https://scilett-fsg.uitm.edu.my/
Vol. 17(2) JUNE 2023

ISSN: 1675-7785
eISSN: 2682-8626

DOI: 10.24191/sl.v17i2.22750

Lactic Acid Production from Potato Waste Using Response Surface Methodology

Zafirah Zenol Abidin[1], Nurul Diyana Abdullah Sham[1] and Siti Roshayu Hassan[1,2*]

[1]Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia

[2]Advance Industrial Biotechnology Cluster (AdBiC), Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia

Corresponding author: *roshayu.h@umk.edu.my*

Accepted: 1 April 2023; Published: 21 June 2023

ABSTRACT

Lactic acid is an organic acid produced by microorganisms that ferment in glucose. *Lactobacillus spp*. is a type of lactic acid bacteria used to ferment lactic acid. *Lactobacillus spp*. are rod-shaped, gram-positive bacteria that do not produce spores. Because of the increased energy production, a carbohydrate or glucose source is essential for glycolysis and fermentation processes. Lactic acid is now widely used in pharmaceutical, food, and biodegradable polymer industries. Hence, this study aims to analyze the effect of two different parameters on the yield of lactic acid by using Response Surface Methodology (RSM). Secondly, this study also determined lactic acid concentration from potato waste using simultaneous saccharification and fermentation. It evaluated the effect of different concentrations of enzymes and the reaction time of fermentation on the yield of lactic acid. The SFF method was used in this study to produce lactic acid from potato waste. RSM was tasked with studying two different parameters using CCD. The concentration of enzyme at 1.5 g/L and the time for fermentation on day 1 produced the best result in this study, with the highest lactic acid production yield of 33.2%. These findings suggest that potato waste hydrolysate, like carbon sources, can replace synthetic glucose in producing lactic acid in the presence of enzymes. This process can be scaled up in a pilot plant.

Keywords: *Fermentation, glucose, enzyme, lactic acid, carbohydrate*

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INTRODUCTION

Lactic acid, also known as a 2-hydroxy propanoic acid, $CH_3CHOHCOOH$ is a chemical compound formed in the human body from the breakdown of glucose and glycogen, and it is a hydroxycarboxylic acid that can be produced through the fermentation process [1, 2]. Lactic acid exists in two stereoisomers which are: L- and D-lactic acid. Each of them can become a primary concern in various fields. Lactic acid is a natural organic acid that has long been used in the food, pharmaceutical, clothing, and chemical industries [2].

Lactic acid can be manufactured via fermentation or chemical synthesis, using a wide range of waste products as substrates. Its existence as two stereoisomers makes the utilization of one of them or the racemic mixture of significant importance in several fields. The food and pharmaceutical industries, in particular, prefer the isomer l (+), the only one that the human body can digest. However, the chemical industry requires either one of the pure isomers or a combination of both, depending on the application [2]. Based on Abdel-Rahman *et a*l. [3], the passion for lactic acid has risen dramatically because lactic acid can be used as a monomer to prepare Polylactic acid (PLA) for this several years. Crystalline Poly-L-lactic acid can be used to make fibers and films. Starchy materials are suitable for lactic acid production substrates and require amylolytic enzymes to extract fermentative sugars [4].

Response Surface Methodology (RSM) is a tool that was introduced in the early 1950s and has been used for the approximation and optimization of stochastic models [5]. According to Aydar *et al*. [6], the RSM uses a mathematical and statistical approach for modeling and evaluating a mechanism in which several variables influence the response of interest to optimize the response.

Solanum tuberosum L. is another name for potato, and it is a significant source of global food and the most important agricultural crop for human consumption. Potatoes are widely grown crops that contain primarily starch, sucrose, and glucose, which can be converted into lactic acid [7]. In our country, approximately 60% of potatoes are processed into various foods. This leads to the accumulation of potatoes as a waste product. According to E-Gopala *et al*. [8], the potato industry generates between 12% and 20% of waste and by-products. Potato peels, pulps, and rejected potatoes are waste produced during potato processing.

