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Lactic Acid Production from Old Oil Palm Trunk Sap in the Open Batch, Open Repeated Batch, Fed-Batch, and Repeated Fed-Batch Fermentation by *Lactobacillus rhamnosus* ATCC 10863

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Abstract: The cost of fermentable sugars added as a substrate is one major problem for economic lactic acid (LA) production. Old oil palm trunks (OPT) squeezed sap, the agricultural wastes on replanting and pruning of oil palm (*Elaeis guineensis*), contained mainly glucose and fructose as a potential feedstock to use as a vast carbon source for LA production. To improve the LA yield and productivity, various fermentation modes were performed by *Lactobacillus rhamnosus* ATCC 10863 using OPT sap as a basal medium. A modified constant feed mode of fed-batch and repeated fed-batch fermentation using undiluted OPT sap feed medium can achieve a high average LA concentration of 95.94 g/L, yield of 1.04 g/g, and productivity of 6.40 g/L/h) at 11 h cultivation time. It can also provide open and open repeated batch fermentation with an average LA concentration of 91.30 g/L, yield of 0.87 g/g, and productivity of 3.88 g/L/h at 21 h fermentation time.

Keywords: lactic acid; *Lactobacillus rhamnosus*; fermentation; oil palm trunk; OPT sap; open batch fermentation; open repeated fermentation; fed-batch fermentation; repeated fed-batch fermentation

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1. Introduction

Lactic acid (LA) is widely used in food and related industries as a preservative, natural additive, solvent, curing agent, flavoring agent, and buffering agent, with a global market size of USD 2.9 billion in 2021 expected to expand at a compound annual growth rate of 8.0% from 2022 to 2030 [1].

The use of LA as a starter for the production of polylactic (PLA), a biodegradable and biocompatible biopolymer, drives up demand in the LA market [2]. Approximately 90% of LA is produced industrially worldwide through microbial fermentation as opposed to chemical synthesis [3]. When compared to the chemical method, fermentation has a number of benefits, including the ability to produce optically pure LA from agricultural wastes, low energy consumption, and low environmental impact [4].

Carbon sources are the key factor affecting LA production costs. Most often, a plentiful, affordable raw material, such as agricultural residues and industrial by-products like molasses, acid whey, green alfalfa juice, food wastes, etc., was employed as a substrate for LA fermentation [5–10]. Other fermentation feedstock, such as starchy and cellulosic materials, i.e., tapioca starch, sweet sorghum juice, potato, wheat, rye-barley, malt, rice, xylan-derived sugars, galactan, and lignin were studied for cost-effective LA production processes [3,8,11–13].

Oil palm trunks (OPT) are the agricultural wastes of oil palm (*Elaeis guineensis*) generated from replanting and pruning in the plantation sites. OPT contains fibrous materials which can be used in many industries, such as plywood, laminated veneer lumber, wood-cement composites, gypsum board, pulp and paper making, animal feeds, and other value-added products [14,15]. The squeezed sap from old OPT contained mainly glucose, fructose, sucrose, and small amounts of galactose, xylose, and rhamnose, whose

total amounts varied between 16.97–140 g/L [16–18]. Additionally, oil palm sap contains minerals, vitamins, organic acids, and amino acids [15,19].

Numerous microorganisms have been studied for LA production, such as *Lactobacillus* sp. [6], *Lactobacillus casei* CCDM 198 [20], *Lactobacillus plantarum* [3], *L. rhamnosus* ATCC 10863 [9]. *L. rhamnosus* LA-04-1 [21], *Bacillus coagulans* A534 [8], *Enterococcus mundtii* QU 25 [22], and *Lactococcus lactis* subsp. *lactis* NBRC 12007 [23].

The most frequent methods for LA production are batch, fed-batch, repeated batch, and continuous fermentation. Regarding the nature and variety of substrate, microbial activity, and viscosity of the fermentation broth, many fermentation procedures can be used [5]. Batch mode produces high yields by fully utilizing the substrate when the cost of raw materials is low [9]. However, the substrate and final product inhibition of the traditional batch process resulted in decreased productivity [24]. Different feeding strategies were created to prevent these issues. By inoculating a portion or all of the cells from the previous batch into the next, repeated batch operations entailed several cycles of fermentation. In *Rhizopus oryzae*, repeated batch culture combines the advantages of batch and continuous cultures, such as the feasibility of high product content due to the complete bioconversion of substrate. The one and two-reactor system was also conducted [4]. Additionally, fed-batch fermentation may offer a variety of operating modes depending on the substrate, end products, and microorganisms. The external substrate concentration can be changed with the right feed rate [19]. To feed a suitable medium, repeated fed-batch or cyclic fed-batch can be overcome by process design, and numerous cycles offer without limiting substrate or product. LA production could be carried out via non-sterile fermentation because it minimized the need for equipment, reduced energy usage, simplified the fermentation process, and saved labor [4,25]. Zhao et al. [4] developed open repeated fermentation to synthesize optically pure LA.

