

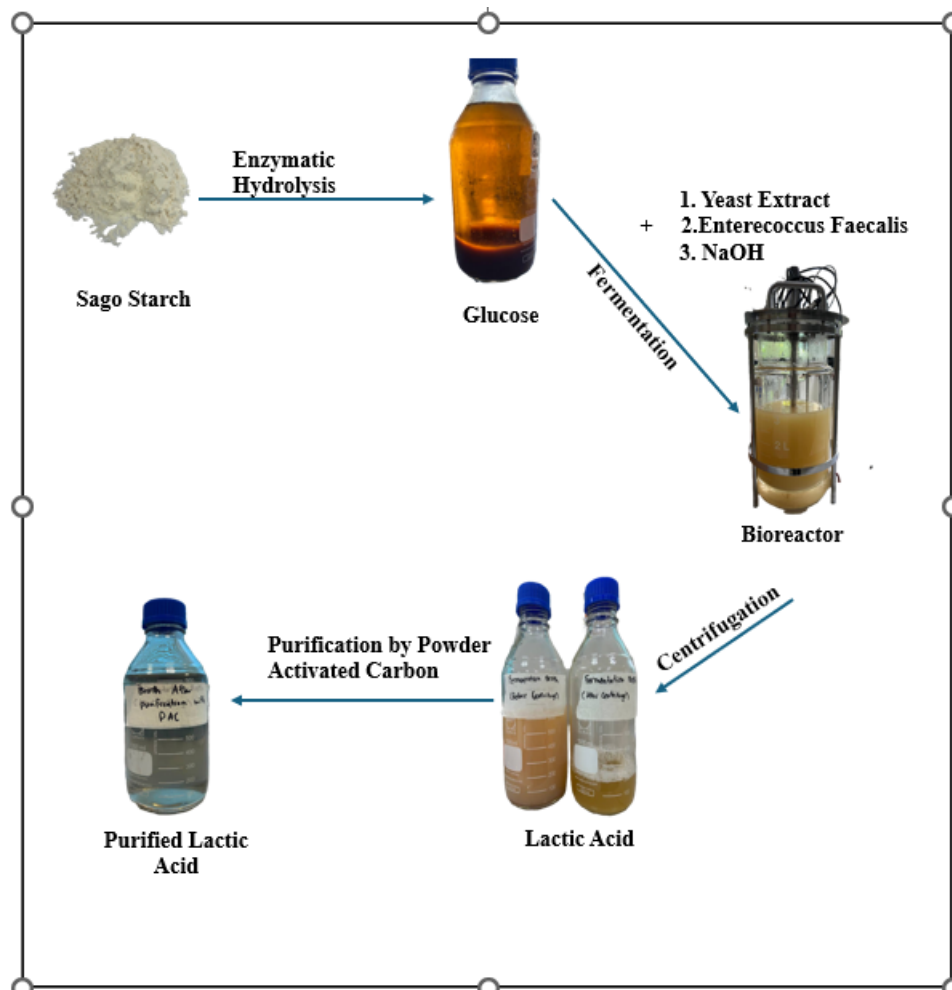
Production of Lactic Acid from Sago Starch Using *Enterococcus faecalis* Strain Isolated from Silage

Mohd Hafizzul Hazzmi Hossen,^a Sariah Abang,^{a,*} Md. Rezaur Rahman ^{a,*} Cirilo Nolasco Hipolito,^b Murtala Namakka,^a Hashimatul Fatma Hashim,^c Dayang SalwaniAwang Adeni,^c Muneera S. M. Al-Saleem,^d Jehan Y. Al-Humaidi ^d and Mohammed Muzibur Rahman ^e

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GRAPHICAL ABSTRACT



Production of Lactic Acid from Sago Starch Using *Enterococcus faecalis* Strain Isolated from Silage

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Production of high-purity lactic acid was achieved through fermentation using *Enterococcus faecalis* (strain 2409), isolated from silage, as the microorganism for converting glucose derived from sago starch. Lactic acid is a valuable organic compound with numerous applications, including food preservation, pharmaceuticals, and the production of biodegradable plastics. The fermentation process was conducted under carefully controlled conditions in a fermenter, with optimal parameters of pH 6.8, 45 °C, and 200 rpm, resulting in a lactic acid concentration of 87 g/L and a purity of approximately 80%. These findings highlight the potential of *E. faecalis* to efficiently produce lactic acid and demonstrate the viability of utilizing renewable resources such as sago starch for sustainable bioprocessing. Additionally, activated carbon was used to purify the fermentation broth, effectively removing impurities, and enhancing the overall quality of the final product. This approach offers an environmentally friendly and cost-effective solution for the commercial production of lactic acid.

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Keywords: *Enterococcus faecalis*; Silage; Lactic acid fermentation; Glucose

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INTRODUCTION

Lactic acid (2-hydroxypropanoic acid) is an organic compound of great industrial and biological significance. It has two optically active forms, L-lactic acid and D-lactic acid. L-Lactic acid is an important metabolite of human metabolism, whereas D-lactic acid may be toxic when it accumulates at a high level, although there is little in humans (Farrokh *et al.* 2018; Pohanka 2020; Xu *et al.* 2022).

Lactic acid can be synthesized by fermentation or chemical synthesis, and each approach provides advantages for industrial applications. In microbial fermentation, the lactic acid bacteria (LAB) convert carbohydrates to lactic acid, and it uses glucose as a substrate from fruit peel sugarcane bagasse and agricultural residues (Jawad *et al.* 2013).

Such a method is attractive due to the utilization of renewable resources and ability to meet sustainability targets (Bouhadi 2017; Wischral *et al.* 2018).

By comparison, chemical synthesis consists of the hydrolysis of lactonitrile to give a racemic mixture of D- and L-lactic acids (Chiu *et al.* 2015). High energy consumption and dependence on non-renewable feedstocks are limiting factors in this process (Chahal and Starr 2006). This is preferable for lactic acid production by fermentation as optically pure lactic acid, particularly in food and pharmaceutical uses (Lux and Siebenhofer 2012). Furthermore, fermentation proceeds at milder conditions and consumes less energy (Rahmayetty *et al.* 2023), as well as the substrates are cheap and renewable making it economically more feasible and eco-logically sustainable than chemical synthesis (Sukaïem, n.d.; Thitiprasert *et al.* 2024).

