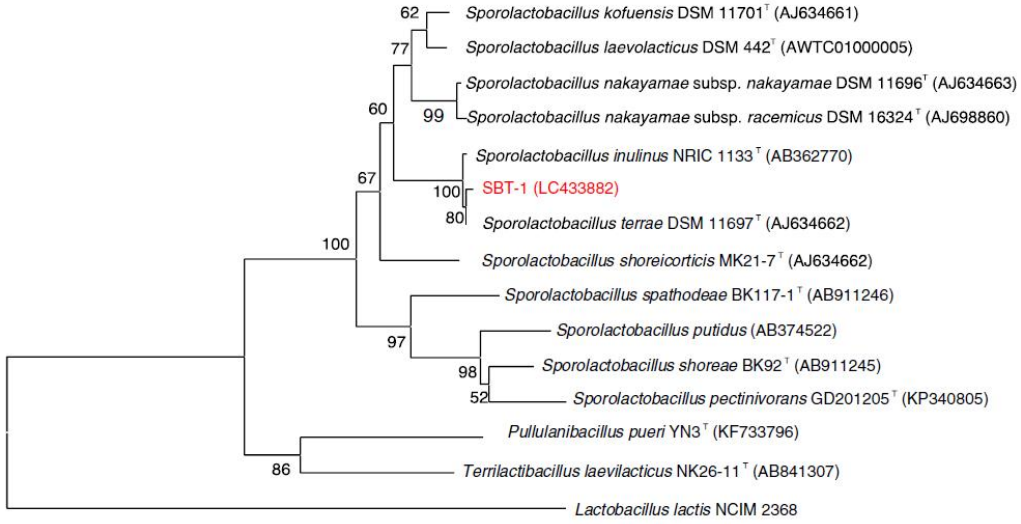


Figure 5 The related in phylogenetic relationship by 16S rRNA gene sequencing of *Sporolactobacillus terrae* SBT-1.

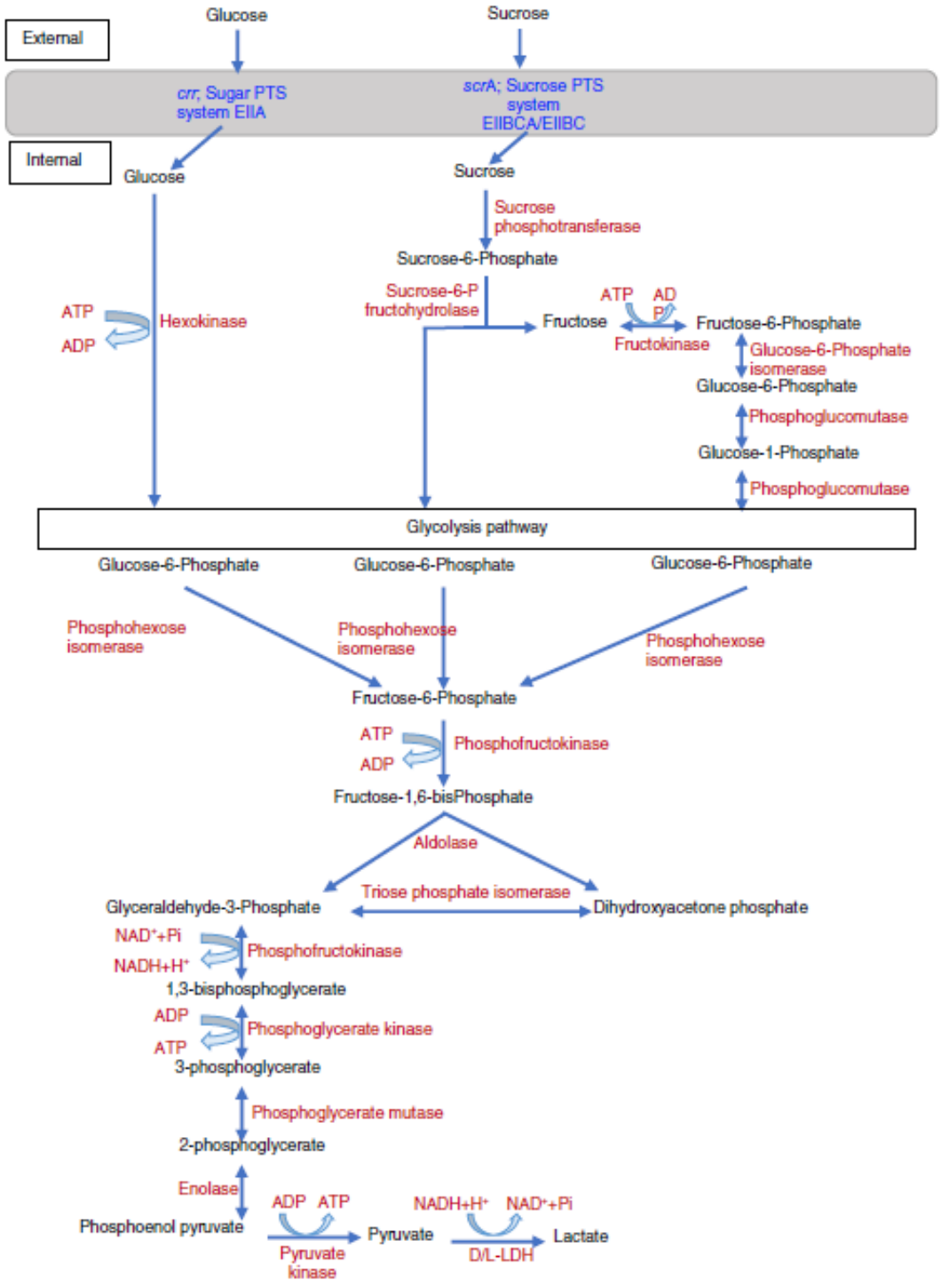


Figure 6 *S. terrae* SBT-1 sugar uptake pathway and key enzyme (Thitiprasert *et al*., 2021)

2.4 Lactic acid application

Due to the properties of lactic acid, it is used as a basic supply in many industries, including the pharmaceutical, textile, chemical, and food industries.

- The food industry

Lactic acid is used in a wide range of foods; approximately 85% of lactic acid demand goes to food-related applications. In many food products, lactic acid is used as an acidulant and preservative agent that is applied for pH control in food products. Moreover, the combination of lactic acid and acetic acid is used as a commercial bactericidal agent in poultry meat processing industries (Vijayakumar *et al*., 2008).

- Beer and wine

In soft drinks and fruit juice products, lactic acid is used as an acidulant to enhance the flavor of the beverages. In comparison to citric acid, lactic acid enhances the flavor and allows the taste to linger (Vijayakumar *et al*., 2008).

- The chemical industry

Lactic acid comprises two functional groups, a carboxylic and a hydroxyl group, which makes lactic acid the highest-potential monomer feed stock. In chemical inversion, lactic acid can be converted into propylene oxide, acetaldehyde, acrylic acid, propanoic acid, 2,3-pentanedione, and dilactide (Vijayakumar *et al*., 2008).

- Textiles

In the silk dying process, lactic acid is used as a dye fixative in woolen printing; it is also used in leather bating steps (Vijayakumar *et al*., 2008).

- The cosmetics industry

Lactic acid is added to cosmetic ingredients as a pH controller and moisturizer. Lactic acid can be also used as a skin brightening agent. Lactic acid is well known as an alpha hydroxyacid (AHA), improving the skin texture, that is commonly applied in anti-aging products (Vijayakumar *et al*., 2008).

- Pharmaceuticals industry

In the pharmaceuticals industry, lactic acid is used as an electrolyte solution. Moreover, lactic acid is used in mineral preparations such as tablets, prostheses, surgical sutures, and drug delivery systems. Since lactic acid is an anti-microbial agent, it is used in anti-acne products. Further, due to the biodegradability of lactic acid polymer, it is used for medical implants, stitches, or screws used in the repair of broken bones *(Vijayakumar et al*., 2008).

2.5 Medium optimization

Temperature, pH, C/N ratio, inoculation, and sterilization are essential parameters for the optimization of the lactic acid fermentation process, and the selection of substrate is one of the keys for medium optimization. The use of cost-effective, sustainable products is preferred. This selection step needs to be carried out before the scaling-up process can be undertaken. In medium optimization, it is essential to understand the role of factors like the use of carbon or nitrogen sources. It should be noted that a minimal growth requirement of the microorganism must be met

in order to achieve appropriate product yield. The preferred formula used for medium optimization should be the one that reduces the production time and cost while improving efficiency and simplifying the process.

- Carbon source optimization

In this study, the supplemented sugars, including glucose, sucrose, raw sugar, and molasses were considered in selecting the carbon source for medium optimization. To develop the medium formula, an understanding of each carbon source is required.

-Glucose

In medium optimization, glucose is one of the potential sugars that can be considered for use in the medium formula. Glucose is the most preferred sugar for lactic acid producing strains. In fact, many studies have used glucose as the sole carbon source for lactic acid fermentation. However, glucose has some disadvantages, one of which is the limitation of carbon catabolite repression, which has led researchers in many studies to try to solve the problem by using an engineered strain (Wang *et al*., 2015).

- Sucrose

The purest product can be obtained from the purest sugar fermentation. The cost of sucrose purification is less than that of glucose purification. Although sucrose is in the group of pure substrates, it the least expensive disaccharide substrate for fermentation. After sucrose molecule hydrolysis, one form of glucose and fructose is obtained.

- Raw sugar

In sugar manufacturing, raw sugar is an important material for the refining and affiliation process. It has a yellowish-covered color because of the appearance of molasses. The quality of raw sugar depends on moisture content, ash content, grain size of the crystal, reducing sugars content, and polarization value. In lactic acid fermentation, Mimitsuka *et al*. (2012) applied raw cane sugar for their lactic acid fermentation study with the reduction of sub-raw material for decreasing the production cost (Mimitsuka *et al*., 2012). The process from cane harvesting to the receipt of raw sugar comprises 5 steps: (i) cane juice extraction, (ii) cane juice clarification, (iii) cane juice evaporation, (iv) crystallization, and (v) centrifugation.

-Molasses

Molasses is a byproduct of sugar processing. It contains glucose, sucrose, and fructose. Molasses has a dark brown color, strong odor, and high f Chemical Oxygen Demand (COD) content as well as Biochemical Oxygen Demand (BOD). Due to its composition, molasses is a potential substrate for fermentation. However, molasses also contains heavy metals, which inhibit microbial growth. To enhance molasses efficiency, it can be pretreated by using sulfuric acid, tricalcium phosphate, potassium ferrocyanide, and EDTA (Vidra *et al*., 2017).

2.6 Oil palm trunk sap as a raw material for lactic acid production

Oil palm is an economically important plant, that can be used in the food, cosmetic and detergent industries. After 20-25 years of growth, the palm tree productivity decreases. This leads to replanting at the plantation site, which requires that the old trees be cut down. This increases oil palm tree waste, especially oil palm trunks (OPT). However, OPT is used as a power generation source, and it can also be used in paper production due to the fiber in oil palm waste, resulting in a valued-added product. The various uses of oil palm waste its subsidiary products are shown in Figure 7 (Dungani *et al*., 2018).