To increase LA yield and productivity using OPT sap as a base substrate, the feasibility of open batch, repeat open batch, fed-batch, and repeated fed-batch fermentation was examined in this study. To lessen the product inhibition, the fed-batch and repeated the fed-batch fermentation's constant feed mode modification to dilute product concentration can be used.

2. Materials and Methods

2.1. Chemicals and Culture Media

Yeast extract, peptone, De Man-Rogosa-Sharpe (MRS) agar, and MRS broth were purchased from Himedia^{TM} (Mumbai, India). A standard 33-element TraceCERT was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other reagents and standards were of analytical grade.

2.2. Oil Palm Trunk (OPT) Sap Preparation

Old oil palm trees (approximately 20 years old) left for replanting, located at Nakhon Si Thammarat, Thailand, were felled down, cut into small logs, and sliced into small pieces around 10 × 1.5–2.0 × 150–200 cm^3. The palm disks were immediately squeezed, passed through a sugar cane pressing machine to get oil palm trunk sap (OPT sap). The OPT sap was centrifuged at 4000 rpm for 40 min at 4 °C and collected at −18 °C for the experiment (Figure 1). The sugar and mineral compositions of OPT sap were determined using the standard methods described by Saelee [26].

2.3. Microorganism and Seed Preparation

A homofermentative LA bacterium, *Lactobacillus rhamnosus* ATCC 10863, was used in this investigation. The microorganism was maintained in MRS broth containing 30% glycerol at −20 °C. For seed preparation, double propagation was performed on MRS broth. First, the 250 µL of *L. rhamnosus* ATCC 10863 stock culture was transferred to 5 mL MRS broth, and static culture was performed by incubation at 40 °C, 48 h. Then, one percentage (*v/v*) was transferred to the MRS seed medium composed of 20 g/L glucose,

10 g/L bacto-tryptone, 10 g/L yeast extract, 2 g/L K_2HPO_4, 5 g/L $CH_3COONa{\cdot}3H_2O$, 0.2 g/L $MgSO_4{\cdot}7H_2O$, and 5 g/L $MnSO_4{\cdot}4H_2O$ [15]. The pH was brought to 7.0 using 6 N NaOH or conc. HCl. The medium sterilization was autoclaved at 121 °C for 15 min. Before being utilized as a seed inoculum, fermentation was carried out in a shake-flask at 150 rpm, 40 °C for 18 h.

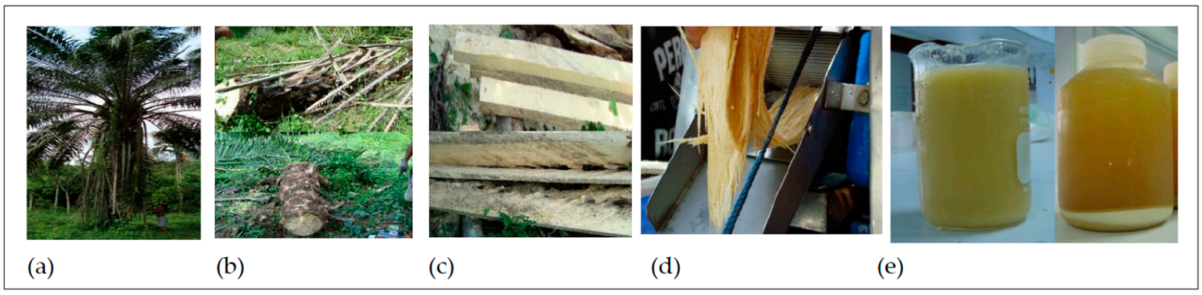

Figure 1. Oil palm tree (**a**), shedding and logging (**b**), slicing to disks (**c**), squeezing (**d**), and oil palm squeezed sap (OPT sap) (**e**).

2.4. Open Batch and Open Repeated Batch Fermentation

Bath fermentation was conducted in a 2 L stirred tank fermentor (Bioflo, New Brunswick Scientific Co., Picataway, NJ, USA) containing a 1.2 L OPT sap base medium. The addition of 10 g/L yeast extract, 5 g/L peptone and trace salts (1.5 g/L $CH_3COONa{\cdot}3H_2O$, 1.5 g/L KH_2PO_4, 1.5 g/L K_2HPO_4, 0.2 g/L $MgSO_4{\cdot}7H_2O$, and 0.05 g/L $MnSO_4{\cdot}4H_2O$) were performed [27]. Non-sterile OPT sap and trace salts were used as a basal medium. Fermentation was operated at 40 °C, with an agitation speed of 200 rpm without aeration. The pH was controlled at 6.5 by using 6N $Ca(OH)_2$. Nitrogen gas was flushed to the OPT sap-based medium before inoculation and after sampling to maintain an anaerobic condition. Prior to the 10% *v/v* inoculation, the $OD_{620\,nm}$ of the pre-seed was adjusted to a constant value of 4. Samples were aseptically withdrawn every 3 h intervals from the fermenter to measure the viable cells and biomass growth at $OD_{620\,nm}$. The culture broth was heated (70 °C, 30 min), centrifuged (10,000× *g*, 10 min) at room temperature (26–29 °C), and filtered through a 0.45 µm filter membrane. The sugars and LA concentration were examined in the clear supernatant, as detailed below.