During fermentation, lactic acid is mainly produced by lactic acid bacteria (LAB). The LAB genera produce lactic acid, which is the main factor of food preservation due to their capability of acidifying which makes them resistant to spoilage organisms (Datta *et al.* 1995; Leroy and Vuyst 2004; Abedi and Bagher Hashemi 2020). In the dairy sector, lactic acid also acts as a preservative and flavor enhancer; for example, it contributes to texture and taste of foods such as yogurt and cheese (Demmelmayer *et al.* 2024; Díaz-Orozco *et al.* 2025).

Besides its application in food industry, lactic acid is attracting increasing interest in biochemistry, pharmaceuticals, and materials science. It is an essential intermediate for biodegradable polymers, notably polylactic acid (PLA) produced by polymerization of lactic acid. The increasing demand of lactic acid, approximately USD 67.9 billion in 2023, on the global scale demonstrates environmentally friendly property and biodegradable aspects that are capable for sustainable material development (Inkinen *et al.* 2011; Demmelmayer *et al.* 2024; Díaz-Orozco *et al.* 2025). Furthermore, current investigations are examining alternative industrial applications of lactic acid and its derivatives such as in medical devices including tissue engineering, drug delivery systems (Inkinen *et al.* 2011; Castañeda-Rodríguez *et al.* 2023).

Enterococcus faecalis is a lactic acid bacteria (LAB) classified as a Gram-positive bacterium that is closely related to *Lactobacillus* and *Lactococcus*. The bacteria are facultatively anaerobic, characterized by their ability to grow under both aerobic and anaerobic conditions. This gives them versatility as species found in different ecological niches ranging from the human intestinal tract, fermented foods and places modified by human (Kahieshesfandiari *et al.* 2021; Faris and Atya 2023; Rodríguez-Lucas and Ladero 2023).

The application of *E. faecalis* in the fermentation of lactic acid from glucose has emerged due to its ability to metabolize carbohydrates, including glucose, into lactic acid. Studies have shown that certain strains of *E. faecalis* can effectively ferment glucose, resulting in the production of lactic acid as a principal product (Fan and Hou 2019). The Gram-positive nature of *E. faecalis* contributes to its suitability for fermentation under acidic conditions, with optimal temperature ranges reported between 30 and 43 °C, which facilitates efficient lactic acid production (Abdel-Rahman *et al.* 2019).

Research indicates that *E. faecalis* utilizes a homofermentative pathway, primarily converting glucose to lactic acid while yielding a relatively high molar yield of ATP in the process (Sayed *et al.* 2020). Specific strains of *E. faecalis* demonstrate adaptability to various substrates, including waste materials rich in glucose, which allows for the efficient conversion of renewable resources into valuable products like lactic acid (Yuan *et al.* 2018). This economic and ecological aspect of utilizing *E. faecalis* in fermentation

processes is increasingly relevant in the context of sustainable bioprocessing (Tarraran and Mazzoli 2018).

In laboratory settings, the metabolic pathways of *E. faecalis* during fermentation have been studied, revealing that the bacterium typically utilizes the Embden-Meyerhof-Parnas pathway for lactic acid production, generating about 2 moles of ATP per mole of glucose (Sayed *et al.* 2020). *Enterococcus faecalis* demonstrated notable fermentation efficacy not only in pure glucose solutions but also in more complex substrates, including lignocellulosic hydrolysates and cheese whey permeate (Yuan *et al.* 2018; Dosuky *et al.* 2022). The production of lactic acid *via* fermentation with *E. faecalis* can be enhanced through operational modifications, including cell recycling and pH control, and optimizing conditions for lactic acid biosynthesis (Barroso *et al.* 2024).

Furthermore, the dynamics of *E. faecalis* in mixed fermentations with other LAB positively influence lactic acid production under various fermentation conditions (Wang *et al.* 2019). The acidic environment generated by lactic acid production promotes not only the survival of *E. faecalis* but also enables other LAB species to thrive, which demonstrates cooperative interaction that enhances fermentation quality (Atuna *et al.* 2025). It has been found that *E. faecalis* LX10 is able to secrete 28.8 g/L of lactic acid, the highest among other strains working under harsh conditions (Zhang *et al.* 2022). This fermentative capacity is useful for conventional food applications, such as cheese and sausage production, but also indicates an opportunity for in converting whey and other waste products to valuable by-products (Ćirić *et al.* 2020; Ziadi *et al.* 2020).

One significant advantage of *E. faecalis* in lactic acid fermentation is its efficiency in producing lactic acid. Studies have demonstrated that *E. faecalis* can effectively produce lactic acid from diverse substrates, such as cheese whey and agricultural feedstocks (Ziadi *et al.* 2020). This ability to ferment various carbon sources diversifies potential applications and enables the use of inexpensive raw materials, contributing to economic feasibility. For instance, *E. faecalis* RKY1 has been successfully employed in fermentation setups to convert substrates like wheat and barley into significant yields of lactic acid (Ziadi *et al.* 2020). Moreover, *E. faecalis*'s capacity to thrive in diverse environmental conditions exemplifies its adaptability. This resilience allows for its natural presence in various fermentation environments, from dairy products to plant-based substrates (Pérez *et al.* 2019).

Enterococcus faecalis can also increase the production of lactic acid when exposed to specific substances. These substances have been shown to upgrade the activity of lactate dehydrogenase in *E. faecalis* V583, leading to high lactic acid production (Biaggini *et al.* 2017). In addition, *E. faecalis* contributes to reduction in pH of fermented products which leads to the formation of acidic environment that prevents growth of pathogenic bacteria thus enhancing safety and control food quality (Nnabuike *et al.* 2024). In addition, *E. faecalis* could ferment with glycerol, which indicates potential to valorize biodiesel waste to produce lactic acid, while reducing waste management costs (Zou *et al.* 2017; Ćirić *et al.* 2020).

