RASĀYAN *J. Chem.*
Vol. 18 | No. 1 |380-389| January - March | 2025
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

OPTIMIZATION OF LACTIC ACID FERMENTATION FROM BAGASSE USING *Lactobacillus casei* B75: A STATIC FERMENTATION APPROACH

Rossy Choerun Nissa[1,2,✉], Finna Fitriyani Soleha[3], Assyifa Junitasari[3], Maya Irmayanti[4], Hidawati[5], Deddy Triyono Nugroho Adi[2], Yeyen Nurhamiyah[2], Akbar Hanif Dawam Abdullah[2], Safri Ishmayana[6] and Dadan Sumiarsa[6]

[1]Department of Biotechnology, Graduate School, Universitas Padjajaran, Bandung, 40132, Indonesia
[2]Research Center for Biomass and Bioproducts, National Research and Innovation Agency, Jl. Raya Bogor km. 46 Cibinong, Bogor,16911, Indonesia
[3]Department of Chemistry, Faculty of Science and Technology, Sunan Gunung Djati State Islamic University, Jl. A.H. Nasution No.105 Cibiru, Bandung, 40614, Indonesia
[4]Master Program Agro-industrial Technology, Faculty of Agro-Industrial Technology, Universitas Padjadjaran, Jl. Raya Bandung-Sumedang km. 21, Jatinangor, 45363, Indonesia
[5]Research Center for Environmental and Clean Technology, National Research and Innovation Agency, Bandung, 40135, Indonesia
[6]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjajaran, Jl. Raya Bandung-Sumedang km. 21, Jatinangor, 45363, Indonesia
✉Corresponding author: rossy19001@mail.unpad.ac.id

ABSTRACT

Bagasse, a by-product of the sugar industry, contains cellulose and hemicellulose that can be converted into glucose and xylose through acid hydrolysis, offering a valuable carbon source for lactic acid fermentation. This study aims to optimize lactic acid production from bagasse hydrolysate under static fermentation conditions using *Lactobacillus casei* B75. The effects of inoculum concentration (5–15%), substrate concentration (5–15 mg/mL), and fermentation time (12–72 hours) on reducing sugar content and lactic acid yield were evaluated using a Response Surface Methodology (RSM) - Box-Behnken Design (BBD). Optimal conditions—15% inoculum, 15 mg/mL substrate, and 72-hour fermentation—resulted in 0.37 mg/mL of reducing sugar and 9.72 mg/mL of lactic acid. These findings demonstrate the potential of *L. casei* to efficiently convert bagasse hydrolysates into lactic acid, supporting the sustainable use of agricultural residues for biochemical production. The study highlights the effectiveness of RSM - BBD in optimizing fermentation, offering a viable approach for utilizing low-cost biomass in value-added product generation.

Keywords: Lactic Acid, Bagasse Hydrolysates, RSM-BBD, Static Fermentation, Lactic Acid Bacteria.
RASĀYAN *J. Chem.*, Vol. 18, No. 1, 2025

INTRODUCTION

Lactic acid production from lignocellulosic biomass has attracted significant global interest due to its extensive applications in the food, pharmaceutical, and chemical sectors.[1,2] Lignocellulosic biomass, such as bagasse, is rich in cellulose and hemicellulose, making it a cost-effective feedstock for lactic acid fermentation.[3] Bagasse can be converted into monomeric sugars through chemical and biochemical processes. Chemical methods, such as hydrolysis with acids like sulfuric or hydrochloric acid, effectively break down the complex structure of bagasse, releasing fermentable sugars.[4] These sugars serve as substrates for lactic acid bacteria during fermentation, where microbial processes are preferred due to their sustainability and the milder conditions required compared to chemical synthesis.[5]

Lactobacillus casei is widely used in lactic acid production due to its ability to utilize diverse substrates and adapt to various fermentation strategies. It thrives at 37°C and tolerates a wide pH range, making it ideal for efficient, cost-effective, and sustainable industrial processes. The fermentation process optimizes