When the maximum $OD_{620\,nm}$ was reached close to the stationary phase, open repeated batch fermentation was carried out by removing the fermentation broth and replacing it with a nonsterile OPT sap medium. Open repeated batch fermentation was performed in the following cycles using the same non-sterile open conditions. The fermentation broth was taken off until there was only 0.2 L left. The following cycle began with the operation of three cycles of fermentation after filling 1.2 L of fresh OPT sap with a nutrient supplement without sterilization.

2.5. Fed Batch and Repeated Fed Batch Fermentation

For fed-batch fermentation, undiluted OPT sap was used as the main substrate. A 10% seed formulation was inoculated into a 2 L fermenter (BioFlo, New Brunswick Scientific Co., USA). The medium was sterilized at 110 °C for 10 min. Without aeration, fermentation was conducted with temperature, agitation, and pH automatically controlled at 40 °C, 200 rpm, and 6.5 (6 N $Ca(OH)_2$), respectively. The medium was flushed with nitrogen gas before inoculation and intermittently applied after sample collection. Fed-batch fermentation started with batch fermentation mode in a 2 L fermenter containing 0.4 L OPT sap-based medium. Ten percent of pre-seed was aseptically added, and fermentation was performed using a modified constant rate fed-batch fermentation. The feeding was initiated at 12 h of cultures and fed-batch performed by a pulse feed of 0.2 L feed medium at the beginning of the fed-batch mode followed by a constant feed of 0.6 L of an OPT sap fermentation

medium at a volumetric feed rate, resulting in the final volume of 1.2 L. The feed was stopped after 9 h.

For repeated fed-batch fermentation, after the 1st batch, 800 mL of fermentation broth was drawn off until the 0.4 L volume remained. The next cycle was started by adding feed medium until the total volume was 1.2 L. Then, 3 cycles of fed-batch fermentation were performed. The feeding medium contained the same composition as the fermentation medium. Five mL samples were taken every 3 h for analysis.

2.6. Kinetic Parameters Calculation

LA concentration (g/L) was calculated from the volumetric fermentation broth and the total LA produced at the end of fermentation. The total sugars (g/L) or residual sugars (g/L) were expressed by the sum of glucose, fructose, and sucrose in the volumetric fermentation broth at test time. The total sugar consumption (g/L) was calculated as the initial sugars (g/L) minus the residual sugars (g/L). In the batch mode, the LA yield, Y_{LA}, was calculated based on the total LA produced per gram of total sugar consumed (g/g) as Equation (1), with P^F_{LA} representing the LA concentration at the end of fermentation, P^I_{LA} denoting the LA concentration at the beginning of fermentation, S^I_{TOTAL} denoting the total sugars concentration detected at the beginning of fermentation (g/L), and S^F_{TOTAL} denoting the total sugar concentration detected at the end of fermentation (g/L). The LA productivity (Q_{LA}, g/L/h) was calculated using the ratio of LA concentration (g/L) to the entire fermentation time (h) as Equation (2), with t denoting the fermentation time (h).

$$Y_{LA}(g/g) = \frac{\text{Lacic acid produced (g)}}{\text{Total sugars consumed (g)}} = \frac{\Delta P_{LA}}{\Delta S_{TOTAL}} = \frac{P^F_{LA} - P^I_{LA}}{S^I_{TOTAL} - S^F_{TOTAL}} \quad (1)$$

$$Q_{LA}\ (g/L/h) = \frac{\text{Lactic acid concentration (g/L)}}{\text{Fermentation time(h)}} = \frac{\Delta P_{LA}}{t} = \frac{P^F_{LA} - P^I_{LA}}{t} \quad (2)$$

In repeated modes, LA yield (Y_{LA}) (g/g) was calculated as Equation (3). LA productivity (Q_{LA}) (g/L/h) was calculated as Equation (4), where V_F, V_{FE}, and V_I were the total final volume, total feed volume, and total initial volume (L) of each cycle, respectively. P_F and P_I represented the final and initial concentrations of total LA in the fermentation broth (g/L) of each cycle, respectively. S_F, $S_{FE,}$ and S_I represented the final, feed, and initial concentrations of total sugars in the fermentation broth (g/L), respectively, and t represented the fermentation time (h).