The lactic acid market is growing rapidly due to high demand from industries such as bioplastics and food production. One of the major factors behind this is the increasing demand for polylactic acid (PLA), a biodegradable plastic obtained from lactic acid that has been increasingly employed in packaging and medical devices (Liu *et al.* 2016; Solehah Din *et al.* 2021). Globally, growth of LA has been estimated at 18.7% CAGR (2019–2025) owing to its relevance in sustainable and environmentally friendly production (Solehah Din *et al.* 2021; Castillo-Martínez *et al.* 2022;).

With the development of fermentation technology, the lactic acid titer was correspondingly increased. Technologies such as dual-phase fermentation, and utilization of different substrates such as food wastes, agro- residues have been helpful for efficient lactic acid production (Acedos *et al.* 2022; Sugahara *et al.* 2022 Rahim *et al.* 2023). These developments are not only environmentally friendly, but they also cut down the production costs, thus enhancing commercial profitability (Pau *et al.* 2022). Furthermore, the utilization of renewable resources to produce LA corresponds with the trend of bio-based chemicals. Accordingly, lactic acid is one of the main building blocks in an emerging biobased economy (Dusselier *et al.* 2013; Komesu *et al.* 2017).

Currently there is no clear evidence of studies that specifically report lactic acid production from sago starch using an *Enterococcus faecalis* strain isolated from silage. However, the available literature indicates several closely related findings. *E. faecalis* has been shown to produce lactic acid from various starch-based substrates, with some studies mentioning sago starch in a broader context, though not involving silage-derived strains (Ziadi *et al.* 2020).

E. faecalis is commonly present in silage microbiota and plays a role in lactic acid formation during ensiling. Strains isolated from silage have been investigated for their fermentation capabilities, but not specifically with sago starch as the substrate (Guo *et al.* 2022; Li *et al.* 2018; Chen *et al.* 2017; Liptáková *et al.* 2017).

There are reports of *E. faecalis* being used to produce lactic acid from alternative substrates such as glycerol and cheese whey, including in immobilized systems. These studies demonstrate the organism's versatility, yet they do not involve silage-derived strains applied to sago starch (Dosuky *et al.* 2022; Ziadi *et al.* 2020). In lactic acid fermentation by LAB, glucose functions as more than just a basic nutrient; it serves as the primary substrate for lactic acid synthesis. Therefore, measuring glucose content is essential to quantify substrate consumption and correlate it directly with lactic acid production yield.

In summary, although related studies are available—namely (i) the use of sago starch as a substrate with *E. faecalis* and (ii) the application of silage-derived *E. faecalis* in lactic acid fermentation, none of the cited references explicitly integrate all three aspects: sago starch utilization, lactic acid production, and a silage-derived *E. faecalis* strain.

Accordingly, this study explores the potential of a silage-derived *Enterococcus faecalis* strain as a functional microorganism for lactic acid fermentation. Its fermentation performance is evaluated to determine its suitability and effectiveness as a candidate for industrial-scale lactic acid production.

EXPERIMENTAL

Materials and Methods

Sago starch was bought from local market. *Enterococcus faecalis* (strain DSM 20478), originally isolated from silage, was purchased from the Leibniz Institute, DSMZ—German Collection of Microorganisms and Cell Cultures (GmbH). Reagents such as 3,5-dinitrosalicylic acid ($\geq 98\%$), activated carbon charcoal (powder, particle size 100), chloroform (ACS, ISO, Reag. Ph Eur.) yeast extract (technical grade), Tryptic Soy Agar (TSA) and Tryptic Soy Broth (TSB) were purchased from Sigma-Aldrich. Sodium hydroxide 230 pellets ($M = 40.00$ g/mol) were purchased from System ChemAR. 110,000

U/ml Glucoamylase was purchased from Sunson Industry Group, China. The food grade α -amylase was procured from Jiangsu Rui Kang Lai Technology Co., Ltd. China.

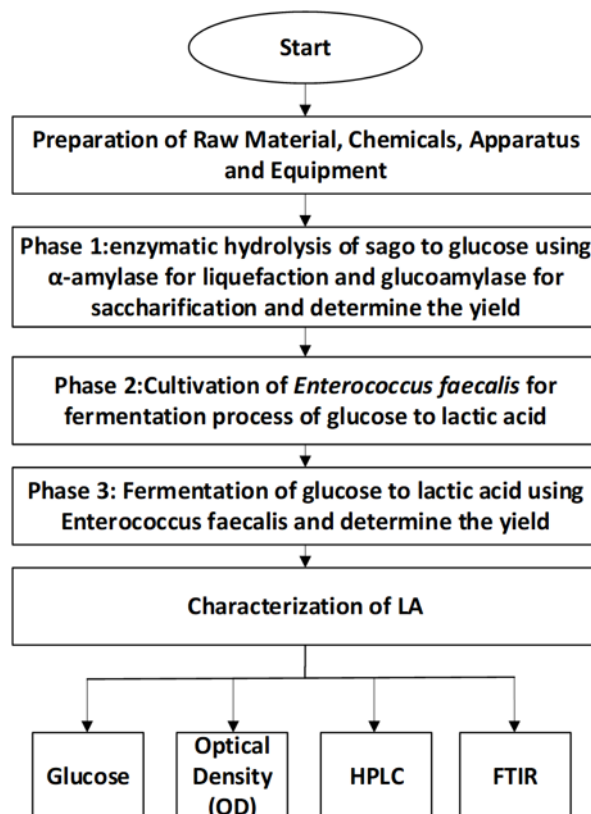


Fig. 1. Research flowchart

Enzymatic Hydrolysis

Enzymatic hydrolysis of sago starch was carried out according to the method of (Sunaryato *et al.* 2013) with minor changes, consisting of two steps: liquefaction and saccharification. Sago starch (100 g) was mixed with 800 mL of water at the liquefaction stage, and the pH was adjusted to 6.5 using 1 M NaOH. Following this, 10 g α -amylase enzyme from *Bacillus amyloliquefaciens* was incorporated to the mixture and allowed to heat for 140 min at 95 °C under constant agitation using an overhead stirrer (200 rpm). The liquid product was subsequently applied in saccharification. For saccharification, the temperature was reduced to 60 °C by natural cool at room temperature and pH was adjusted approximately to 4.3 with 1 N HCl. Subsequently, 5 mL of glucoamylase enzyme (*Aspergillus niger* and pullulanase from *Bacillus licheniformis*) were added, and the mixture was incubated at 60 °C for 24 h under stirring at speed of 200 rpm. Impurities and unhydrolyzed starch from the mixture were removed by centrifugation with Kubota 2800 centrifuge (Japan) after incubation.