In this study, potato waste was used to produce glucose which the lactic acid bacteria require to produce lactic acid. The simultaneous saccharification and fermentation (SSF) process with RSM determination was investigated further. It involves the process of enzymatic hydrolysis of potato waste and the fermentation process to produce lactic acid to evaluate the effect of different enzyme concentrations and time fermentation on the yield of lactic acid. The hydrolysis process involves the breakdown of the polymer of the potato waste into the most straightforward sugar assisted by the enzymatic reaction. Using potato waste as a substrate in conjunction with the presence of enzyme *and L. plantarum,* a lactic acid bacterium, will aid in the experiment's saccharification and fermentation (SSF) method. This type of bacteria was thought to be the most important for lactic acid production.

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EXPERIMENTAL

Fresh and rotten potatoes were collected from the UMK cafeteria kitchen, Jeli Campus. *The materials needed to conduct the study were the Lactobacillus plantarum* strain, MRS agar, MRS broth, alpha-amylase (Diastase), and methanol grade HPLC.

Preparation of Raw Material

1.5 kg of fresh potato and 1.5 kg of rotten potato were ground with a laboratory blender and mixed with distilled water until they became slurry. Both samples were mixed. Then, the sample was homogenized and autoclaved in an Erlenmeyer flask at 121°C for 2 hours to avoid contamination. After that, the sample was stored in the freezer at -20°C for further use.

Media Preparation

Lactobacillus plantarum was inoculated and fermented in De Man, Ragosa, and Sharpe (MRS) broth. 55 g of MRS broth powder was dissolved in 1 L of distilled water and thoroughly mixed. The mixture was then autoclaved at 121°C for 2 hours before being incubated at 35°C for 24 hours.

Inoculum Preparation

The *Lactobacillus plantarum* strain was obtained from the UMK laboratory. The inoculum preparation for *L. plantarum* was performed by inoculating a full slant culture in a 500 mL flask with 100 mL of sterile growth medium containing MRS broth. The flask was then incubated at 37°C for 18 hours with shaking at 160 rpm.

Lactic Acid Production Using SSF Method

In this study, Simultaneous Saccharification and Fermentation (SSF) processes were operated with 13 runs according to Central Composite Design (CCD). 20 mL of potato waste and alpha-amylase were added into the flask containing 5 mL inoculum broth and MRS broth. The volume of alpha-amylase and MRS broth was based on the value given in the CCD.

Design of Experiment Using Response Surface Methodology (RSM)

This study used a central composite design model to evaluate the parameter effect in lactic acid production from potato waste during the fermentation process. Two independent variables were conducted in this study: enzyme concentration and fermentation time, with the response being lactic acid yield. The "Numeric factor" was set to 2 in the central composite design, based on the number of factorials that need to be tested

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in this experiment. The maximum and minimum value of each factor is shown in Table 1. The central composite design model generated 13 sets of experimental runs, as shown in Table 2.

Table 1: Parameter used for lactic acid

Parameters	**Range**	
	Low	**High**
Concentration of enzyme (g/L)	1	1.5
Time for fermentation (days)	1	3

Table 2: Experimental response using the central composite design model

Std	**Run**	**Factor 1** **Concentration of yeast extract (g/L)**	**Factor 2** **Time for fermentation (day)**
2	1	1.5	1
9	2	1.25	2
6	3	1.60	2
5	4	0.90	2
8	5	1.25	3.5
3	6	1.0	3
4	7	1.5	3
10	8	1.25	2
1	9	1.0	1
7	10	1.25	0.6
12	11	1.25	2
11	12	1.25	2
13	13	1.25	2

Measurement of Lactic Acid Content

High-Performance Liquid Chromatography (HPLC) was used to analyze lactic acid. 20 µL of the sample was injected into a Diamonsil C18 column with a temperature of 30°C. The mobile phase was 100% methanol with a 1 mL/min flow rate. The detection was accomplished using a prominent Liquid Chromatograph (LC-20AT). The ultraviolet (UV) detection rate was 254 nm, and each sample took 15 minutes to analyze.