$$Y_{LA}\ (g/g) = \frac{V_F P_F - V_I P_I}{V_I S_I + V_{FE} S_{FE} - V_F S_F)} \quad (3)$$

$$Q_{LAP}\ (g/L/h) = \frac{V_F P_F - V_I P_I}{V_F \text{ x } t} \quad (4)$$

2.7. Analytical Methods

Sugar composition in fresh OPT sap was analyzed by a High Performance Liquid Chromatography (HPLC) system (Waters, Milford, MA, USA), 300 × 7.8 mm Animex HPX-87P column (Biorad, Hercules, CA, USA). Deionized water (DI) as a mobile phase was isocratically performed at 0.6 mL/min, and the column oven was set at 80 °C. The Refractive index (RI) detector was set at 50 °C. Before a 10 µL injection, samples were diluted with DI to the proper dilution and filtered using a 0.45 µm filter. The external standards of 1%, D(-)-fructose, D-(+)-glucose anhydrous, D(+) galactose, sucrose, D-(+)- maltose monohydrate, L-rhamnose monohydrate, and arabinose were used for quantification. Unknown concentration was calculated by a linear relationship plot between the peak area and known concentrations of standards [26].

LA and sugars concentrations of fermentation broth were determined using HPLC (Waters, USA), with Animex HPX 87H column (300 × 7.8 mm) (Biorad, USA) with RI

detector (Waters 2410, Waters, USA). The eluent 0.005 M H_2SO_4 (0.6 mL/min), detector, and column were set at 50 °C. Before a 10 µL injection, samples were diluted with DI to the proper dilution and filtered using a 0.45 µm filter. Different concentrations of 1% L (+) lactic acid, ethanol, D-(+)-glucose anhydrous, D(-)-fructose, and sucrose were used as a calibration standard. To determine the correlation coefficient (r^2), slope (a), and y-intercept values, a linear regression of standards was plotted.

Using an inductively coupled plasma optical emission spectrometer (ICP-OES, Varian 730-ES, Belrose, NSW, Australia), mineral contents were examined. Nitric acid (2%) was used to dilute 10 mL of OPT sap, which was then left overnight. Each sample underwent centrifugation, filtration, and dilution in DI to the proper concentration. TraceCERT, 33 elements (Sigma-Aldrich, St. Louis, MO, USA) of standards, aluminum (Al), boron (B), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), nickel (Ni), phosphorous (P), potassium (K), sodium (Na), sulfur (S), and zinc (Zn) were used.

The absorbance at 620 nm ($OD_{620\ nm}$) (spectrophotometer UV-1601, Shimadzu Co., Tokyo, Japan) was measured to estimate bacterial growth. Viable cells (cfu/mL) were examined using the spread plate technique on MRS agar with anaerobic incubation at 40 °C in an aerobic jar.

3. Results and Discussion

3.1. Some Chemical Compositions of OPT Sap

Some chemical compositions of OPT sap are shown in Table 1. In this investigation, the predominant sugars were glucose and fructose, which were 55.69 ± 1.42 and 40.10 ± 2.35 g/L, respectively. Additionally, trace amounts of cellobiose (2.037 ± 0.09 g/L) and sucrose (3.89 ± 0.44 g/L) were discovered in OPT sap. The high total free sugar content value of 101.71 ± 3.31 g/L was higher than that reported by other studies [15,28,29]. However, Noparat et al. [29] observed that the top of OPT had 18.06 ± 1.6 g/L of total sugars. Juice from oil palm fronds (OPF) was found to have a high average amount of sugars of 76.09 ± 2.85 g/L [30]. Similar main sugar component values were found, with glucose having the largest content [28,29]. Yamada et al. [31] also reported on the composition of the glucose, fructose, and sucrose found in OPT juice. In contrast to this study, sucrose initially dominates the sugars in OPT sap but rapidly declines at the beginning of storage. Because sucrose was decomposed into glucose and fructose, the amount of these two sugars increased while sucrose was reduced in the OPT sap. The composition of OPT juice varies with the variety, soil, climate, locality, processed method, and storage time [18]. OPT sap also contained some minerals, such as P (0.257 ± 0.009 g/L), Na (0.257 ± 0.080 g/L), K (1.676 ± 0.417 g/L), Mg (0.503 ± 0.145 g/L), Ca (0.488 ± 0.150 g/L), and S (0.694 ± 0.218 g/L) (Table 1).

Table 1. Chemical characteristics of OPT sap.

Compositions	Concentrations (g/L)
Sugars	
Glucose	55.69 ± 1.42
Fructose	40.10 ± 2.35
Sucrose	3.89 ± 0.44
Cellobiose	2.037 ± 0.09
Minerals	
P	0.257 ± 0.009
Na	0.257 ± 0.080
K	1.676 ± 0.417
Mg	0.503 ± 0.145
Ca	0.488 ± 0.150
S	0.694 ± 0.218

Table 1. *Cont.*

Compositions	Concentrations (g/L)
Al	0.003 ± 0.002
Mn	0.009 ± 0.007
Fe	0.022 ± 0.011
Ni	0.0036 ± 0.001
Cu	0.0014 ± 0.001
Zn	0.0045 ± 0.004

Values are given as mean ± standard deviation (SD) from triplicate determinations.