Bacteria Cultivation

Bacteria thawing

An ampoule of *Enterococcus faecalis* was reconstituted with 0.5 mL Tryptic soy broth (TSB) medium. The pellet was rehydrated in the laminar flow hood for at most 30 min. The contents were then mixed gently with an inoculation loop. Half of the mixture

was homogenized in 5 mL of TSB in a test tube for bacteria stock culture and the other half was streaked on the appropriate agar. The broth culture and agar plate was incubated at 37 °C for 24 h in an oven incubator to enable bacterium thawing and growth during the use of these stock cultures and inoculum preparation.

Stock culture preparation

The bacterial stock culture was prepared using a sterile 20% glycerol solution mixed with sterile TSB solution. Following thawing, the bacterial culture was incubated at 37 °C for 24 h to ensure optimal growth prior to stock preparation. A total of 800 µL of the incubated liquid culture was aliquoted into each 50 sterile 2 mL cryovials. Subsequently, 200 µL of sterile 20% glycerol solution was added to each vial and gently mixed, resulting in a final glycerol concentration of 20%. The cryovials were then sealed and stored at −80 °C for long-term preservation. For enhanced freezing efficiency, the cryovials were initially placed at −20 °C for 24 h before being transferred to −80 °C. To revive the bacterial culture, cryovials were rapidly thawed either in a 37 °C water bath or at room temperature. The thawed culture was then streaked onto an agar plate or inoculated into a liquid medium for further cultivation.

Pre-inoculum Preparation

The method for pre-inoculum, inoculum, and media preparation was adapted from (Nolasco-Hipolito *et al.* 2019) with some modifications. The stock culture was thawed at room temperature and activated in 5 mL culture medium containing TSB. The culture medium was incubated at 37 °C for 24 h in an incubator oven.

Inoculum Preparation

After 24 h of incubation, the activated pre-inoculum culture of *E. faecalis* was transferred to 200 mL of culture medium containing 10 g/L yeast extract and 30 g/L glucose. The culture was placed in a 250 mL Erlenmeyer flask and incubated at 37 °C for an additional 24 hours. Following incubation, the culture was centrifuged using a high-speed centrifuge (Kubota 2800, Japan) at 4000 rpm and 37 °C for 5 min to collect the bacterial cells.

Culture Media Preparation for Fermentation Process

The media was prepared by mixing 100 g/L of glucose and 10 g/L of yeast extract in 1L bottle. The media was stirred for 12 min using magnetic stirrer. Then, the media was autoclaved at 121 °C for 20 min in an autoclave (Hirayama HICLAVE HVE-50, Japan).

Fermentation in Bioreactor

The cell pellet (5.0 g) harvested from the inoculum preparation, together with 4 L of a glucose-yeast extract mixture (100 g/L glucose and 10 g/L yeast extract per 1 L), was introduced into a 5 L bioreactor (Sartorius Biostat B Plus 5, Germany) with a working volume of 4 L. The fermentation parameters, including pH, temperature, and NaOH consumption, were continuously controlled and monitored in real time. The fermentation was conducted at 45 °C to slow growth but maintain glucose conversion to lactic acid, effectively uncoupling growth from production with an agitation speed of 100 rpm, while the pH was maintained at 6.86 by the automated addition of 10 M NaOH. *Enterococcus faecalis* is a thermotolerant microorganism with a rapid adaptive response to heat stress. In this study, the temperature increased from 37 to 45 °C to promote shift from biomass

formation to lactic acid production, thereby maintaining or enhancing lactic acid yield (Sun *et al.* 2020). The fermentation process lasted for 48 hours, and the total consumption of 10 M NaOH was recorded. After fermentation, the broth was centrifuged at $4000 \times g$ for 20 min at 24 °C to separate the cells using a Kubota 2800 centrifuge (Japan).

Color Removal by Activating Carbon

Powdered activated carbon (PAC) was employed to purify lactic acid from the fermentation broth by adsorbing impurities, including residual sugars, proteins, and polyphenols, which were responsible for the dark coloration of the broth. A total of 15 g of PAC was added to 1 L of clarified fermentation broth, and the mixture was stirred at room temperature for 1.0 h using a magnetic stirrer. The mixture was then centrifuged at $4000 \times g$ for 10 min at 24 °C to separate the solid PAC from the liquid phase. The resulting supernatant, which contained the sodium lactate solution, was collected for further processing.

Analytical Method

Cell concentration was measured at 600 nm using a UV-1800 double-beam spectrophotometer (Shimadzu, Kyoto, Japan) with a 10 mm path length cuvette.

Fermentation metabolites, including lactate, acetate, and formate, were quantified by high-performance liquid chromatography (HPLC), as shown in Fig. 1, following the method described by Talluri *et al.* (2013). The analysis was performed using a Shimadzu LC20 system equipped with a refractive index detector (RID-10A). Briefly, culture samples were centrifuged at $4,000 \times g$ for 20 minutes to obtain the supernatant. The supernatant was then filtered through a 0.45 μm nylon membrane and injected into a 300×7.8 mm Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) maintained at 60 °C. A 5.0 mM H_2SO_4 solution was used as the mobile phase.

Fourier-transform infrared spectroscopy (FTIR) was employed to characterize lactic acid in the fermentation broth, with transmittance spectra recorded over the 400 to 4000 cm^{-1} range at a resolution of 2 cm^{-1} using a Thermo Scientific Nicolet iS5 FT-IR spectrometer.