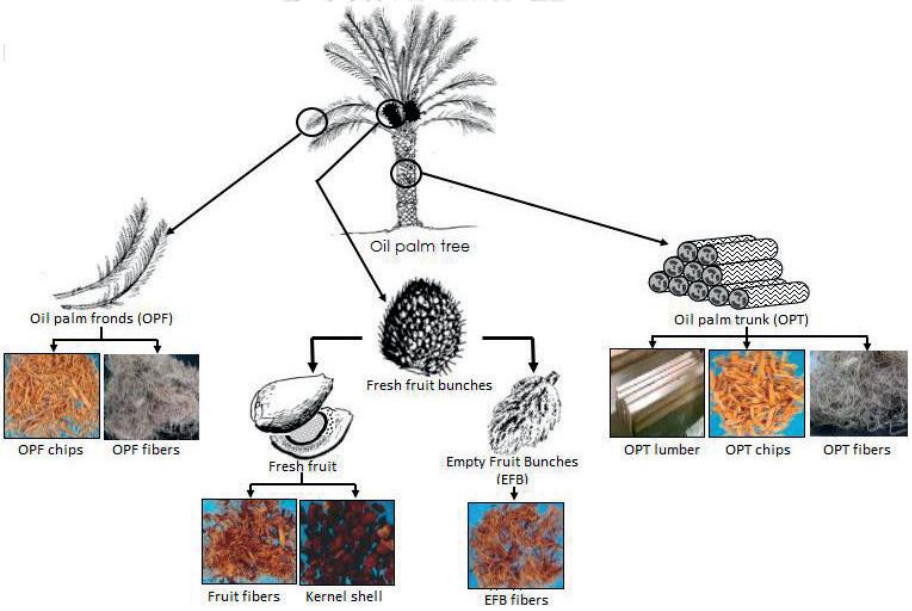


Figure 7 The oil palm waste and subsidiary product (Dungani *et al.*, 2018)

Among South and Southeast Asian countries like Malaysia, Sri Lanka, Philippines, and Indonesia, the biggest oil palm producer is Indonesia, followed by Malaysia and Thailand (Dallinger, 2011). In Thailand, over 60 oil palm crushing mills are in operation. Approximately 90% of oil palm plantations in Thailand are found in the Southern provinces, although expansion is prominent in the eastern area, especially in most parts of Chonburi and Trat. In 2008, the provinces which were most dominant were Krabi, Surat Thani, and Chumphorn (Dallinger, 2011).

The step of tapping the palm sap is briefly described in three steps (Figure 8). The first step is to fell the old oil palm tree, after which the terminal bud is cut. In the second step, the oil palm trunk is cut and chipped into small pieces. In the third step, the shredded oil palm trunk is squeezed by a screw press machine. Following the end of the process, the palm fiber is recycled as a biomass fuel; the palm sap is the waste product of oil palm fiber production.

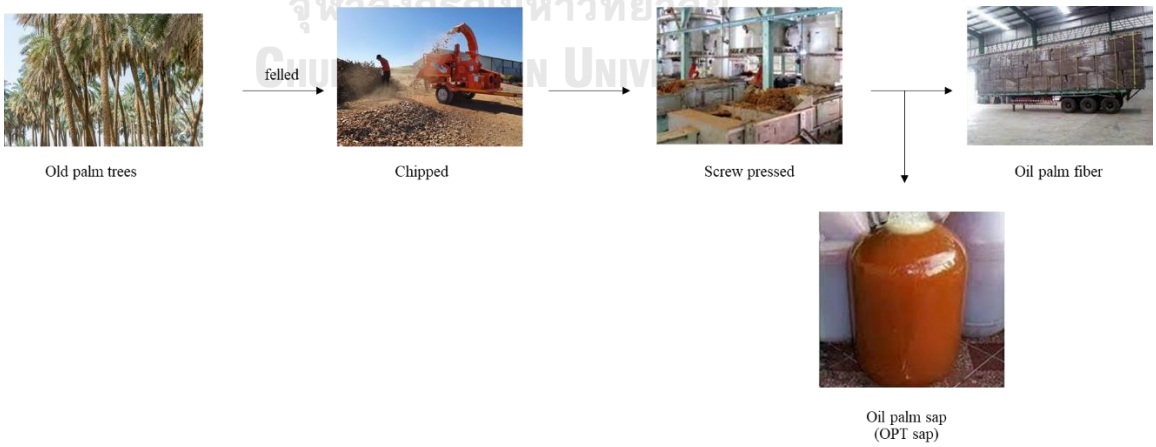


Figure 8 Oil palm sap production

2.6.1 The composition of the palm sap

Generally, palm sap contains sucrose, glucose, and fructose to an upper limit of about 12%. The quality of the sap varies (Table 3), probably due to the natural composition on the sap, the tapping method, the analysis method, and the period of the year that palm saps are tapped (Okafor, 1978). Fresh palm sap has an oyster white color, and the pH is acidic. The palm sap contains carbohydrates, amino acids, proteins, minerals, vitamins, and phenolic compounds (Hebbar *et al*., 2018). Palm sap can be naturally fermented by microorganisms, and Hebbar *et al*. (2015) found that there is a relationship between the sugar content and pH. Initially, lactic acid fermentation is obtained, followed by alcoholic fermentation, and then acetic acid fermentation. Palm sap has a plenty of substrate for microbial growth, especially bacteria and yeast. The largest genera are *Lactobacillus*, *Acetobacter*, *Sarcina*, *Streptococcus*, *Leuconostoc*, *Bacillus*, *Zymomonsa*, *Brevibacterium*, *Micrococcus*, *Serratia*, *Corynebacterium*, *Pediococcus*, *Klebsiella*, *Saccharomyces*, and *Candida* (Zongo *et al*., 2020).

Table 3 The sugar content in oil palm sap

Total sugar (%)	**Glucose (%)**	**Sucrose (%)**	**Fructose (%)**	**reference**
23.77–71.89	-	-	-	(Phaichamnan *et al*., 2010)
-	4.01–24.13	59.15–84.37	4.44–23.55	(Naknean & Meenune, 2011)
-	3.00–9.00	70.00–79.00	3.00–9.00	(Arcieri, 2014)

Generally, palm sap is used for production of beverages such as palm wine by the fermentation from yeast. The product, of course, is alcohol. However, palm sap is used in this study for acetic acid production and lactic acid production (Chooklin *et al*., 2011). The research studies palm sap fermentation to produce ethanol and lactic acid, as shown in Table 4. Based on palm sap characterization, this raw material can be readily utilized by microorganisms, indicating that palm sap demonstrates good potential as a raw material for fermentation.

Table 4 The fermentation by using oil palm sap as a substrate

Strain	Product	Yield	Experiment scale	Reference
Lactobacillus casei TISTR 1500	Lactic acid	0.78 g/g	Flask	(Chooklin *et al*., 2011)
Saccharomyces cerevisiae Kyokai no.7	Ethanol	0.94 g/g	Flask	(Akihiko Kosugi *et al*., 2010)
Lactobacillus lactis ATCC19435	Lactic acid	0.90 g/g	Flask	(Akihiko Kosugi *et al*., 2010)
Clostridium beijerinckii PS-3	Hydrogen	140.9 ml H_2/g	Flask	(Noparat *et al*., 2011)
Saccharomyces cerevisiae	Ethanol	0.46 g/g	2.5 L stirred tank	(Samsudin & Don, 2015)
Actinobacillus succinogenes 130Z	Succinic acid	0.50-0.55 g/g	Flask	(Bukhari *et al*., 2019)
Clostridium acetobutylicum DSM 1731	Butanol	0.35 g/g	glass serum bottle	(Komonkiat & Cheirsilp, 2013)

CHAPTER 3

MATERIAL AND METHODS

3.1 Apparatus and Chemicals

3.1.1 Apparatus

Apparatus	Model	Manufacturer	Country
Autoclave	KT-40L	ALP Co.,Ltd.	Japan
Centrifuge	7000	Kubota	Japan
Electronic balance	ML204/01	Mettler Toledo AG	Switzerland
Electronic balance	ML3002E/01	Mettler Toledo AG	Switzerland
High-Performance Liquid Chromatography	Shimadzu LC-10A	Shimadzu Co., Ltd.	Japan
High-Performance Liquid Chromatography	20A	Bara Scientific Co., Ltd.	Thailand
High-Performance Liquid Chromatography	40A	Bara Scientific Co., Ltd.	Thailand

Apparatus	Model	Manufacturer	Country
Hot air oven	UL-80	Memmert Co., Ltd.	Germany
Laminar flow hood clean bench	NK system	International Scientific Supply	Thailand
Microcentrifuge	F-45-12-11	Eppendorf	Germany
pH meter	AB15	Fisher Scientific, Ltd.	Singapore
Rotary incubator shaker	G25	New Brunswick Scientific Co., Inc.	USA
Spectrophotometer	UV-1280	Shimadzu	Japan
Stirred tank bioreactor	FM1, FM4	B.E. Marubishi Co., Ltd.	Thailand

3.1.2 Chemicals

Chemicals	**Manufacturer**	**Country**
Calcium carbonate ($CaCO_3$)	Honeywell	USA
Copper sulfate ($CuSO_4$)	Merck	Germany
Dextrose ($C_6H_{12}O_6$)	Pure Chem Co., Ltd.	Thailand
D-Fructose Extra pure ($C_6H_{12}O_6$)	Loba Chemie Pvt., Ltd.	India
Di-Potassium hydrogen phosphate anhydrous (K_2HPO_4)	Carlo Erba	Italy
Ethanol (C_2H_5OH)	Merck	Germany
Glucose ($C_6H_{12}O_6$)	Siamchai Chemical	Thailand
Hydrochloric acid (HCl)	Merck	Germany
Iron (II) sulfate heptahydrate ($FeSO_4.7H_2O$)	Sigma	Germany

Chemicals	**Manufacturer**	**Country**
Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$)	Riedel-de Haen	Germany
Molasses	MH FOOD	Paraguay
Peptone	Fluka	France
Potassium chloride (KCl)	Merck	Germany
Potassium phosphate monobasic (KH_2PO_4)	Merck	Germany
Raw sugar	Lin	Thailand
Sodium chloride (NaCl)	Ajax Finechem Pty, Ltd.	Australia
Sodium hydroxide (NaOH)	Grand Chemical	Thailand
Sucrose ($C_{12}H_{22}O_{11}$)	Mitr phol	Thailand
Sulfuric acid (H_2SO_4)	Sigma	Germany
Yeast extract	BioSpringer	France
Calcium carbonate ($CaCO_3$)	Honeywell	USA

3.2 Methodology

3.2.1 Characterization of oil palm trunk sap

The chopped oil palm trunk was dewatered by using a screw press machine then discharged into a sterile plastic bottle. The oil palm trunk sap (OPTsap) that was collected in a plastic bottle was sterilized at 121°C for 15 minutes by using an autoclave at Nitto-Freshco Biofuel Company Limited. The oil palm trunk sap supported by Nitto-Freshco Biofuel Company Limited was sent to characterize the composition at ALS Laboratory Group (Thailand) for vitamins, minerals, amino acids, and fiber. Before using the oil palm sap in every experiment, the sap was centrifuged at 10000 g at 4°C for 10 minutes to separate the sediment out of the sap. Then the sap was autoclaved and determined for sugar content before being stored in the cold room at 4°C. The sugar content in the sap was determined by high-performance liquid chromatography (HPLC).