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Functional Group of Lactic Acid Quantification

Fourier Transform Infrared Spectroscopy (FTIR) method was used to determine the lactic acid quantification. The absorbance spectra (650 to 4000 cm-1) were recorded by using an FTIR spectrometer (IR-Prestige) equipped with a horizontal attenuated total reflectance (ATR) accessory with a single reflection. The spectral resolution is 4 cm-1, and 128 scans were accumulated for each spectrum.

RESULTS AND DISCUSSION

Analysis of the CCD Model

The 2FI model was chosen for this study because the standard deviation for the response was 0.1935, the lowest value among the models. Furthermore, the correlation coefficient, the adjusted R2 value for this study, was 0.8241, which is close to 1, indicating that the model is significant. In this experiment, the R2 value for the response was more significant than the adjusted and predicted R2 value generated by this model. Table 3 shows that the 2FI model was the best-fit model for this study response, which is lactic acid yield, and that the cubic model was aliased because it was far away to fit the response for this study.

Table 3: Model statistic for yield lactic acid

Source	Std. Dev	R^2	Adjusted R^2	Predicted R^2	Press	Remarks
Linear	0.3100	0.4985	0.3981	-0.0170	1.95	
2FI	0.1935	0.8241	0.7655	0.6219	0.7245	Suggested
Quadratic	0.1751	0.8880	0.8080	0.5943	0.7774	
Cubic	0.1649	0.9291	0.8298	0.8562	0.2756	Aliased

The Yield of Lactic Acid

The factors of the study were divided into two categories: low and high. For this study, the coded factors -1 and +1, which represent the lowest and highest values for the factors generated by CCD, were used. The design experiment with coded factors is shown in Table 4.

Table 4: Design experiment with coded factors

Factors	Parameter	Units	Coded factors	
A	Concentration of enzyme	g/L	-1	+1
B	Time for fermentation	Days	-1	+1

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Table 5 displays the results of 13 RSM experiments using the CCD. The highest yield of lactic acid produced in this experiment was 33.2% using 1.5 g/L alpha-amylase concentration and one day of fermentation time. This study's lowest lactic acid yield was 1.8% using 1.25 g/L alpha-amylase concentration and two days of fermentation time.

Table 5: Response value with coded factors

Run	Factor 1 A: Concentration of alpha-amylase (g/L)	Factor 2 B: Time for fermentation (days)	Response 1 The yield of lactic acid (%)
1	1.5	1	33.2
2	1.25	2	26.0
3	1.60	2	12.0
4	0.90	2	23.2
5	1.25	3.5	26.4
6	1.0	3	25.8
7	1.5	3	1.26
8	1.25	2	25.2
9	1.0	1	26.4
10	1.25	0.5	18.0
11	1.25	2	26.6
12	1.25	2	1.8
13	1.25	2	21.4

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Interaction Between Concentration of Enzyme and Time for Fermentation on Lactic Acid Yield

Figure 1(a) and Figure 1(b) show the interaction effect of the concentration of enzyme α-amylase (A) and time for fermentation (B) on the yield of lactic acid. Based on Figure 1, the interaction of enzyme concentration and time for fermentation was higher at a concentration of 1.5 g/L of the enzyme, and the time for fermentation is 24 hours or one day. The yield of lactic acid produced was 1.66 mg/L. Besides that, the lowest yield of lactic acid produced was 0.09 mg/L at a concentration of enzyme 1.25 g/L, and the time for the fermentation was two days. This shows that the time taken for the fermentation process takes 24 hours, and if a higher concentration of alpha-amylase is used, a higher yield of lactic acid will produce. The enzyme availability per unit substrate increases as the enzyme concentration increases. More mass transfer and enzyme transport occur at the surface of the starch granules. Reducing sugar production resulted from the breaking down the α-1,4-glycosidic bond in the middle of the amylose and amylopectin chains of the starch from the potato waste. As a result, total reduced sugar yields increase [9].