RESULTS AND DISCUSSION

Based on Table 1, the moisture content of the sago starch samples obtained from the experiment ranged between 12.0% and 12.7%, with a mean value of 12.3%. The low variation observed among the three replicates indicates good analytical precision and reflects effective control of the drying process. This moisture range consists of reported values for native sago starch, which typically lies between 12% and 13%, as documented by (Ningrum *et al.* 2023).

The moisture content of sago starch, measured at approximately 12%, reflects its native state, which is not ideal for fermentation. Moisture content is crucial for lactic acid fermentation, as it influences starch hydration, enzymatic accessibility, and the metabolic activity of LAB. While 12% moisture is suitable for storage stability, it is too low for effective fermentation. Studies show that moisture levels between 20% and 25% promote starch gelatinization, enhance enzymatic hydrolysis, and improve LAB metabolic efficiency, resulting in higher lactic acid production (Adawiyah *et al.* 2017; Puspita Dewi *et al.* 2022). Excessively high moisture can lead to microbial spoilage, while insufficient

moisture restricts LAB growth and metabolic activity (Dong and Yuan 2024). Therefore, adjusting moisture to the range 20% to 25% provides the ideal conditions for effective LAB fermentation and enhanced lactic acid production.

Table 1. Moisture Content

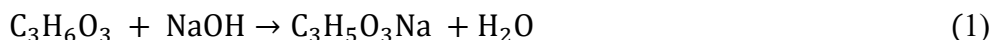
No.	Weight of Plate	Weight of Sample	Weight of Plate + Sample	Final Weight	Moisture Content (%)
Sample 1	3.80	5.00	8.80	8.16	12.72
Sample 2	4.30	5.00	9.10	8.70	11.98
Sample 3	3.79	5.00	8.79	8.19	12.10
Mean					12.26

Lactic Acid Production

This section describes the theoretical calculation used to estimate the amount of 10 M NaOH required to completely neutralize the lactic acid produced during fermentation.

The neutralization reaction between LA and NaOH is assumed to follow a 1:1 molar stoichiometry, as shown in Eq. 1. This indicates that one mole of lactic acid reacts with one mole of NaOH to form sodium lactate and water.

The total production of LA was determined by multiplying the weight of NaOH consumed at any time and for considering factor, $F = 0.69$. The value of conversion factor (F) was calculated based on stoichiometric equation of the reaction as given in Eq. 2:



It is known that the NaOH molar mass is 40 g/mol, lactic acid molar mass is 90 g/mol, and NaOH density ρ is 1.3 g/mL. Thus, the volume of 1 g of 10 M NaOH = $\frac{\text{mass}}{\text{density}} = \frac{1\text{g}}{1.3\text{g/mL}} = 0.77\text{mL}$

$$\begin{aligned} \text{Gram of NaOH per mL of 10 M NaOH} &= \frac{400 \text{ g NaOH}}{1000 \text{ L}} = \frac{0.4\text{g}}{\text{mL}} \\ \text{Equivalent factor NaOH to Lactic acid} &= \frac{\text{Lactic acid molar mass}}{\text{NaOH molar mass}} = \frac{90}{40} \\ &= 2.25 \end{aligned}$$

$$\text{Conversion factor (F)} = 0.7692 \frac{\text{mL}}{\text{g}} \times 0.4 \frac{\text{g}}{\text{mL}} \times 2.25 = 0.692 \quad (2)$$

Theoretical mass consumed for 100% conversion of lactic acid:

$$\begin{aligned} \text{Mass of NaOH consumed} &= \frac{\text{Mass of starch (g)}}{\text{Conversion factor}} \\ \text{Mass of NaOH consumed} &= \frac{400\text{g}}{0.692} = 578.03\text{g} \end{aligned}$$

$$\text{Volume of NaOH consumed} = 608 \text{ mL}$$

The molar masses of NaOH and LA are 40 g/mol and 90 g/mol, respectively. Based on this stoichiometric relationship, an equivalent factor of 2.25 is obtained by dividing the

molar mass of LA by that of NaOH (90/40). This factor represents the mass of lactic acid neutralized per unit mass of NaOH.

The density of NaOH is given as 1.3 g/mL, allowing the volume of NaOH corresponding to 1.0 g to be calculated as 0.769 mL. For a 10 M NaOH solution, which contains 400 g/L of NaOH, the concentration is equivalent to 0.4 g/mL. Combining the density-based volume conversion, solution concentration, and equivalent factor yields a conversion factor (F) of 0.692, which relates the volume of 10 M NaOH to the mass of lactic acid neutralized.

Using this conversion factor, the theoretical mass of NaOH required for complete (100%) neutralization of LA is calculated by dividing the estimated mass of lactic acid (assumed to be equivalent to 400 g) by the conversion factor. This results in a required NaOH mass of 578.03 g.

Therefore, it is theoretically estimated that 578.03 g of 10 M NaOH solution is required to achieve complete neutralization of the LA produced from 4 L of hydrolysate. This calculation provides a theoretical baseline for NaOH consumption, which may differ from experimental values due to process inefficiencies, impurities, or incomplete conversion during fermentation.

Table 2. Real Consumption

	Weight (g)
Bottle	565.17
Bottle + NaOH (Initial)	1851.82
Bottle + NaOH (Final)	1325.61
Consumption	526.21

$$\text{Yield} = \frac{\text{Consumed NaOH}}{\text{Theoretical}} \times 100$$

$$\text{Yield} = \frac{526.1 \text{ g}}{578.03 \text{ g}} \times 100 = 90.8\%$$

This result indicates that approximately 90.8% of the theoretical NaOH requirement was utilized during the neutralization of lactic acid. The deviation from 100% conversion may be attributed to factors such as incomplete neutralization, experimental handling losses, the presence of other buffering components in the fermentation broth, or inaccuracies in the estimation of lactic acid concentration. Nevertheless, the high yield obtained suggests that the neutralization process was largely efficient and closely aligned with theoretical expectations.