3.2. Open Batch Fermentation and Open Repeated Batch Fermentation

To examine the economics of LA production, open batch LA fermentation of OPT sap by *L. rhamnosus* ATCC 10863 was studied (Figure 2). The maximum LA concentration was 93.99 ± 2.91 g/L at 15 h of cultivation, which resulted in a yield of 0.95 g/g and an LA productivity of 6.27 g/L/h. This was greater than Ouyang et al.'s study [32], which produced LA with an average productivity of 1.04 g/L/h and a yield of 74.5% using open fermentation of lignocellulosic hydrolyzates by *Bacillus* sp. strain NL01. Since *L. rhamnosus* ATCC 10863 requires a temperature of 40 °C for optimal growth, this may not be appropriate for other bacteria. Additionally, a non-sterilized or open fermentation technique could delay the Millard reaction and the development of furfural compounds, which generally proceed during sterilization [4]. According to Abdel-Rahman et al. [33], *E. mundtii* QU 25 produced a maximum LA concentration of 132 g/L at a yield of 0.853 g/g and productivity of 6.99 g/L/h. under non-sterile conditions. Open batch fermentation of OPT sap by *L. rhamnosus* ATCC 10863 rapidly devoured sugar during 15 h of incubation, the remaining sugars less than 4.72 ± 0.73 g/L, and completely utilized sugar at 21 h of cultivation. LA concentration, yield, and production efficiency were decreased to 91.30 g/L, 0.88 g/g, and 4.35 g/L/h at 21 h cultivation. A high cell concentration of 9.15×10^{11} cfu/mL was attained using the open batch mode, which was in accordance with a high cell growth rate as measured by the OD_{620nm} at 32.60 ± 2.79. This suggested that *L. rhamnosus* had no trouble consuming the fermentable sugars present in OPT sap, such as glucose and fructose. Glucose (57.43 g/L initially) and fructose (44.70 g/L initially) were consumed concurrently, but fructose was more rapidly and completely utilized at 9 h (Figure 2). According to Senedese et al. [9], *L. rhamnosus* ATCC 10863 most frequently used glucose as a substrate, with a maximum LA of 16.5 g/L at 14 h batch fermentation. Additionally, adopting thermophile fermentation decreases the cost of industrial LA manufacturing [34].

LA generation was impacted by inoculums and LA buildup. Batch fermentation was carried out repeatedly to increase LA productivity over batch culture. Because certain cells from the final culture broth are frequently reused, repeated batch culture offers several advantages over batch culture. These benefits include reducing the time needed to clean and sterilize the fermenter, skipping the time needed to prepare the seeds, and reducing the time needed for the primary culture by increasing the initial inoculation volume [4].

Figure 3 depicts the results of four batches of open repeated batch fermentation of OPT sap by *L. rhamnosus* ATCC 10863. Compared to the batch cycle, the first cycle's fermentation period was cut by 18 h. According to the aforementioned non-sterile batch fermentation, each fermentation cycle took place over 21 h. The highest LA concentrations were 94.15, 99.08, 84.80, and 85.60 g/L for the first batch through the third repeated cycle, respectively (Table 2). This is due to the amount of LA at the beginning of fermentation, which was 103.19–112.81 g/L. The maximum yield and productivity for the LA were achieved by the first open batch, which had values of 0.91 g/g and 4.31 g/L/h, respectively. The LA productivity and yield were 4.65, 3.26, and 3.28 g/L/h, and 0.82, 0.77, and 0.95 g/g, respectively, in the first, second, and third repeated cycles. Unfermented sugars remained after fermentation until the medium was refilled with fresh medium. This might lead to an accumulation at the start of fermentation for each cycle, equating to a decreased yield and productivity. In this investigation, the four batches, respective average LA, yield, and

productivity values were 91.30 g/L, 0.87 ± 0.08 g/g, and 3.88 ± 0.71 g/L/h. Compared to the experiment by Abdel-Rahman et al. [33], which was conducted for 11 runs with the same yield (0.761–0.832 g/g glucose consumed). Zhao et al. [4] suggested that high cell density did not always result in an improvement in LA production since the number of viable cells declined while the number of non-viable cells increased in the latter cycles of open repeated batch fermentation.