3.2.2 Culture preparation

Sporolactobacillus terrae SBT-1 stock culture was grown on a GYP agar slant (10 g/L of glucose, 5 g/L of yeast extract, 5 g/L of peptone, 0.25 g/L of KH_2PO_4, 0.25 g/L of K_2HPO_4, 20 g/L of agar, 5g/L of $CaCO_3$ and 10 mL of salt solution (40 g/L of $MgSO_4.5H_2O$, 2 g/L of $MnSO_4.5H_2O$, 2 g/L of $FeSO_4.7H_2O$ and 2 g/L of NaCl), pH 6.8 adjusted by 10M NaOH) at 37°C for 24 hours under anaerobic condition by using W-zip pouch with AnaerobicPack-Anaero (Mitsubishi Gas Chemical) on the inside.

For preculture preparation, 1% v/v inoculum size of 24 hours *S. terrae* SBT-1 culture was grown in 50 mL GY broth (10 g/L of glucose, 15 g/L of yeast extract, 4

g/L of NH_4Cl, 0.25 g/L of KH_2PO_4, 0.25 g/L of K_2HPO_4, 5 g/L of $CaCO_3$, and 10 mL of salt solution, pH 6.8 adjusted by 10M NaOH) in 250 mL flask which was T-type silicone plugged, incubated at 37°C and shaking at 200 rpm for 6 hours under the anaerobic condition as mentioned above.

3.2.3 Optimization of fermentation medium in flasks

To optimize the fermentation medium, 50% v/v of preculture was inoculated in the fermentation medium. The fermentation medium, OPTsap mixed sugar, consists of sucrose 15 g/L, glucose 10 g/L, and fructose 15 g/L, which were supplemented by 80 g/L of 4 different sugar, containing raw sugar, glucose, sucrose, and molasses. The pH was controlled by adding 80 g/L of $CaCO_3$ to each flask. This experiment was performed at 37°C and shaking at 150 rpm under the anaerobic condition for 48 hours. The sample was collected every 6 hours. Then determined pH by pH meter and cell density by spectrophotometer at 600 nm.

3.2.4 Optimization of fermentation medium in 5L fermenter

3.2.4.1 culture preparation for 5L fermenter

The 5L fermenter was prepared, the pH probe and the temperature probe were calibrated by using a fermenter controller (B.E. Marubishi, Thailand, Co., Ltd.). The fermenter contained 1.75 L of modified GY medium in which glucose was replaced by OPTsap. Then was sterilized at 121°C for 20 minutes. After the fermenter was left to cool down at room temperature, the OPTsap was added after sterilization and controlled pH at 6.8 by 10M NaOH.

To prepare the starter culture for the 5L fermenter, this method starts with preculture preparation steps in flasks as same as 3.2.2. by using a

modified GY medium for growth. After incubation for 6 hours, the preculture was transferred by using 10%v/v inoculum size into a medium in a fermenter. The preculture was incubated at 37°C, with agitation at 300 rpm and non-air-feeding conditions for 5 hours. The preculture was 50% v/v of working volume in the fermenter.

3.2.4.2 Fermentation in 5L fermenter

After preculture was grown, the fermentation medium was added to the fermenter. Fermentation medium composition consists of OPTsap supplemented with 4 different sugar, containing raw sugar, glucose, sucrose, and molasses to get a final concentration of 120g/L. The fermentations were performed at 37°C with an agitation rate of 300 rpm and under non-air feeding conditions. Then determined pH by pH meter and cell density by spectrophotometer at 600 nm.

3.2.5 Preparation of sample and HPLC analysis

To collect the fermentation broth, the sample was acidified by using 1M HCL and was centrifuged at 10000 g for 5 minutes to separate cells and supernatant. The supernatant was diluted with distilled deionized (DDI) water. The HPLC system (LC-20AT, Shimadzu Co. Ltd., Japan) was equipped with an Aminex HPX-87H column at 45°C (Aminex HPX-87H ion exclusion organic acid column; 300 mm × 7.8 mm) and a refractive index (RID-20A) by using 0.005 M H_2SO_4 as a mobile phase, the flow rate was 0.6 mL/min. by using lactic acid, acetic acid, sucrose, glucose, fructose and ethanol as a standard solution. The product concentration was determined for production yield (Y p/s).

$$Production\ yield\ (Y\ p/s) = \frac{product\ concentration\ (g)}{Substrate\ consumed\ (g)}$$

To determine the optical purity of D-lactic acid (% ee), the chiral column (Sumipack, Sumichiral OA5000) was set at 40°C by using 1 mM $CuSO_4$ as a mobile phase, the flow rate was 1.0 mL/min and the UV detector was 245 nm. DL-lactic acid was applied as a standard solution.

$$Optical\ purity\ (\%ee) = \frac{\text{D-lactic acid} - \text{L-lactic acid}\ (peak\ area)}{\text{D-lactis acid} + L - \text{lactic acid}\ (peak\ area)}$$

3.2.6 Data analysis

All the data will be represented the results of three independent experiments and be expressed as the mean values ± standard deviations (SDs). Analysis of variance (one-way ANOVA) followed by Duncan's test for testing differences among means will be conducted using SPSS version 22 (IBM Corp., Armonk, NY, USA). Differences will be considered significant at $P < 0.05$.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Characterization of oil palm trunk sap

The oil palm sap was sent to ALS (ALS Laboratory Group, Thailand) in order to analyze its composition. Table 5 shows the composition, including amino acid profile, food and vitamins, moisture, and metal content. Figure 9 illustrates the composition breakdown of oil palm sap. Moisture comprises the largest proportion of the composition at 59.69%, followed by sugar at 29.94%, while food and vitamins accounted for 9.57%, and total amino acid and metal just 0.80%. There was variation found; amino acid, mineral, and vitamin content in palm sap depended on the day that palm sap was preserved, and the percentages would significantly decrease due to protein degradation and the production of microorganisms (Jolly *et al*., 2006).

The sugar ratio in palm sap depends on the species of a palm tree; in this study, the sugar content was determined by HPLC. The sugar content of OPT sap comprises sucrose, glucose, and fructose, which are fermentable sugars. Table 5 shows that the total sugar content in OPT sap was 55.37 g/L, and due to the unhygienic tapping process, alcoholic and acidic fermentation was found in the palm sap. Generally, OPT sap is used for fermented products such as toddy, tuba, or tuak, which is a fermented palm wine commonly consumed in Asia and Africa (Jolly et al., 2006). In biological technology, Kosugi *et al*. (2010) also study ethanol production using oil palm trunk sap as a substrate fermented by sake brewing yeast strain (Kosugi et al., 2010), and lactic acid production using *Lactobacillus lactis* ATCC19435. They reported that OPT sap contains readily fermentable sugar, which

provides the same efficiency as using glucose from the MRS medium. Moreover, according to Kunasundari *et al.* (2017), palm sap was used for lactic acid production. In that research, palm sap was fermented by *Bacillus coagulans* strain 191, which provided a lactic acid yield of 53%, and productivity was 1.56 g/L·h. This study pretreated the oil palm sap by using the activated charcoal, acidic, and alkaline precipitation resulting in the reduction of metal element, and organic and phenolic compounds, but also the loss of some total sugar content (Kunasundari *et al*., 2017). To compare this with OPT sap in this study, without any pretreatment steps, the inhibitor compound, e.g., metal, was obtained in a few concentrations (0.8%).

This indicates that OPT sap, which, without any pretreatment step contains the amino acid, mineral, and vitamin content, can be considered as a potential raw material for lactic acid fermentation.

Table 5 Characterization of oil palm sap

Oil palm sap composition	
Amino acid profile	**Concentration (g/L)**
Aspartic acid	0.123
Cystine	0.021
Glutamic Acid	0.045
Glycine	0.024
Histidine	0.074
Hydroxylysine	0.000

Oil palm sap composition	
Amino acid profile	**Concentration (g/L)**
Hydroxyproline	0.000
Isoleucine	0.009
L-Alanine	0.041
L-Arginine	0.091
Leucine	0.018
Lysine	0.053
Methionine	0.011
Phenylalanine	0.018
Proline	0.021
Serine	0.038
Threonine	0.022
Tryptophan	0.023
Tyrosine	0.019
Valine	0.013
Food and vitamins	**Concentration (g/L)**
Ash	1.180
Biotin	0.000
Calories (Include dietary fiber)	5.426
Carbohydrates (Include dietary fiber)	9.115
Dietary Fiber (Total)	0.949

Oil palm sap composition	
Food and vitamins	**Concentration (g/L)**
Fat	0.256
Folic acid	0.000
Pantothenic acid	0.000
Protein	0.767
Vitamin B1	0.000
Vitamin B12	0.000
Vitamin B3 (Niacin)	0.001
Vitamin B6 (as Pyridoxine)	0.000
Vitamin C	0.000
Moisture	110.382
Metals	**Concentration (g/L)**
Calcium	0.071
Iron	0.007
Magnesium	0.087
Manganese	0.001
Phosphorus	0.058
Potassium	0.539
Selenium	0.000
Sodium	0.055
Zinc	0.001

Oil palm sap composition	
Sugar	**Concentration (g/L)**
Sucrose	15.69
Glucose	23.60
Fructose	16.08
Total sugar	55.37

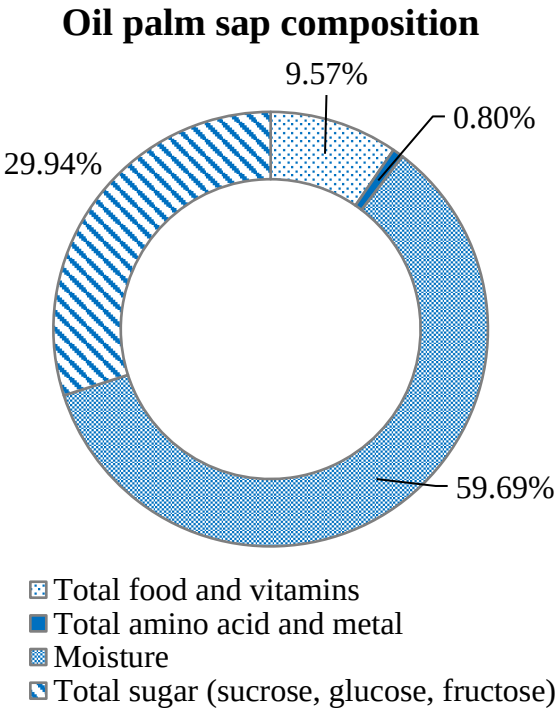


Figure 9 Percent composition of oil palm sap

4.2 Optimization of fermentation medium in flasks

Using different types of sugar not only provides cost effectiveness in designing the fermentation medium, but also facilitates the identification of the most efficient substrate for lactic acid fermentation. According to the situation that OPT sap does not supply. The OPT sap model solution was applied, then supplemented with 4 different sugars (glucose, sucrose, raw sugar, and molasses) at a concentration of 120 g/L to be fermented by *S. terrae* SBT-1. The results are shown in the next section.