Various factors, including substrate availability and environmental conditions such as pH, can affect fermentation efficiency. Lactic acid bacteria thrive best under specific pH levels and nutrient conditions, which, if not met, can lead to a slowdown in metabolic activity and, consequently, a drop in lactic acid production (Lee *et al.* 2015). For example, at certain growth rates, LAB can produce a mixture of end products such as acetate or ethanol instead of lactic acid, as hydrogen production tends to be favoured when ATP yield from substrate-level phosphorylation is optimal (Regueira *et al.* 2020; Winder *et al.* 2022).

In addition, the presence of byproducts and their concentration can significantly impact the fermentation pathway. Accumulation of metabolites, including lactate itself, has been shown to alter the redox environment within the cell, which may inhibit further lactate production (Kelly *et al.* 2018). Higher concentrations of extracellular lactate can promote its uptake and conversion back to pyruvate, consequently reducing the net lactic acid production during fermentation (Kelly *et al.* 2018). Furthermore, LAB species vary in their metabolic pathways; some may favor heterofermentative pathways, resulting in a broader spectrum of fermentation products rather than exclusively lactic acid (Santamaría *et al.* 2018).

Lactic Acid Analysis

Based on Table 2, The lactic acid concentration of 87 g/L achieved through fermentation with *E. faecalis* exhibited a highly efficient fermentation process, consistent with current literature on LA production by various strains of LAB. *Enterococcus faecalis* is widely recognized as a proficient LA producer across different fermentation conditions. For example, studies by Wee *et al.* (2004, 2006) reported lactic acid concentrations ranging from 24 to 93 g/L when *E. faecalis* was used to ferment wood hydrolysate and corn steep liquor, with yield variations depending on substrate composition and fermentation parameters.

It is also important to note that Subramanian *et al.* (2014) indicated that specific mutations and strain variations in *E. faecalis* can influence its acid production efficiency, suggesting that bioengineering efforts could further enhance lactic acid yields. The type of fermentation technique, whether batch or repeated batch, also plays a crucial role in determining final LA concentrations. For example, Mokhtar *et al.* (2024) emphasized the superior efficiency of repeated-batch fermentation systems using *E. faecalis* RKY1, which yield higher substrate conversion rates compared to continuous fermentation methods.

Additionally, *E. faecalis*'s ability to produce lactic acid from a wide range of substrates underscores its versatility. Literature supports that *E. faecalis* can ferment not only glucose but also more complex carbohydrates, efficiently converting them into lactic acid (Doi 2018). Findings by Dosuky *et al.* (2022) further support this, showing that different *E. faecalis* strains and substrates can produce comparable or higher lactic acid concentrations under controlled conditions. The strain from silage produced 87 g/L of lactic acid when fermented with glucose derived from sago starch under optimized conditions of pH 6.8, 45 °C, and 200 rpm. This result demonstrates the strain's efficient ability to convert glucose into lactic acid.

When compared to other strains, the *E. faecalis* from silage fell between the highest lactic acid yields and those at lower levels. For example, *E. faecalis* used with wheat bran hydrolysate produced 149 g/L of lactic acid under different conditions (Yoshimune *et al.* 2016), while other strains such as *E. faecalis* RKY1 achieved a lower yield of around 6.37 g/L/h under specific conditions (Reddy *et al.* 2016). These comparisons indicate that while the strain isolated from silage does not produce the highest lactic acid concentration, it is still highly competitive, producing a substantial amount of lactic acid from renewable resources such as sago starch. The findings suggest that with further optimization of fermentation processes or genetic modifications, this strain could effectively compete in large-scale industrial lactic acid production, highlighting its potential for sustainable biotechnological applications.

Table 3. Experimental Data

Strain Used	Medium Used for Fermentation	Parameters for Fermentation	Lactic Acid Produced (g/L)	Purity (%)
<i>Enterococcus faecalis</i> (isolated from silage)	Glucose from Sago Starch	pH 6.8, 45 °C, 200 rpm	87	80

Optical Density (OD) Analysis

The first stage of fermentation was initiated with a substrate containing 100 g/L glucose and 10 g/L yeast extract. The growth phases observed were as follows: the lag phase occurred between 0 and 12 h, the exponential phase was observed from 12 to 40 h, the deceleration phase occurred between 40 and 45 h, the stationary phase was seen between 45 and 60 h, and the death phase began after 65 h, corresponding to a fermentation process with an initial substrate concentration of 100 g/L.

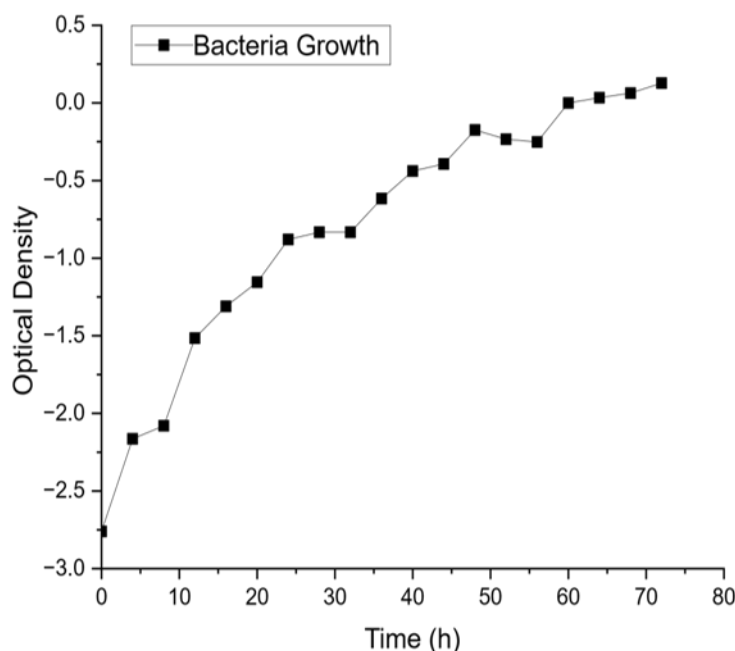


Fig. 2. Bacterial growth curve, where the X-axis represents time (h) and the y-axis shows the natural logarithm of optical density, indicating the cell density of bacteria

Optical density analysis at 600 nm (OD_{600}) is a key tool for monitoring microbial growth in fermentation, particularly for LAB such as *Lactobacillus* and *Streptococcus*. It offers a fast, non-invasive way to track biomass concentration, which is crucial for optimizing fermentation conditions and enhancing product yields. OD_{600} measures changes in optical density that reflect microbial biomass and metabolic activity, helping to monitor lactic acid production. Studies have shown its effectiveness in tracking LAB growth and lactic acid yield, with research demonstrating its ability to provide valuable insights into fermentation dynamics (Fan *et al.* 2016; Ma *et al.* 2021).