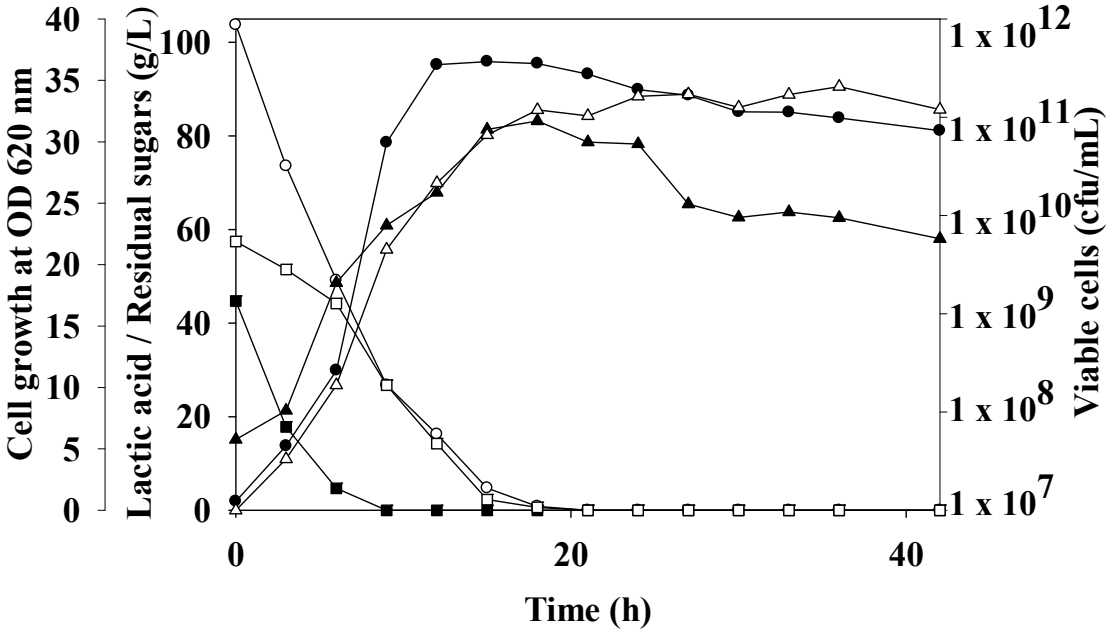

Figure 2. Open batch fermentation of oil palm trunk sap by *L. rhamnosus* ATCC 10863. Culture was performed in a 2 L fermenter containing 1.2 L working volume at a temperature of 40 °C, agitation speed of 200 rpm, and pH 6.5. Lactic acid (●), residual sugars (○), glucose (□), fructose (■), viable cells (▲), and cell growth at OD_{620nm} (Δ) was indicated.

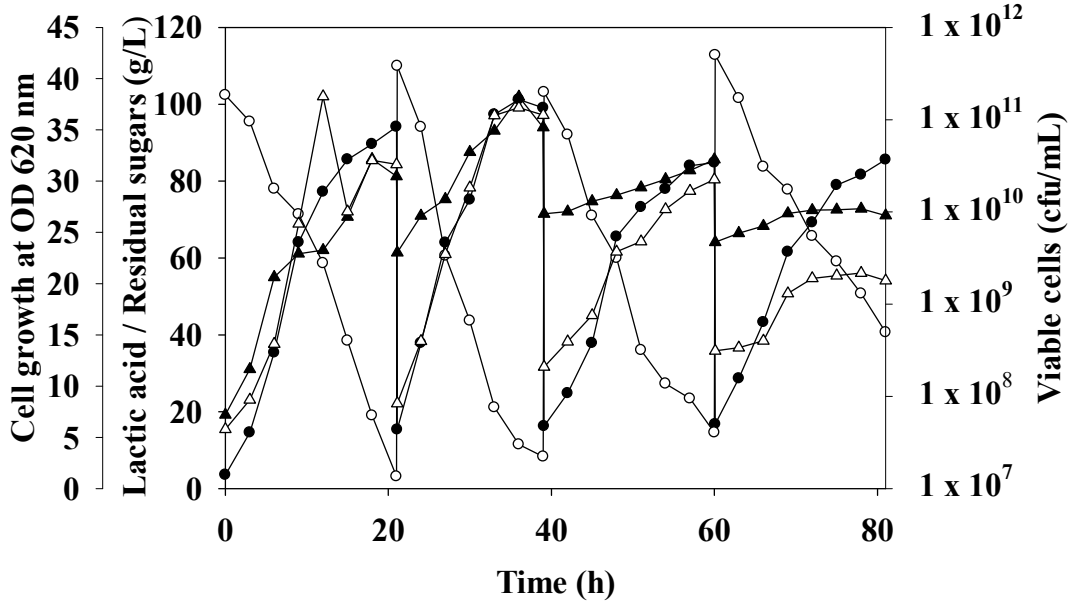

Figure 3. Open repeated batch fermentation of oil palm trunk sap by *L. rhamnosus* ATCC 10863. Culture was performed in a 2 L fermenter containing 1.2 L working volume at a temperature of 40 °C, agitation speed of 200 rpm, pH 6.5. Lactic acid (●), residual sugars (○), viable cells (▲), and cell growth at OD_{620nm} (Δ) was indicated.