The productivity of lactic acid bacteria, *L. plantarum,* in producing lactic acid increases as the sugar level rises. *L. plantarum* uses reducing sugar as a nutrient to generate energy to yield lactic acid during fermentation [10]. Within 24 hours, the lactic acid bacteria in the fermentation process can consume enough substrate to generate energy to yield lactic acid. The lactic acid concentration was reduced at two days of fermentation because there was no more substrate presence. The lactic acid bacteria had a death phase with the decreased substrate [11]. The yield of lactic acid is also affected by the availability of nutrients, carbon, temperature, pH, and ethanol. During the simultaneous saccharification and fermentation (SSF) process, the inhibitors may have presented that caused the significant reduction in the amount of lactic acid produced. The compounds in the potato that contained the inhibitors slowed the enzyme's activity. The activity of the inhibitors slowed starch degradation and reduced the glucose yield for lactic acid bacteria consumption [12].

Determination of Functional Group in Lactic Acid

Fourier Transform Infrared Spectroscopy (FTIR) analysis determined the functional groups for each sample of different enzyme concentrations. According to Figure 2, the corresponding spectra for the functional group of the lactic acid spectrum are Aliphatic Primary Amides and Primary Aliphatic Alcohol. Primary Aliphatic Amides are carboxamides in which the amide linkage is directly bonded to an aliphatic system, whereas Primary Aliphatic Alcohol is an organic compound containing one or more hydroxyl groups (-OH). The number of R groups attached to the carbon with the hydroxyl group determines the aliphatic alcohol. The alcohol is present in this experiment because of the fermentation process. During the fermentation process, the lactic acid will produce ethanol. In this experiment, ethanol is present due to the addition of the enzyme alpha-amylase. Because potato waste contains starch, alpha-amylase is essential for this experiment. Starch is a polysaccharide substrate that microorganisms cannot directly utilize.

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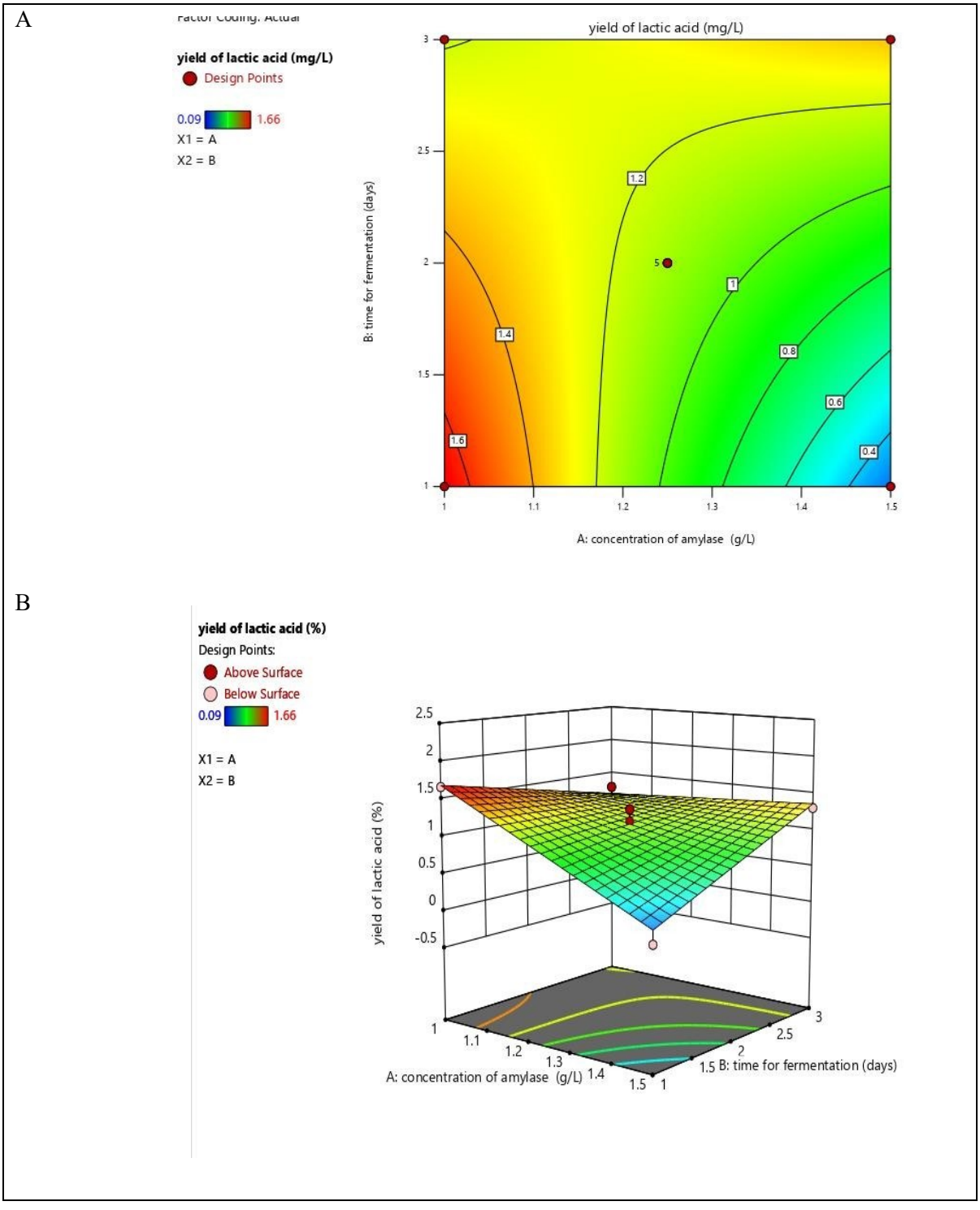