A study has indicated that *E. faecalis* can be successfully cultured to specific OD values, such as 0.252, utilizing growth media such as MRS broth, with precise control over fermentation conditions including temperature and medium composition (Alghazaly and Alshareef 2019; Bohora and Kokate 2017). This optimal growth tuning is essential as it

determines metabolic output and the efficiency of fermentation processes (Rosalina and Aprilia 2021). Using OD readings in conjunction with other microbiological techniques, researchers can delineate the growth phases of *E. faecalis* and assess the efficacy of antimicrobial agents (Pargaputri *et al.* 2017; Deviyanti *et al.* 2024).

HPLC Analysis

The chromatogram in Figs. 3 and 4 displays three distinct peaks, each representing different compounds in the sample, with Peak #3 being the most prominent. Peak #1, with a retention time of 2.830 min, has an area of 4492 and a height of 357 mV, contributing 4.3% to the total area and 8.5% to the total height. Peak #2, occurring at 3.59 min, has a larger area of 15562 mV.min⁻¹ and a height of 537 mV, accounting for 15.0% of the area and 12.83% of the height. Peak #3, with a retention time of 5.53 min, is the dominant component, with an area of 83500 mV.min⁻¹ and a height of 3300 mV, representing 80.6% of the total area and 78.7% of the total height. In summary, Peak #3 is the most abundant compound, while Peaks #1 and #2 contribute less significantly. The total area is 10400 mV.min⁻¹, and the total height is 4190 mV.

Table 4. HPLC Data on Lactic Acid Fermentation

Sample	Original Concentration (g/L)	Purity (%)
Fermentation Broth	87.26	80.64
After PAC	83.93	80.07

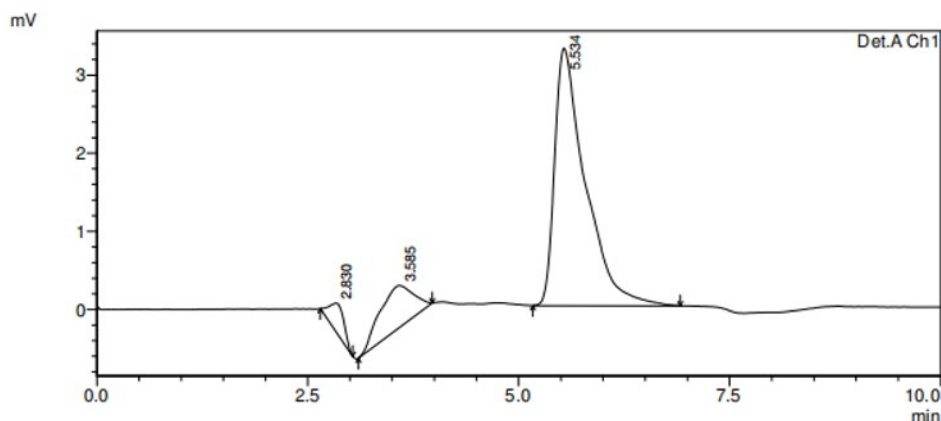


Fig. 3. HPLC analysis on fermentation broth

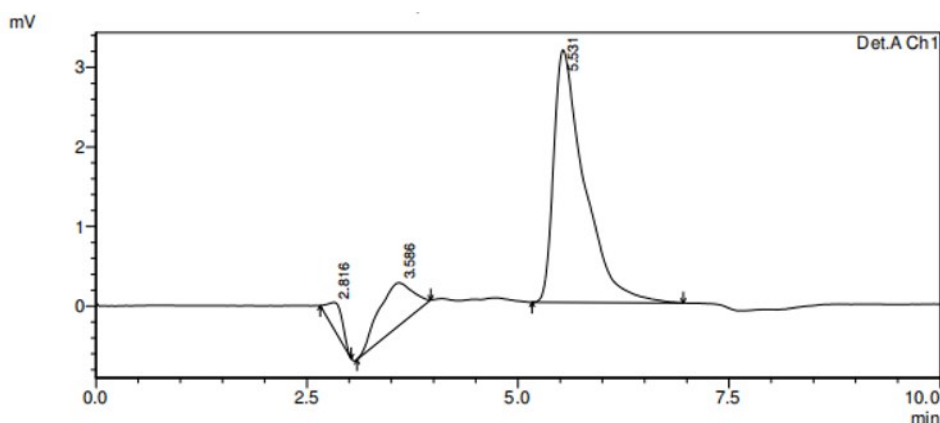
The chromatogram displays three peaks at retention times of 2.816, 3.586, and 5.531 minutes, corresponding to different compounds in the sample. Peak #1, with an area of 4170 mV.min⁻¹ and a height of 326 mV, contributes 4.15% of the total area and 8.08% of the total height, indicating a low concentration. Peak #2, having an area of 15800 mV.min⁻¹ and a height of 540 mV, contributes 15.8% to the area and 13.4% to the height, representing a moderate concentration. Peak #3, the dominant compound, has the largest area of 80300 mV.min⁻¹ and the highest peak height of 3173 mV, making up 80.1% of the total area and 78.6% of the total height. The total area under the peaks equals 100300 mV.min⁻¹, with a total height of 4040 mV, indicating that Peak #3 is the most abundant in the sample. The peaks are well-separated, suggesting proper column performance.