Table 2. Kinetics of open repeated batch fermentation of oil palm trunk sap by *L. rhamnosus* ATCC 10863.

Kinetic Parameters	First Cycle Open Batch Fermentation	First Cycle Open Repeated Batch Fermentation	Second Cycle Open Repeated Batch Fermentation	Third Cycle Open Repeated Batch Fermentation	Average
Lactic acid (g/L)	94.15	99.08	84.80	85.60	91.30
Q_{LA} (g/L/h)	4.31	4.65	3.26	3.28	3.88
Y_{LA} (g/g)	0.91	0.82	0.77	0.95	0.87
Initial broth volume (L)	1.2	0.5	0.5	0.5	0.5
Final broth volume (L)	1.2	1.2	1.2	1.2	1.2
Fermentation time (h)	21	18	21	21	21
Initial viable cells (cfu/mL)	6.32×10^7	3.63×10^9	9.53×10^9	4.7×10^9	2.94×10^8

Q_{LA} = lactic acid productivity (g/L/h), Y_{LA} = Lactic acid yield (g/g).

3.3. Fed Batch and Repeated Fed Batch Fermentation of OPT Sap

Fed-batch fermentation patterns of LA production, sugars consumption, and biomass growth of *L. rhamnosus* ATCC 10863 using concentrated OPT sap with nutrient supplementation are shown in Figure 4. It was started with 0.4 L OPT sap-based medium in batch fermentation and changed to fed-batch after the 12 h cultivation. Then, a modified constant feed mode was performed. Herein, 0.2 L of feed medium was pulse-fed at the start of the batch feeding process, and 0.6 L of OPT sap fermentation medium was delivered continuously at a rate of 67.7 mL/h to create a 1.2 L working volume. The feed was stopped after 9 h. For 9 h of fermentation, this feeding strategy may sustain total sugar levels between 6.49 and 8.57 g/L with high cell concentrations between 4.29×10^9 and 1.69×10^{10} cfu/mL. When the fed-batch mode was finished, product efficiency increased to 7.19 g/L/h, which was greater than batch mode (6.05 g/L/h). Compared to batch fermentation, the yield in the fed-batch mode was marginally lower. However, *L. rhamnosus* B103 with pH-stat fed-batch fermentation produced 143.7 g/L of LA from dairy waste, with a productivity of 1.17 g/L/h [24]. Repeated fed-batch fermentation is currently a good tactic for improving LA fermentation as it lowers operating costs, because no seed purchase improved LA fermentation, which inhibited the higher LA production rate in fed-batch mode [6,34].

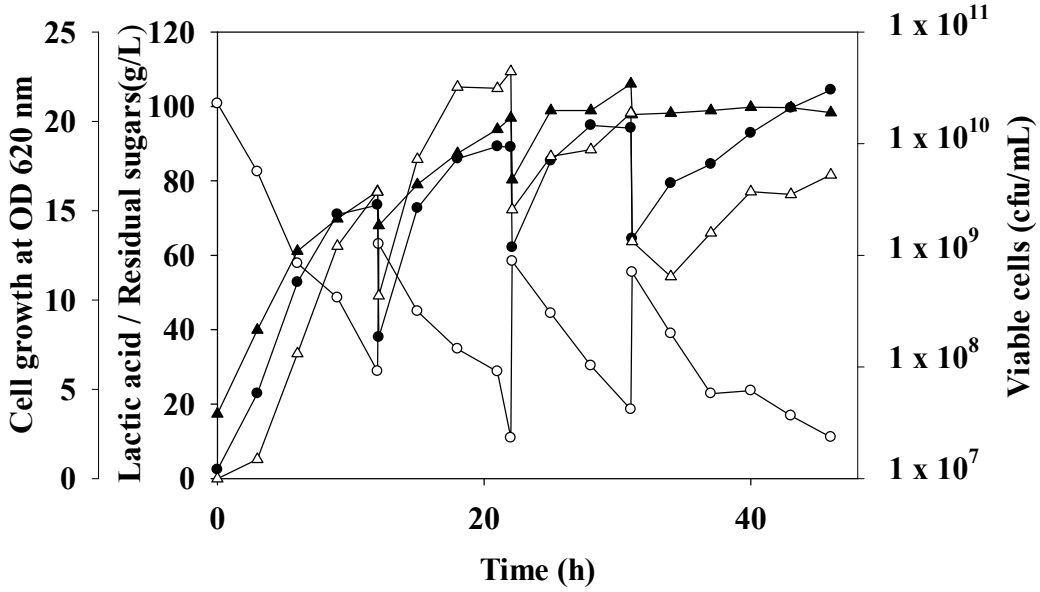

Figure 4. Fed-batch and repeated fed-batch fermentation for lactic acid production by *L. rhamnosus* ATCC 10863 using undiluted oil palm trunk sap with nutrient supplementation. Cells cultivated in 2 L fermenter containing 1.2 L working volume with 0.8 L OPT sap feed medium. A 0.2 L of feed medium was added at the beginning of feeding; the other was constant fed at the rate of 67.7 mL/h. Fermentation was performed at a temperature of 40 °C, agitation speed of 200 rpm, pH 6.5. Lactic acid (●), residual sugars (○), viable cells (▲), and cell growth at OD 620 nm (Δ) was indicated.