4.2.1 Fermentation of lactic acid by *S. terrae* SBT-1 in OPT sap model solution with supplementation of glucose at 120 g/L

Figure 10 shows the fermentation kinetics of *S. terrae* SBT-1 in OPT sap model solution that was supplemented with glucose at a concentration of 120 g/L. After 24 h, the fermentation was complete, and no residual sugar content was found. The consumption rates of glucose and fructose were 3.84 and 0.88 g/L·h respectively. At 24 h the lactic acid production by *S. terrae* SBT-1 reached a peak concentration of 99.70 g/L. This condition provides the highest lactic acid productivity at 4.15 g/L·h and lactic acid yield at 0.87 g/g. Cell density, found by measuring optical density at 600nm, was 21.68 (Table 6). The optical purity (%ee) was 98.13%.

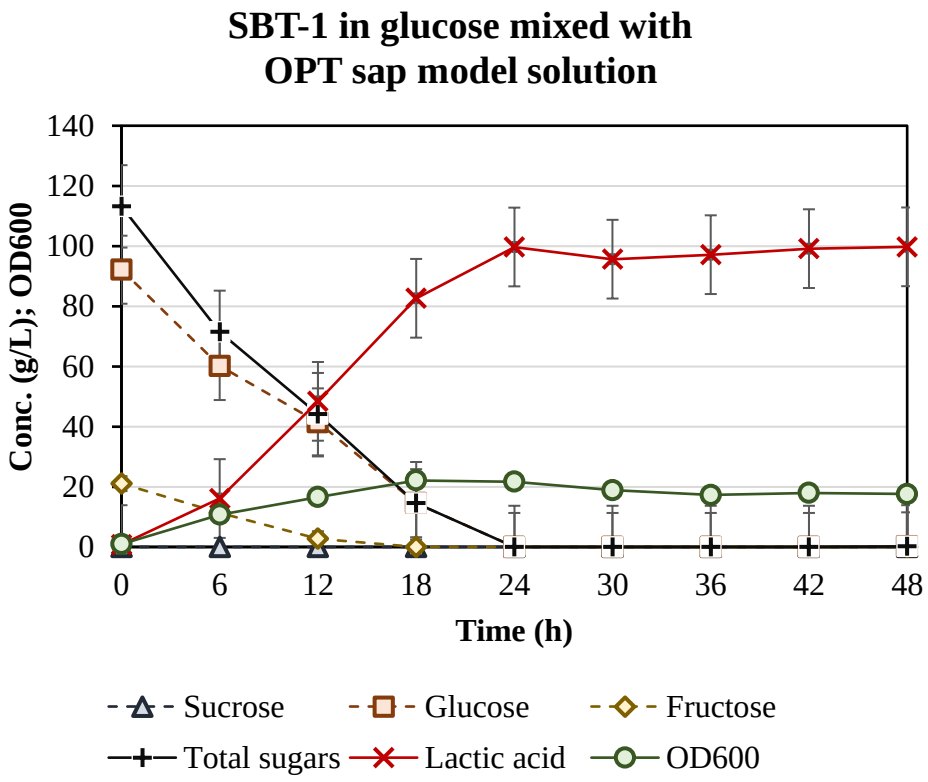


Figure 10 Fermentation of *S. terrae* SBT-1 in OPT sap model solution with supplementation of glucose at 120 g/L

4.2.2 Fermentation of lactic acid by *S. terrae* SBT-1 in OPT sap model solution with supplementation of sucrose at 120 g/L

Figure 11 shows the fermentation kinetics of *S. terrae* SBT-1 in OPT sap model solution with sucrose serving as an additive sugar at a concentration of 120 g/L. At 18 h, the sucrose was completely consumed. Sucrose, glucose, and fructose consumption rates were 0.45, 2.55, and 2.46 g/L·h respectively. Fermentation was complete at 24 h, with no residual sugar detected. The lactic acid production increased significantly from 0 h to 24 h, and lactic acid production by *S. terrae* SBT-1 at complete fermentation time was 104.70 g/L. The lactic acid yield was 0.87 g/g, and the productivity was 4.36 g/L·h. At 24 h, cell density, found by measuring optical density at 600nm, was 20.98 (Table 6). The optical purity (%ee) was 96.89%.

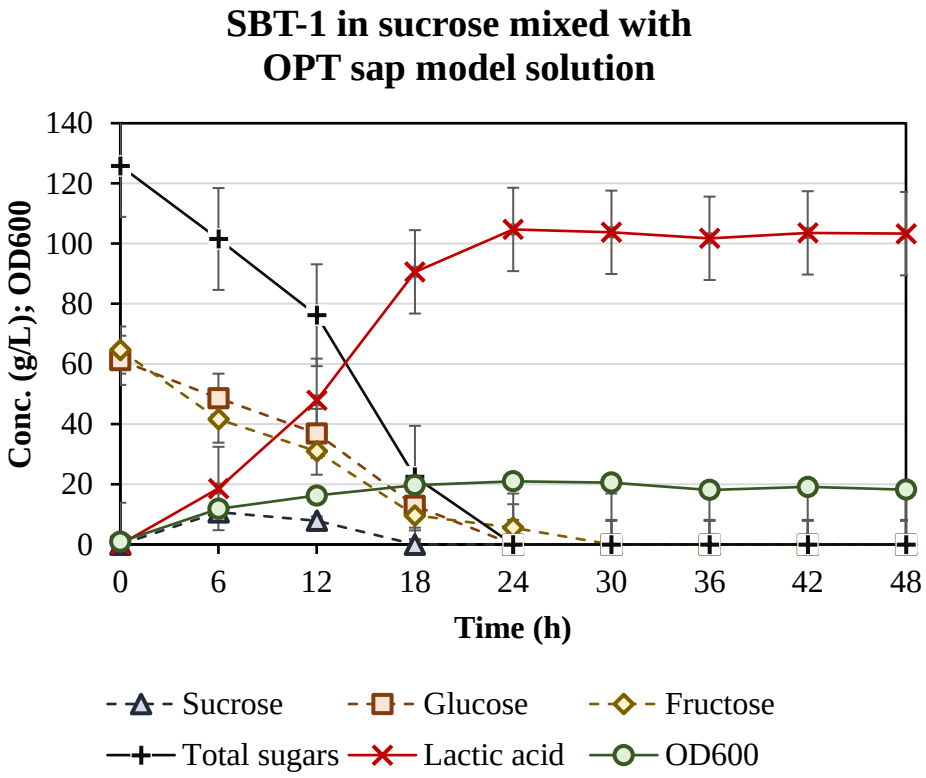


Figure 11 Fermentation of *S. terrae* SBT-1 in OPT model solution with supplementation of sucrose at 120 g/L

4.2.3 Fermentation of lactic acid by *S. terrae* SBT-1 in OPT sap model solution with supplementation of raw sugar at 120 g/L

Figure 12 shows the fermentation kinetics of *S. terrae* SBT-1 in OPT sap model solution with raw sugar added at a concentration of 120 g/L. Fermentation was completed in 24 h, with no residual total sugar detected. Sucrose, glucose, and fructose consumption rates were 0.38, 2.34, and 2.05 g/L·h respectively. The lactic acid was produced at a high rate from 0 h to 18 h, and at complete fermentation time, lactic acid was 103.95 g/L. The lactic acid yield was 0.86, and the productivity was 4.33 g/L·h. Cell density at the end of fermentation time, measured by optical density at 600nm, was 20.63 (Table 6). The optical purity (%ee) was 99.20%.

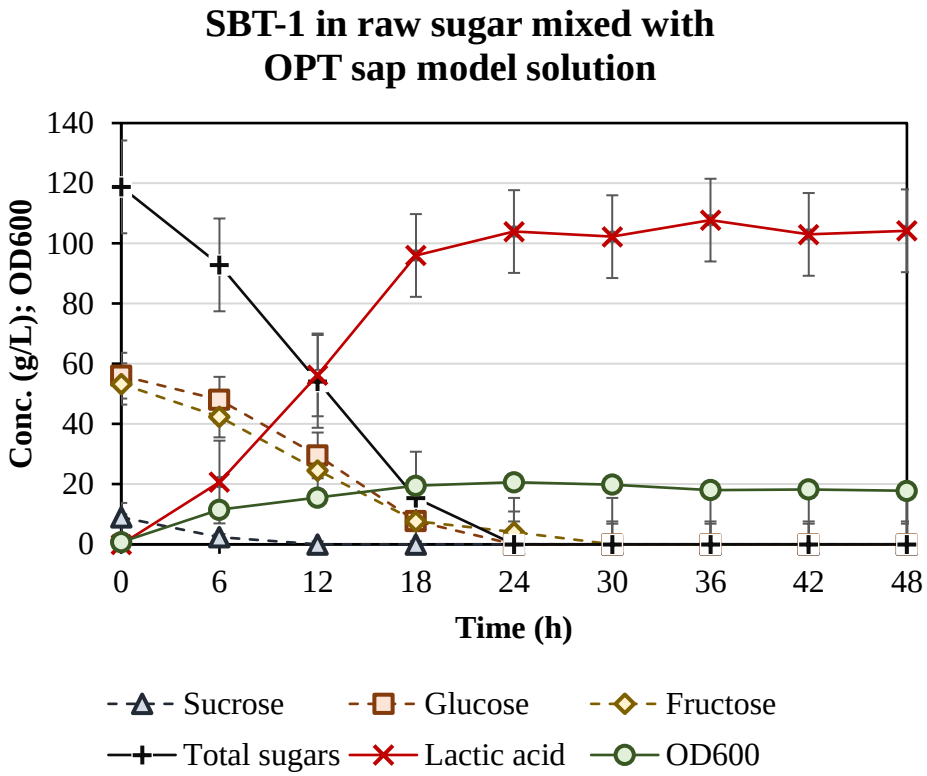


Figure 12 Fermentation of *S. terrae* SBT-1 in OPT sap model solution with supplementation of raw sugar at 120 g/*L*

4.2.4 Fermentation of lactic acid by *S. terrae* SBT-1 in OPT sap model solution with supplementation of molasses at 120 g/L

Figure 13 shows the fermentation kinetics of *S. terrae* SBT-1 in OPT sap model solution, with molasses added at a concentration of 120 g/L. Fermentation was complete at 36 h. At the end of the sampling time, 48h, residual sugar was detected at a concentration of 14.90 g/L. Sucrose, glucose, and fructose consumption rates were 0.31, 1.32, and 1.12 g/L·h respectively. The lactic acid production by *S. terrae* SBT-1 at complete fermentation time was 89.42 g/L. The lactic acid yield was 0.87, and the productivity was 2.42 g/L·h. Cell density, found by measuring optical density at 600nm, was 21.10 (Table 6). The optical purity (%ee) was 99.13%.