Figure 1: (A) 2D contour plot and (B) 3D Response surface of concentration of enzyme α-amylase (A) and time for fermentation (B)

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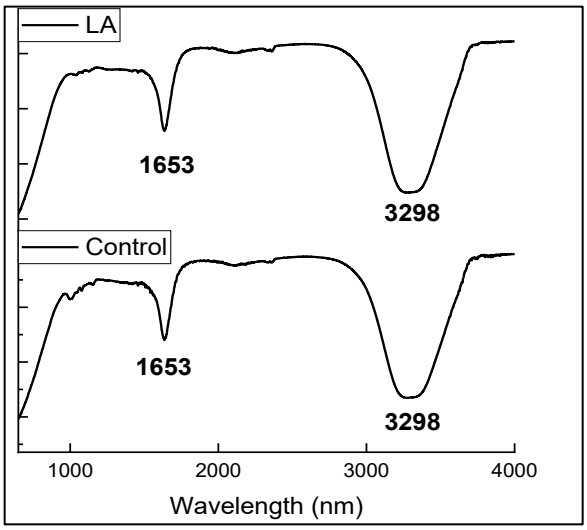

Figure 2: Wavelength of FTIR analysis

CONCLUSION

The lactic acid concentration from potato waste was determined using simultaneous saccharification and fermentation. The effect of enzyme concentration and time for fermentation to yield lactic acid was also evaluated. Simultaneous saccharification and fermentation (SSF) were conducted by adding homogenized potato waste, inoculum broth, enzyme α-amylase, and MRS broth into the flask. The two independent variables, α-amylase concentration (g/L) and fermentation time (days), were determined using RSM software. The study discovered that *Lactobacillus plantarum* produces 33.2% of lactic acid from potato waste via simultaneous saccharification and fermentation (SFF). In addition, the response alpha-amylase concentration and time for fermentation resulted in 1.5 g/L of alpha-amylase and 1-day time for fermentation being the most significant parameter influencing lactic acid production.

ACKNOWLEDGMENTS

The authors wish to acknowledge financial support from Malaysia's Fundamental Research Grant Scheme (FRGS) (Ref no. FRGS/1/2021/TK0/UMK/02/6).

AUTHOR'S CONTRIBUTION

Nurul Diyana Abdullah Sham conducted the research and wrote the article. Zafirah Zenol Abidin revised the article and arranged highlighted relevant theories. Siti Roshayu Hassan designed the research and supervised the research progress.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted without any self-benefits or commercial or financial conflicts and declare the absence of conflicting interests with the funders.

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