In the following cycle of fed-batch fermentation, a modified repeated fed-batch fermentation was carried out for 9 h. For the first batch, first fed-batch, second fed-batch, and third fed-batch cycles, respectively, the maximal LA production values were 73.49, 89.25, 94.16, and 104.42 g/L. In the first batch, the first fed-batch, the second fed-batch, and the third fed-batch cycles, respectively, there were 28.80, 28.76, 18.56, and 0 g/L of total sugars left at the end of the cycle. The highest cell density was 1.34×10^{10}, $3.44 \times 10^{10,}$ and 2.11×10^{10} cfu/mL for the first fed-batch, second fed-batch, and third fed-batch cycle, respectively. The sugar levels were fairly low (10.90 to 18.56 g/L) at the end of each fed-batch fermentation medium. During the first 9 h of cultivation, the sugar concentration was rapidly reduced, and after 15 h of cultivation, the last fed-batch cycle, it was totally consumed.

Kinetic parameters of three cycles of repeated fed-batch fermentation for LA production of OPT sap by *L. rhamnosus* ATCC 10863 were investigated (Table 3). The maximum volumetric LA productivities for the first fed-batch, second fed-batch, and third fed-batch cycles were 7.19, 7.16, and 4.86 g/L/h, respectively. These first and second fed batches had a greater fermentation rate than the initial batch (6.05 g/L/h). In the first cycle, the highest LA productivity was attained. This study showed that adding feed medium at the beginning of fermentation helps to prevent dilution of the culture broth, which could alter the growth rate and by-product accumulation in the culture broth [35]. According to Boontawan et al. [36], the fed-batch fermentation had a slightly lower glucose consumption rate than the previous fermentation. In addition, inadequate glucose utilization dramatically decreased fermentation performance. LA is the cause of this inhibition. Both bacterial development and LA production would greatly benefit from removing lactate ions from the fermentation broth. Nolasco-Hipolito et al. [37] emphasized that increasing the feed amount of glucose free-solution eliminated the end-product inhibition by LA. This inherent feature makes it easier to maintain LA concentrations between 35 and 40 g/L, which batch fermentation does not impede. As a result, it is feasible to boost the formation of LA by increasing the glucose concentration in the feed medium. In this investigation, the repeated fed-batch procedures combined nutrient supplementation with low concentrations of undiluted OPT sap to lengthen the period of productive batch fermentation and prevent substrate and catabolite suppression. This also suggested that initial sugar and LA concentrations should be taken into account to increase the productivity and yield of LA.

Table 3. Kinetics of fed-batch and repeated fed-batch fermentation of oil palm trunk sap by *L. rhamnosus* ATCC 10863.

Kinetic Parameters	Batch Culture	First Cycle Fed-Batch Fermentation	Second Cycle Fed-Batch Fermentation	Third Cycle Fed-Batch Fermentation	Average of Fed-Batch Fermentation
Lactic acid (g/L)	73.49	89.25	94.16	104.42	95.94
Q_{LA} (g/L/h)	6.05	7.19	7.16	4.86	6.40
Y_{LA} (g/g)	0.99	0.94	1.21	0.98	1.04
Q_S (g/L/h)	6.11	7.57	5.91	4.95	6.14
Initial broth volume (L)	1.2	0.4	0.4	0.4	0.4
Final broth volume (L)	1.2	1.2	1.2	1.2	1.2
Fermentation time (h)	12	9	9	15	11
Initial viable cells (cfu/mL)	3.80×10^7	8.17×10^9	3.44×10^{10}	2.12×10^{10}	2.12×10^{10}

Q_{LA} = lactic acid productivity (g/L/h), Y_{LA} = Lactic acid yield (g/g), Q_S = substrate utilization rate (g/g).

4. Conclusions

To increase the LA yield and productivity of undiluted OPT sap by *L. rhamnosus* ATCC 10863, various fermentation procedures were investigated. With 11 h of fermentation time for the production of LA as opposed to 21 h for repeated open batch fermentation, a modified constant feed mode of fed-batch and repeated fed-batch fermentation might increase yield, productivity, and short time. Additionally, when employing OPT sap as a cheap, abundant carbon source, all these techniques, including open batch and open repeated batch fermentation, could aid in extending the LA fermentation. However, utilizing cheap nitrogen sources as supplements would be worth considering.

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