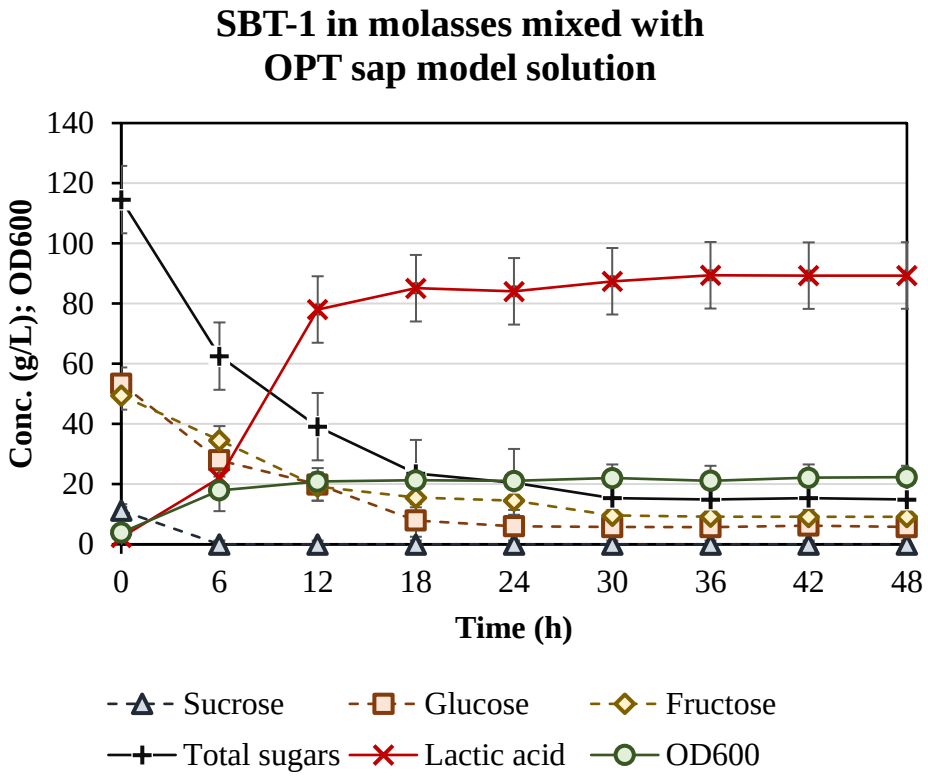


Figure 13 Fermentation of *S. terrae* SBT-1 in OPT sap model solution with supplementation of molasses at 120 g/L

Table 6 Fermentation kinetics of lactic acid by *S. terrae* SBT-1 using OPT model solution with the supplementation of raw sugar, sucrose, glucose and molasses

Fermentation kinetics	**OPT sap + Glucose**	**OPT sap + Sucrose**	**OPT sap + Raw sugar**	**OPT sap + Molasses**
Highest lactic acid concentration (g/L)	99.70±0.30	104.70±0.05	103.95±0.05	89.42±1.05
Fermentation time (h)	24	24	24	36
Initial sugar concentration (g/L)	113.20±0.70	125.80±0.40	118.80±0.81	114.57±0.91
Residual total sugar concentration (g/L)	0.00±0.00	0.00±0.00	0.00±0.00	14.90±0.04
Sucrose consumption rate (g/L·h)	0.00±0.00	0.45±0.02	0.38±0.00	0.31±0.02
Glucose consumption rate (g/L·h)	3.84±0.02	2.55±0.01	2.34±0.01	1.32±0.01
Fructose consumption rate (g/L·h)	0.88±0.01	2.46±0.01	2.05±0.01	1.12±0.01
Max OD	21.68±0.02	20.98±0.02	20.63±0.27	21.10±0.33
Yp/s	0.87±0.00	0.87±0.00	0.91±0.02	0.87±0.02
Yx/s	0.18±0.00	0.16±0.00	0.16±0.00	0.22±0.00
Productivity (g/L·h)	4.15±0.01	4.36±0.00	4.33±0.08	2.42±0.03*
Optical purity %ee	98.13±0.45	96.89±0.84	99.20±0.31	99.13±0.01

Statistical significance was determined using a post hoc test followed by Tukey's honestly significant difference. The difference is indicated as significant (*$p < 0.05$). *OPT sap model solution supplemented by molasses provided the significant difference

Glucose is a practical substrate for many fermentations due to the fact that it is a reducing sugar that can enhance yield and productivity in the fermentation. Generally, glucose is the most preferred sugar for microbes that produce lactic acid (Wang *et al.*, 2015). Moon *et al.* (2012) used glucose at a concentration of 200 g/L, fermented by *Lactobacillus paracasei* subsp. *paracasei* CHB212; the lactic acid concentration was 192 g/L, productivity was 3.99 g/ L·h, and yield was 0.96 g/g (Moon *et al*., 2012). Moreover, by using glucose as a substrate, *Bacillus subtilis* MUR1 produced lactic acid at 99.3 g/L and 183.2 g/L within 12 and 52 h of fermentation, respectively. The substrate conversion yield was 98.5% (Gao *et al*., 2012). Even glucose can give high lactic acid production, but using glucose has the limitation of carbon catabolite repression, which has a negative effect on utilization of other sugars (Wang *et al.*, 2015). In this study, batch fermentation supplemented by glucose also provided high lactic acid yield, and productivity at 0.87 g/g and 4.15 g/L·h, with no glucose repression found.

Sucrose was used as a carbon source for lactic acid fermentation, both in the mixed sugar study and as the sole carbon source. Sucrose is a one of the more inexpensive carbon sources because it can be derived from sugar cane or beet molasses. It would be advantageous to be able to ferment lactic acid from this cost-effective material in order to reduce production cost. Wang *et al.* (2012) used sucrose as a substrate for lactic acid fermentation by using engineered *Escherichia coli* as a lactic acid producer (Wang *et al*., 2012). Their experiment produced lactic acid at 85 g/L by fermenting 100 g of sucrose, and exhibited productivity at 1.0 g/L·h. However, sucrose has been reported as a substrate that can cause glucose suppression. In the case of sucrose being hydrolyzed into glucose and fructose, and sugar uptake rate was

slower than the hydrolyzed rate (Thompson & Chassy, 1981). In this study, by using *S. terrae* SBT-1 as a lactic acid producer strain, sucrose was uptaken via a sucrose specific phosphotransferase system (PTS). Therefore, in the high concentration of sucrose, lactic acid fermentation was not repressed (S. Thitiprasert *et al*., 2021).

Raw sugar is an important material for sugar manufacturing because it is used for refined sugar production. Raw sugar is sucrose with a mixture of impurities such as ash and color coating on the crystal. Raw sugar is an interesting substrate for fermentation due to the raw sugar price, which is lower than white or refined sugar or glucose, as reported by the Office of the Cane and Sugar Board, Thailand. Mungkalasiri *et al.* (2018), used raw sugar in an experiment related to ethanol production. They considered raw sugar as an alternative substrate by evaluating the optimum quantity of raw sugar in proportion to molasses (Mungkalasiri *et al*., 2018). This research presented the economic results from the mixture of raw sugar and molasses; it was found that 20%: 80% provides the greatest profit. In our study, the batch fermentation using raw sugar also provides high lactic acid yield and productivity at 0.87 g/g and 4.33 g/L·h respectively. This indicates that raw sugar has the potential to improve fermentation efficiency and reduce production costs. Thus, raw sugar is an attractive substrate for lactic acid fermentation.

Molasses, a sugar industry byproduct, has been used in many research studies as a cheap raw material. In lactic acid fermentation, molasses plays a part in the culture medium as a carbon source due to the fermentable sugar content, including sucrose, glucose, and fructose, in this substrate. Molasses contains both organic and inorganic inhibitors that can block cell metabolization, such as nitrogen compounds,

organic acids, amino acids and heavy metals (Xu & Xu, 2014). To reduce the inhibition from molasses, some studies pretreat the molasses before applying it in the fermentation process, but this step increases the production cost. In a lactic acid fermentation study, Vidra *et al*. (2017) use *Lactobacillus casei* and *Lactobacillus sp.* MKT878 to ferment the sugar content in molasses; the result showed lactic acid yield between 55-80 g/g, even with the effect of inhibition from heavy metal. Additionally, Kunwar *et al*. (1995) also used *Sporolactobacillus cellulosolvens* to ferment molasses into lactic acid; the result showed the lactic acid yield coefficient at 0.79 (Kanwar *et al*., 1995). In our study, the addition of molasses fermented by *S. terrae* SBT-1 during the batch fermentation provided higher lactic acid production yield, at 0.87 g/g, with some unfermentable sugar remaining.

In this study, the fermentation kinetics in flask by *S. terrae* SBT-1 are shown in Table 6. The productivity from the glucose, sucrose and raw sugar supplemented batch was significantly better than molasses condition (Table 6). The consumption rate of sucrose, glucose, and fructose under every condition is shown in table 6. In every batch fermentation, the sucrose consumption rate was the slowest, while the glucose and fructose consumption rates were similar, except for the supplementation of glucose experiment, in which the glucose consumption rate was the highest. This can be explained by the *S. terrae* SBT-1 sugar uptake pathway. It should be noted that *S. terrae* SBT-1 is phosphoenolpyruvate-dependent phosphotransferase system (PTS) associated. All sugars need to be converted into glucose-6-phosphate to be utilized by the glycolysis pathway; glucose was phosphorylated by hexokinase enzyme to form glucose-6-phosphate. However, more enzymes are involved in the sucrose uptake metabolic pathway. Sucrose-6-P from phosphorylation produced fructose and

glucose-6-phosphate by sucrose-6-P hydrolases, and fructose was hydrolyzed by glucose-6-phosphateisomerase (Reid & Abratt, 2005; S. Thitiprasert et al., 2021). This reveals that glucose and fructose are equally preferred in lactic acid fermentation by *S. terrae* SBT-1., In fact, similar results in glucose and fructose consumption rates from *S. terrae* SBT-1 were obtained, unlike most bacteria, which prefer glucose for fermentation (Wang *et al*., 2015). This represents the ability to ferment cane sugar from *S. terrae* SBT-1, making lactic acid fermentation by this strain a potentially useful alternative.

To conclude, the results indicate that *S. terrae* SBT-1 has the ability to ferment OPT sap model solution with the supplementation of glucose, sucrose, raw sugar, or molasses at a concentration of 120 g/L, the raw sugar supplemented batch provide the highest lactic acid production yield at 0.91 g/g. In addition, *S. terrae* SBT-1 can produce a high lactic acid concentration. *S. terrae* SBT-1 was found to be the preferred bacteria since it was highly efficient as a lactic acid producer. Moreover, the similar consumption rate of glucose and fructose indicates that *S. terrae* SBT-1 can ferment cane sugar. Therefore, the lactic acid fermentation results from this strain provide an acceptable basis for moving on to the next experiment, scaling up the 5L fermenter process.