Table 5. Peak Table on Fermentation Broth

Peak#	Ret. Time (min)	Area (mV.min ⁻¹)	Height (mV)	Area (%)	Height (%)
1	2.83	4492	357	4.34	8.51
2	3.59	15562	537	15.02	12.83
3	5.53	83524	3295	80.64	78.66
Total		103579	4189	100.00	100

A study by Nassos *et al.* (1984) reported a retention time of approximately 4.77 min for lactic acid when using an Aminex HPX-87H ion exclusion column with a mobile phase of 0.005 N H₂SO₄. Similarly, Srivastava *et al.* (2014) found retention times for lactic acid in the range of 5 to 7 min, depending on the mobile phase and column used. This supports the identification of Peak #3 as lactic acid, as its retention time of 5.53 min falls within the typical range reported for lactic acid in various studies.

Furthermore, other studies, such as those by Hasegawa *et al.* (2003), reported lactic acid retention times in the range of 6 to 7 min, providing additional validation for the observed retention time of Peak #3. These studies confirm that variations in retention times can be influenced by factors such as the chromatographic column, mobile phase composition, and detection methods.

**Fig. 4.** HPLC analysis on fermentation broth after purification with PAC**Table 6.** Peak Table on Fermentation Broth After Purification with PAC

Peak#	Ret. Time (s)	Area (mV.min ⁻¹)	Height (mV)	Area (%)	Height (%)
1	2.82	4168	326	4.15	8.08
2	3.59	15828	540	15.78	13.37
3	5.53	80334	3173	80.07	78.55
Total		100329	4039	100	100

In summary, the chromatographic data, including the prominent peak at 5.53 min (Peak #3), aligns with the expected retention times for lactic acid in HPLC, supporting its identification as the primary compound in the sample. The consistency of retention time across multiple studies underscores the reliability of HPLC for lactic acid quantification in complex mixtures, such as fermentation broths.

FTIR Analysis

The FTIR spectrum reveals key absorbance peaks that correspond to specific functional groups in the sample. The broad peak around 3288 cm^{-1} is indicative of the O-H stretching vibration, typically associated with hydroxyl groups found in alcohols or phenols. This broadness suggests the presence of hydrogen bonding, which is characteristic of water or alcohol. The broadness of the peak suggests hydrogen bonding, which is typical for alcohol or carboxylic acids. At approximately 1634 cm^{-1} , the spectrum shows a peak that is often linked to the C=C stretching vibration in alkenes or the C=O stretching vibration in carbonyl groups, such as those found in aldehydes, ketones, or carboxylic acids.

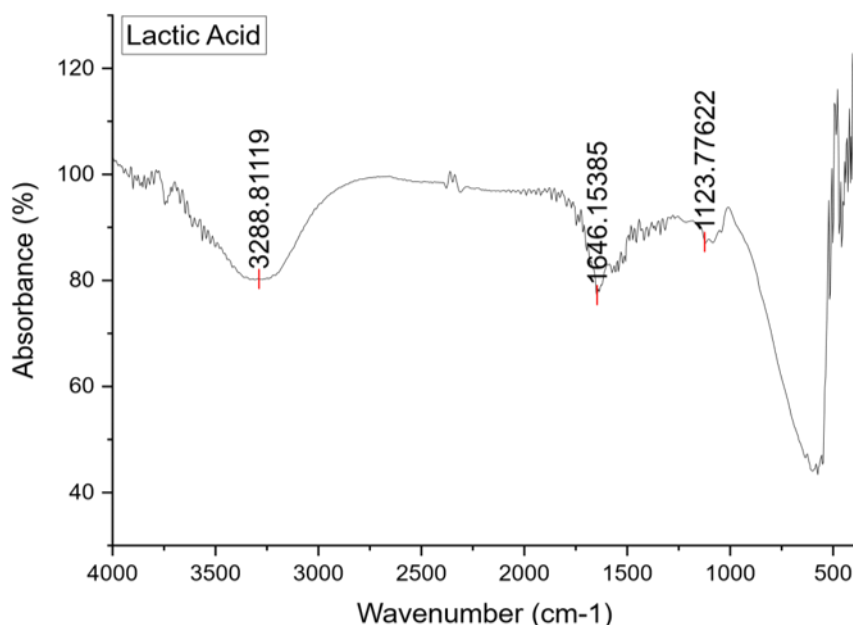


Fig. 5. FTIR result of Lactic acid from fermentation broth

This absorbance suggests the presence of unsaturation or a carbonyl functional group. Finally, the peak around 1118 cm^{-1} corresponds to the C-O stretching vibration, commonly observed in alcohols, ethers, or phenols, indicating the presence of ether or alcohol groups. The sharp rise at the lower wavenumber region near 500 cm^{-1} may point to the presence of other functional groups or possibly an artifact related to the sample. The information provided by these peaks helps to identify the chemical composition and functional groups in the sample.

A prominent absorbance band appears around 3379 cm^{-1} , which is associated with the O-H stretching vibrations, indicating the presence of alcohol functional groups within the lactic acid molecule (Ortinero *et al.* 2017). At approximately 1612 cm^{-1} , the stretching of the carbonyl (C=O) group is observed, signifying the carboxylic acid nature of lactic acid (Ortinero *et al.* 2017). Additionally, an important peak around 1080 cm^{-1} corresponds to the C-O stretching vibrations, providing crucial insights into the molecular interactions within lactic acid (Ortinero *et al.* 2017). The broader O-H peak commonly observed in fermentation samples also indicates the presence of additional hydroxyl-containing compounds (Tóth and Németh 2022). FTIR spectroscopy is a reliable analytical tool for detecting lactic acid during fermentation, allowing identification through characteristic

absorption bands. It is widely used to monitor fermentation progress and assess broth composition containing lactic acid alongside other organic metabolites (Okano *et al.* 2009; Şahin *et al.* 2009).

CONCLUSIONS

1. *Enterococcus faecalis* isolated from silage proved to be an effective and versatile strain for lactic acid fermentation using glucose derived from sago starch. The results demonstrated that this strain could produce high concentrations of lactic acid, making it a promising candidate for industrial-scale fermentation applications.
2. Furthermore, the successful decolorization of the fermentation broth using activated carbon further validates the economic feasibility of this method.
3. These findings contribute to the growing body of research on sustainable lactic acid production, emphasizing the potential benefits of utilizing renewable resources in biotechnological processes to produce valuable biochemical products such as lactic acid.

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