4.3 Optimization of fermentation medium in the 5L fermenter

In accordance with the results from flask-scale medium optimization studies, the results from OPT sap model solution supplemented by 4 different sugar media demonstrated high lactic acid yields with similar result. To study the scaling-up the process, we decided to investigate fermentation by using OPT sap supplemented with raw sugar, sucrose, glucose, and molasses in a 5 L fermenter.

4.3.1 Fermentation of lactic acid by *S. terrae* SBT-1 in OPT sap with supplementation of glucose at 120 g/L in a 5 L fermenter

The fermentation kinetics of lactic acid production by using *S. terrae* SBT-1 fermented OPT sap adding glucose at a concentration of 120 g/L are shown in Figure 14. At 24 h, the fermentation was complete, and 117.43 g/L of lactic acid concentration was obtained. From 0 h to 18 h, lactic acid productively increased and then remained steady until the end of batch fermentation. The residual sugar was 9.70 g/L. Sucrose, glucose, and fructose consumption rates were 2.81, 2.30, and 0.01 g/L·h, respectively. The lactic acid yield was 0.99 g/g, with productivity at 4.94 g/L·h. Cell density, found by measuring optical density at 600 nm, was 32.07 (Table 7). The optical purity (%ee) of D-lactic acid was 99.41%.

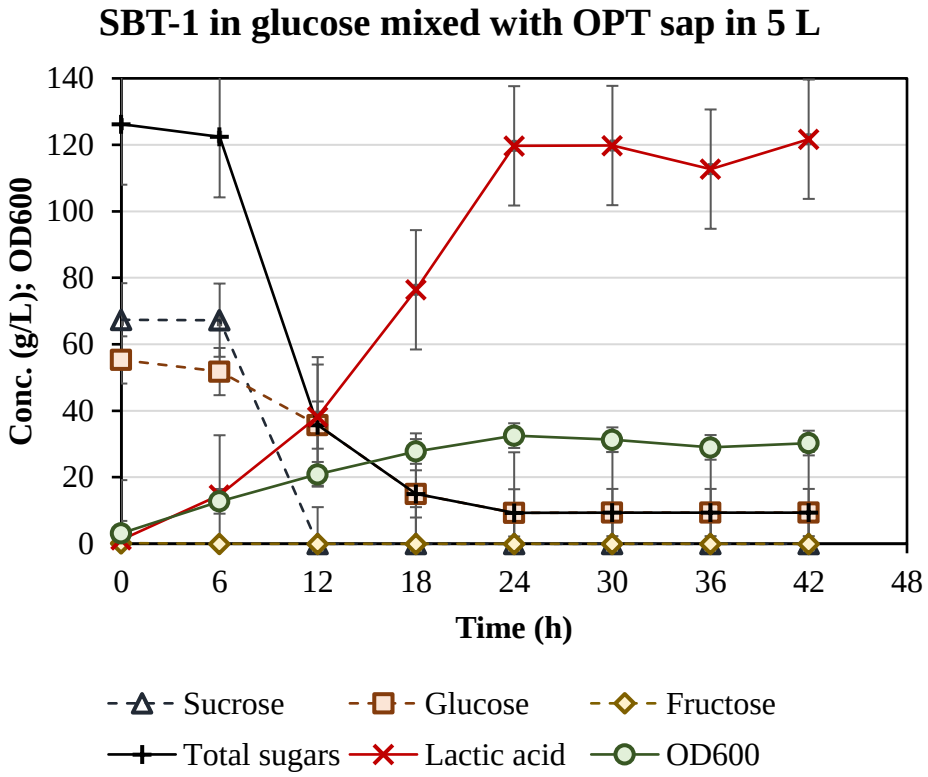


Figure 14 Fermentation of *S. terrae* SBT-1 in OPT sap with supplementation of glucose at 120 g/L in a 5L fermenter

4.3.2 Fermentation of lactic acid by *S. terrae* SBT-1 in OPT sap with supplementation of sucrose at 120 g/L in a 5 L fermenter

Figure 15 shows the fermentation kinetics of OPT sap supplemented with sucrose fermented by *S. terrae* SBT-1. The fermentation was complete at 18 h, and residual sugar concentration at 11.10 g/L was detected. Sucrose, glucose, and fructose consumption rates were 1.11, 2.18, 2.00 g/L·h respectively (Table 7). Lactic acid dramatically climbed from 0 h to 18 h, then reached a peak at 18 h, the concentration was 112.10 g/L. The production yield and the productivity of lactic acid were 1.04 g/g and 6.21 g/L·h respectively. Cell density, found by measuring optical density at 600 nm, was 28.10 (Table 7). The optical purity (%ee) of D-lactic acid was 100.00%.

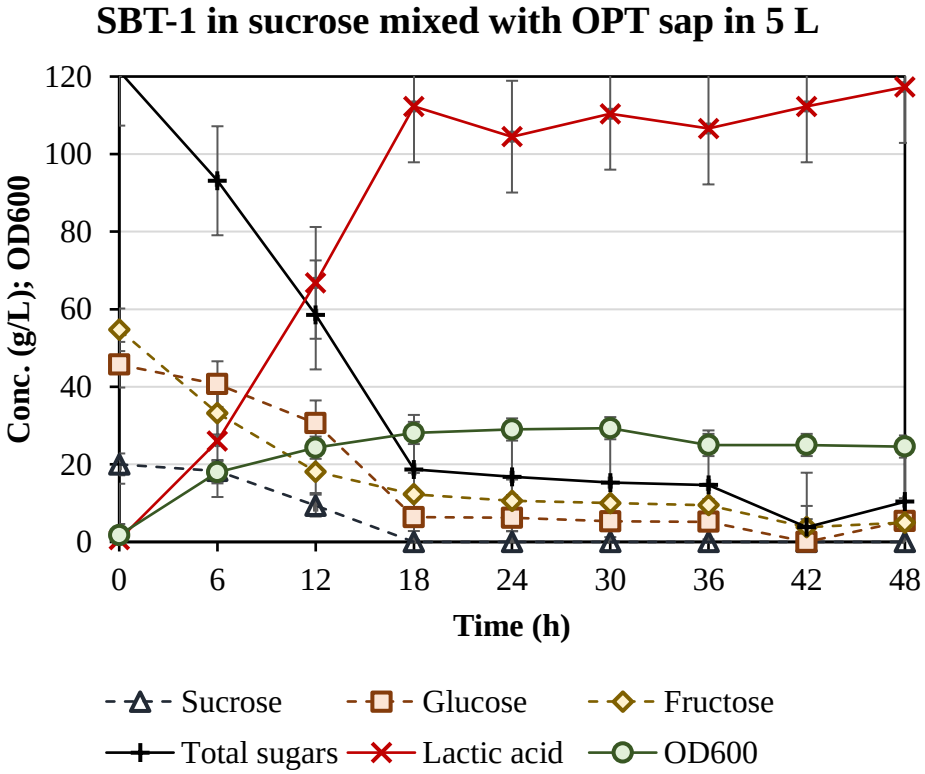


Figure 15 Fermentation of *S. terrae* SBT-1 in OPT sap with supplementation of sucrose at 120 g/L in a 5L fermenter

4.3.3 Fermentation of lactic acid by *S. terrae* SBT-1 in OPT sap with supplementation of raw sugar at 120 g/L in a 5 L fermenter

The kinetics of OPT sap supplemented with raw sugar at a concentration of 120 g/L fermented by *S. terrae* SBT-1 are shown in Figure 16. The highest lactic acid concentration was 115.85 g/L, and fermentation time was 24 h (Table 7). At the end of fermentation time, the residual sugar was 12.81 g/L. From 0 h to 24 h, lactic acid dramatically increased (Figure 16). Sucrose, glucose, and fructose consumption rates were 1.35, 1.76, and 1.66 g/L·h respectively. Lactic acid yield and productivity were 1.02 g/g and 4.78 g/L·h respectively. Cell density, found by measuring optical density at 600 nm, was 27.08 (Table 7). The optical purity (%ee) of D-lactic acid was 99.85%.

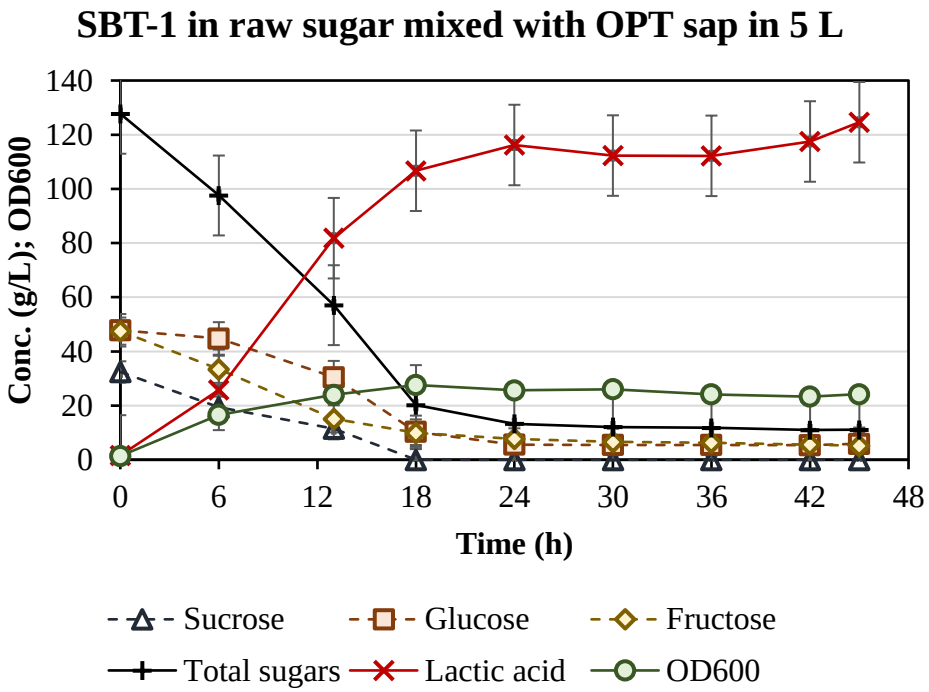


Figure 16 Fermentation of *S. terrae* SBT-1 in OPT sap with supplementation of raw sugar at 120 g/L in a 5 L fermenter

4.3.4 Fermentation of lactic acid by *S. terrae* SBT-1 in OPT sap with supplementation of molasses at 120 g/L in a 5 L fermenter

Figure 17 shows the fermentation kinetics by using OPT sap supplemented by molasses at a concentration of 120 g/L fermented by *S. terrae* SBT-1. The results show the end of fermentation at 36 h, when lactic acid concentration reached a peak at 84.50 g/L. A dramatic increase in lactic acid concentration was found to have occurred from 0 h to 24 h. At the end of the fermentation time, residual sugar was 18.55 g/L. Sucrose, glucose, and fructose consumption rates were 1.39, 1.20, and 1.40 g/L·h respectively. The lactic acid yield and productivity were 0.84 and 3.45 respectively. Cell density, found by measuring optical density at 600 nm, was 28.80 (Table 7). The optical purity (%ee) of D-lactic acid was 100.00%.

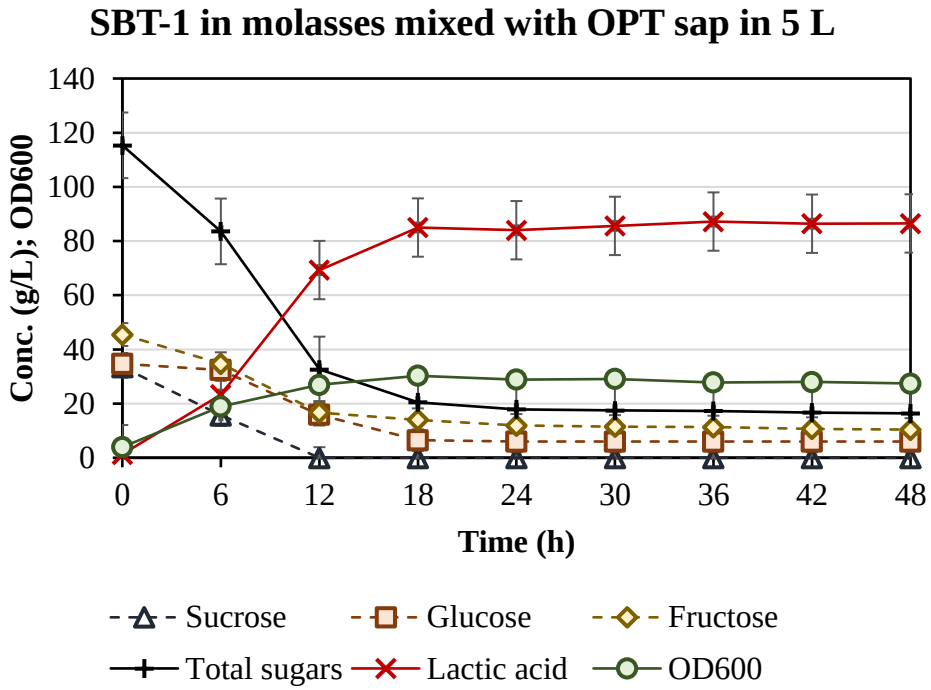


Figure 17 Fermentation of *S. terrae* SBT-1 in OPT sap with supplementation of molasses at 120 g/L in a 5L fermenter

Table 7 Fermentation kinetics of lactic acid by *S. terrae* SBT-1, using OPT model solution with the supplementation of raw sugar, sucrose, glucose, and molasses in 5L fermenter

Fermentation kinetics	**OPT sap + Glucose**	**OPT sap + Sucrose**	**OPT sap + Raw sugar**	**OPT sap + Molasses**
Highest lactic acid concentration (g/L)	117.43±0.48	112.10±0.20	115.85±0.35	84.50±0.50
Fermentation time (h)	24	18	24	24
Initial sugar concentration (g/L)	124.85±0.03	121.12±0.71	126.50±1.23	114.51±0.84
Residual total sugar concentration (g/L)	9.70±0.40	11.10±2.10	12.81±1.80	18.55±1.25
Sucrose consumption rate (g/L·h)	2.81±0.03	1.11±0.29	1.35±0.01	1.39±0.22
Glucose consumption rate (g/L·h)	2.30±0.01	2.18±0.06	1.76±0.14	1.20±0.31
Fructose consumption rate (g/L·h)	0.01±0.03	2.00±0.24	1.66±0.05	1.40±0.16
Max OD	32.07±0.47	28.10±0.65	26.05±0.45	28.37±0.54
Yp/s	0.99±0.01	1.04±0.05	1.02±0.01	0.84±0.01[a]
Yx/s	0.28±0.01	0.23±0.01	0.24±0.02	0.28±0.00
Productivity (g/L·h)	4.94±0.07	6.21±0.03[b]	4.78±0.10	3.45±0.10[b]
Optical purity %ee	99.41±0.59	100.00±0.00	99.85±0.15	100.00±0.00

Statistical significance was determined using a post hoc test followed by Tukey's honestly significant difference. The difference is indicated as significant ($p < 0.05$).

[a] $Y_{p/s}$ from the OPT sap supplemented with molasses condition was significantly different from other conditions

[b] Productivity from OPT sap supplemented with sucrose and molasses conditions were significantly different from other conditions

The fermentation kinetics in the 5 L fermenter are shown in Table 7. In all cases, residual sugar was detected at the end of the fermentation time. In other words, some unfermentable sugar content remained in all the OPT sap supplemented with 4 different sugars, applied in the 5L fermentation platform.

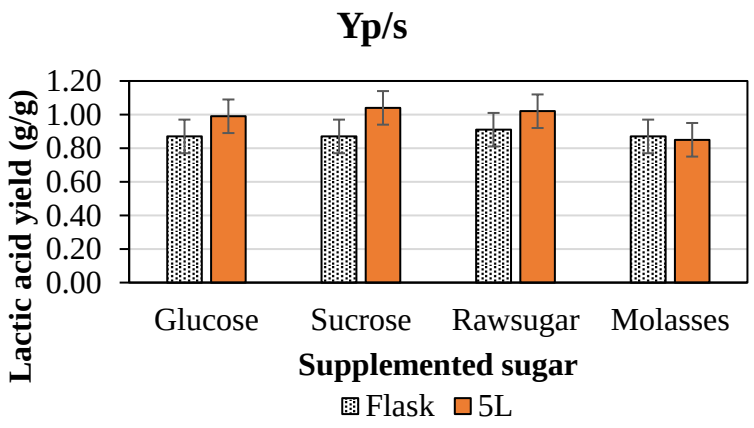
In comparing the results of 5 L batch fermentation, the use of different supplemented sugars resulted in differences in lactic acid yield and productivity. As can be seen in Table 7, in the fermentation medium of OPT sap supplemented by glucose, sucrose and raw sugar at 120 g/L, the lactic acid yields were 0.99, 1.04, and 1.02 g/g respectively. The fermentation using molasses as a supplemented sugar provided the significantly lowest product yield coefficient at 0.84 g/g. Moreover, the productivity from OPT sap supplemented by sucrose batch fermentations was 6.21 g/L·h. The productivity from this condition was significantly higher than others. However, a similar biomass yield was obtained from all experiments.

The fermentation kinetics from flask-scale and 5 L fermenter can be compared. Figure 18 (a) shows the change in lactic acid yield. Fermentation in a 5 L fermenter provided an increase in lactic acid yield in the media supplemented by glucose, sucrose, and raw sugar. However, in the medium supplemented by molasses, a slight decrease in lactic acid yield was found. The same trend was identified from the productivity comparison between flask-scale and 5 L batch fermentation. In considering the effect of the fermentation condition in the 5 L fermenter, it was found that, at 300 rpm of agitation rate, the agitation improves homogeneous mixing in the fermenter. The agitation was found to be a factor that enhances growth and fermentation rates, whether under aerobic or anaerobic conditions (Lee *et al*., 2013).

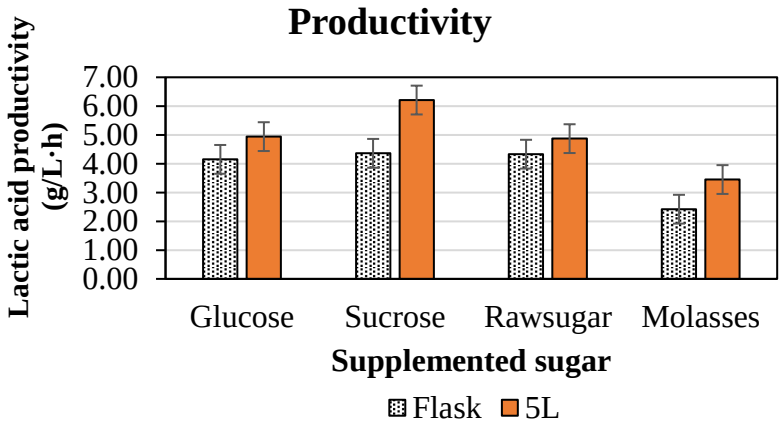
The productivity from the 5 L fermentation batch supplemented with glucose, sucrose, raw sugar, and molasses was improved (Figure 18).

Batch fermentation of OPT sap supplemented by molasses at 120 g/L provided the lowest lactic acid yield in batch fermentation in a 5L fermenter at a significant level. Molasses characteristics, which include some inhibitors, indicate that the fermentation efficiency when using molasses as the supplemented sugar is not preferred in the scale-up process.

(a)



(b)



(c)

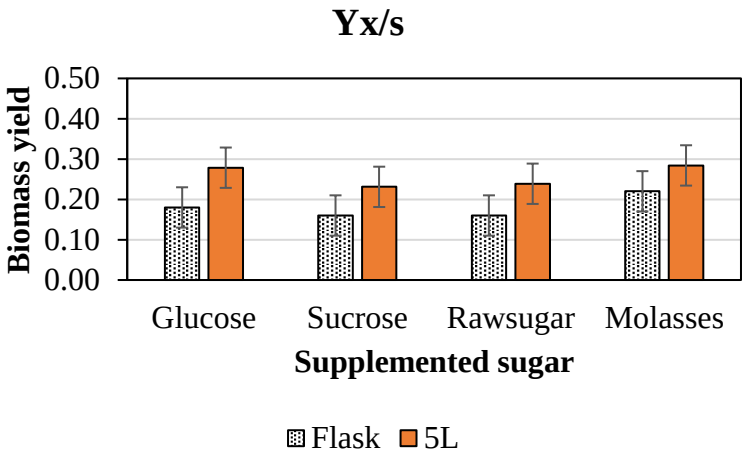


Figure 18 The changing of fermentation kinetics between flask-scale and 5L fermenter

The production cost is one important factor in selecting material to be used as a substrate in fermentation. To reduce the production cost, this study focused on OPT sap, which was applied in preculture media and fermentation media. Table 8 shows the expected cost reduction that results from applying OPT sap in a preculture medium. In using GY medium as a preculture medium, glucose was replaced by OPT sap. The results show that by applying OPT sap in a preculture medium, the production cost was reduced from 18.91 THB/L to 18.55 THB/L. In addition, the fermentation medium provides a difference in production cost due to the difference between supplemented sugar with and without applying OPT sap, as shown in Table 9. The results show a decrease in production cost in the fermentation medium by using OPT sap supplemented with raw sugar, sucrose, glucose, or molasses. The cheapest fermentation medium cost was obtained from raw sugar supplementation. In calculating the total medium cost from preculture and fermentation medium (Table 10), the highest cost-saving percentage from OPT sap supplemented by raw sugar was

obtained at 14.65% (Table 11). For the medium cost, it was required 10.71 THB for 1L fermentation ,and, for the lactic acid concentration from this study, the OPT sap supplemented with raw sugar fermentation batch, which is the most cost-effective fermentation condition, can be estimated; the sale price will be 1216.32 THB/L (Table 12).

Table 8 Preculture medium with using OPT sap and without using OPT sap to replace glucose in the medium composition

Without OPT sap				**With OPT sap**			
Compound	(g/L)	THB/g	THB/L	Compound	(g/L)	THB/g	THB/L
Glucose	10.00	0.03	0.31	OPT sap	10.00	-0.005[a]	-0.05[a]
Yeast extract	15.00	0.86	12.90	Yeast extract	15.00	0.86	12.90
NH_4Cl	4.00	1.28	5.14	NH_4Cl	4.00	1.28	5.14
KH_2PO_4	0.25	1.02	0.25	KH_2PO_4	0.25	1.02	0.25
K_2HPO_4	0.25	1.07	0.27	K_2HPO_4	0.25	1.07	0.27
$CaCO_3$	5.00	0.01	0.04	$CaCO_3$	5.00	0.01	0.04
Total			18.91	**Total**			18.55
Salt solution							
Compound		(g/L)		THB/g		THB/L	
$MgSO_4 \cdot 7H_2O$		40.00		0.75		29.96	
$MnSO_4 \cdot 5H_2O$		2.00		1.50		3.00	
$FeSO_4 \cdot 7H_2O$		2.00		1.28		2.57	
NaCl		2.00		0.32		0.64	
Total (10 mL/L)							0.36
Total price (Without OPT sap)			19.27	**Total price (With OPT sap)**			18.92

[a] The cost reduction by applying OPT sap in preculture medium

Table 9 Fermentation medium cost by directly using sugar and using OPT sap supplemented with sugar as a carbon source for lactic acid fermentation

Without OPT sap				**With OPT sap**			
Compound	(g/L)	THB/g	THB/L	Compound	(g/L)	THB/g	THB/L
Glucose	120	0.03	3.70	Glucose	64.63	0.03	1.99
$CaCO_3$	80	0.01	0.66	OPT sap	55.37	0.00	-0.25
Total			4.35	$CaCO_3$	80	0.01	0.66
				Total			2.40
Compound	(g/L)	THB/g	THB/L	Compound	(g/L)	THB/g	THB/L
Sucrose	120	0.02	1.99	Sucrose	64.63	0.02	1.07
$CaCO_3$	80	0.01	0.66	OPT sap	55.37	0.00	-0.25
Total			2.64	$CaCO_3$	80	0.01	0.66
				Total			1.48
Compound	(g/L)	THB/g	THB/L	Compound	(g/L)	THB/g	THB/L
Raw sugar	120	0.01	1.56	Raw sugar	64.63	0.01	0.84
$CaCO_3$	80	0.01	0.66	OPT sap	55.37	0.00	-0.25
Total			2.21	$CaCO_3$	80	0.01	0.66
				Total			1.25
Compound	(g/L)	THB/g	THB/L	Compound	(g/L)	THB/g	THB/L
Molasses	120	0.02	2.10	Molasses	64.63	0.02	1.13
$CaCO_3$	80	0.01	0.66	OPT sap	55.37	0.00	-0.25
Total			2.75	$CaCO_3$	80	0.01	0.66
				Total			1.54

Table 10 The total cost by apply and non-apply OPT sap in preculture medium and fermentation medium

Without OPT sap				**With OPT sap**			
Preculture price (THB/L)	Fermentation		Total (THB/L)	Preculture price (THB/L)	Fermentation		Total (THB/L)
	Conditions	Price			Conditions	Price	
19.27	Glucose	4.35	23.62	18.92	Glucose	2.40	21.31
	Sucrose	2.64	21.91		Sucrose	1.48	20.39
	Raw sugar	2.21	21.48		Raw sugar	1.25	20.16
	Molasses	2.75	22.02		Molasses	1.54	20.45

Table 11 Cost saving percentage

Conditions	**Cost saving percentage** [b]
OPT sap + Raw sugar	14.65%
OPT sap + Sucrose	13.67%
OPT sap + Glucose	9.77%
OPT sap + Molasses	13.42%

[b] Cost saving percentages were calculated based on the changes in production cost as a result of using OPT sap supplemented with glucose, sucrose, raw sugar , or molasses compared with the medium without using OPT sap and using glucose as a carbon source in a fermentation medium

Table 12 D-lactic acid sale price

OPT sap + Raw sugar	
Lactic acid conc. (g/batch)	**Sale price (THB/L)**
115.85	1216.32

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

D-lactic acid production by *Sporolactobacillus terrae* SBT-1 from the fermentation medium supplemented with the OPT sap was studied. The results in the flask culture suggested that *S. terrae* SBT-1 effectively fermented glucose and sugarcane-based substrates including sucrose, raw sugar, and sugarcane molasses supplemented with OPT sap for lactic acid. *S. terrae* SBT-1 could also ferment sugars at a high concentration of 120 g/L resulted in high final D-lactic acid yield (0.87-0.91 g/g) and productivity (2.42-4.36 g/L·h) as well as the high optical purity (96-99 %ee) at the end of the fermentation; therefore, this isolate was a promising D-lactic acid producing microbe. Compared among the carbon substrates tested in this study, raw sugar was the most preferable that yielded the highest D-lactic acid production performance.

Fermentation process optimization was also conducted in the 5 L stirred fermentor. Instead of using the model OPT sap like those in the flask culture, the actual OPT sap collected from the biomass fuel facility was collected for a supplemented carbon substrates to commercial sugars. The analytical results revealed that the OPT sap contained not only fermentable sugars but also some vitamins, amino acids, and metals at a trace amount that did not harm bacterial growth. The results showed a good fermentation performance. The lactic acid production yield was high (0.84-.1.04 g/g), also the productivity (3.45-6.21 g/L·h). The production yield, productivity, biomass yield and optical purity obtained from the 5 L batch

fermentation was almost higher than the experiment in the flask culture. In addition, agitation played role in improving lactic acid production by providing homogeneity from sufficient mixing. To enhance lactic acid production yield, it would be interesting to investigate the genetic and metabolic which are essential for D-lactic acid fermentation. Moreover, the new fermentation techniques that support the fermenter operation system for example continuous stirred tank reactors is attractive to be applied.

From the results shown in this study, OPT sap, the residue from the biomass fuel processing facility from fell oil palm trunk, could be used as the supplemented carbon substrates together with the commercial carbon feedstocks like glucose, sucrose, raw sugar, and molasses for microbial fermentation. It was observed that supplemented the fermentation medium with OPT sap reduced the production cost. The most effective cost reduction by 14.65% was observed in the fermentation using raw sugar supplemented with OPT sap as the carbon substrates. Thus, OPT sap could be the promising second generation feedstock in the Bio-Circular-Green economy in addition to other biowastes from agriculture that offered the reduction of an environmental footprint and generated value from wastes.

5.2 Recommendations

To apply the fermentation protocol developed in this study, controlling the quality of OPT sap is mandatory. From the previous studies and the results obtained in this study, it was suggested that the sugar concentration and other ingredients that can be either bacterial growth promoter and inhibitor depended on oil palm species, the duration and storage conditions of fell oil palm before processing, the processing

step, and the storage conditions. More information on these parameters should be gathered so that the utilization of OPT sap can become more effective. To further reduce the production cost, medium optimization along with process optimization should also be conducted. This can be done by combining the OPT sap together with other second-generation carbon feedstocks, for example, cellulosic sugars or other sugar rich plant biomass.

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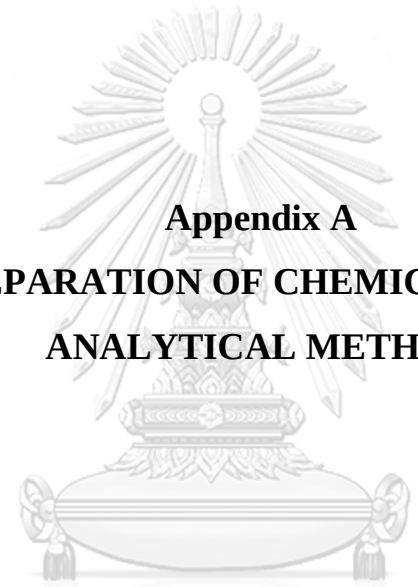
Appendix A
PREPARATION OF CHEMICALS AND ANALYTICAL METHOD



A 1 Medium composition

A 1.1 GYP agar medium

Glucose	10	g/L
Yeast extract	5	g/L
Peptone	5	g/L
KH_2PO_4	0.25	g/L
K_2HPO_4	0.25	g/L
$CaCO_3$	5	g/L
Agar	20	g/L
Salt solution	10	mL/L

A 1.2 GY medium

Glucose	20	g/L
Yeast extract	3.5	g/L
NH_4Cl	3	g/L
KH_2PO_4	0.5	g/L
K_2HPO_4	0.5	g/L
$CaCO_3$	5	g/L
Salt solution	20	mL

A 1.3 Salts solution (per 10 mL)

$MgSO_4 7H_2O$	400	mg
$MnSO_4 5H_2O$	20	mg
$FeSO_4 7H_2O$	20	mg
NaCl	20	mg

All media were adjusted at pH 6.8 and autoclaved at 121ºC for 15 min.

A 1.4 Fermentation medium in flask

OPT sap model solution

Sucrose	15	g/L
Glucose	10	g/L
Fructose	15	g/L
$CaCO_3$	80	g/L
Supplemented sugar	80	g/L

(glucose, sucrose, raw sugar, molasses)

A 1.5 Fermentation medium in 5L fermenter

OPT sap (concentration at 55.37 g/L)	1.75	L
$CaCO_3$	80	g/L
Supplemented sugar	64.63	g/L

(glucose, sucrose, raw sugar, molasses)

A2 High Performance Liquid Chromatography determining

High performance liquid chromatography (HPLC) was used to analyze the composition of fermentative broth including glucose, sucrose, fructose, lactic acid, acetic acid, and ethanol. Samples were diluted with double distilled water (DDI water) . After that diluted the supernate, 15 μL of the sample were automatically injected into an exclusion organic acid column (Biorad, Aminex HPX-87H ion exclusion organic acid column; 300mm x7. 8mm) maintained at 45ºC in a column oven (Shimadzu-CTO-6A). Sulfuric acid at the concentration of 0.005 M was used as a mobile phase at a flow rate of 0.6 mL/min. An RI detector (Shimadzu-RID-6A) was set at the range of 200 to detect the organic compounds. A standard containing 2 g/ L of each component was injected as a reference to determine the sample concentration in range of 0 to 2 g/L (preparation follows below).

Concentration (g/L)	Standard 2 g/L (μl)	DDI water (μl)
0.25	125	875
0.5	250	750
1.0	500	500
1.5	750	250
2.0	1000	-

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