http://doi.org/10.31788/RJC.2025.1819129

lactic acid yield and concentration by controlling key parameters such as substrate concentration, nutrients, temperature, pH, inoculum size, fermentation time, and oxygen levels. [6–8] The fermentation of various substrates by *L. casei* has been extensively studied, including fruit waste, bagasse, cassava starch hydrolysate, date pomace, and whey.[9–11] *L. casei* is well-adapted to metabolize different carbon sources and can effectively process sucrose at concentrations as high as 100 g/L.[12] Optimal concentration ranges, however, vary depending on the substrate. For example, simple sugars like glucose or lactose generally require lower concentrations (approximately 10–20 g/L) to prevent inhibition, while more complex substrates, such as agricultural residues, can tolerate higher concentrations, up to 55 g/L.[13] Most lactic acid fermentations using various Lactobacillus cultures are conducted over 48–72 hours to achieve effective yields. [14] However, the use of bagasse with *L. casei* remains limited. The application of numerical optimization techniques, such as Response Surface Methodology (RSM), has proven effective in identifying optimal conditions for lactic acid production, significantly reducing production costs. [15–17] RSM - BBD are widely used for optimizing fermentation processes by systematically varying key parameters, such as substrate concentration, inoculum concentration, and fermentation time, to determine the optimal conditions for lactic acid production using bagasse hydrolysate as a substrate. This study uniquely applied RSM - BBD for static fermentation, a relatively underexplored approach. Furthermore, integrating fermentation processes to maximize yield while minimizing resource consumption improves lactic acid production's sustainability and enhances process efficiency and economic feasibility of lignocellulosic feedstocks.

EXPERIMENTAL

Materials

Bagasse was obtained from a state-owned Indonesian agricultural company in Bandar Lampung, Indonesia. *Lactobacillus casei* B75*,* sourced from the Indonesian Culture Collection (InaCC). De man, rogosa, Sharpe (MRS) Broth, MRS Agar, yeast extract, and 3,5-dinitro salicylic acid were purchased from Himedia, India. L-lactic Acid standard, D-glucose standard, hydrochloric acid (HCl), glycerol, potassium sulfate, peptone, yeast extract, ammonium sulfate, dipotassium hydrogen phosphate, magnesium sulfate heptahydrate, potassium dihydrogen phosphate, and calcium carbonate were purchased from Merck, USA.

Preparation Microorganism and Culture Medium

L. casei B75, a prominent L (+) lactic acid producer, was preserved in microtubes with MRS medium supplemented with 20% glycerol at -80°C. Before inoculation, the strain was twice propagated in an MRS medium at 37°C for 24 hours under static conditions.

Preparation and Cultivation of Lactobacillus Casei B75 for Experimental Use

Rejuvenation of *L. casei* B75 began by inoculating a loopful of glycerol stock onto slanted MRS agar, followed by incubation at 37°C for 24–48 hours. A loopful of culture from the agar plate was inoculated within 50 mL of MRS broth and incubated at 37°C for 20–24 hours, resulting in a bacterial concentration of 19 × 10^9 CFU/mL.

Bagasse Hydrolysis

Based on a modified version of Laopaiboon's method[18], acid hydrolysis was performed by treating 5 g of bagasse with 100 mL of 1.4% HCl solution. The hydrolysate was prepared by heating the mixture at 121°C for 90 minutes and filtering it afterward.

Optimization Using Box-Behnken Design and Response Surface Methodology

The development of the experimental design was performed using Design Expert (version 9.0.6.2), employing a RSM – BBD. A quadratic model was applied with three independent variables: fermentation time (A, 12-72 hours), inoculum concentration (B, 5-15%), and substrate concentration (C, 5-15 mg/mL) across 17 experimental runs. The study focused on two response variables: reducing sugar content (Y_1) and lactic acid content (Y_2). The effects of the independent variables on these responses were analyzed with 95% confidence ($\alpha = 0.05$), incorporating hypothesis testing, model evaluation, validation, and optimization. Table-1 shows the factors and corresponding levels used in the BBD experimental setup.

Table-1: Experimental Factors and Levels in the BBD

Factors	Codes	Levels		
		-1	0	1
Time (Hours)	A	12	42	72
Inoculum Concentration (% v/v)	B	5	10	15
Substrate Concentration (mg/mL)	C	5	10	15

Optimization of Lactic Acid Fermentation with Bagasse Hydrolysate

30 mL substrate volume of bagasse hydrolysate, with concentrations ranging from 5 to 15 mg/mL, was prepared in a 50 mL Falcon tube. Each sample was added with 1.5% yeast extract, 0.05% dipotassium hydrogen phosphate, 0.2% ammonium sulfate, 1.0% peptone, 0.05% potassium dihydrogen phosphate, 0.005% magnesium sulfate heptahydrate, and 0.4% calcium carbonate. The medium underwent sterilization in an autoclave at 121°C for 15 minutes and was subsequently allowed to cool to ambient temperature. Subsequently, the inoculum *L. casei* B75 was added, and fermentation was performed at 37°C under static conditions at pH 6.0.

Quantification of Reducing Sugars (RS) and Lactic Acid (LA) Concentration

The 3,5-dinitrosalicylic acid (DNS) assay was utilized to quantify reducing sugars.[19] With D-glucose as the standard ($R^2 = 0.99$). Measurements of absorbance at 540 nm were conducted employing an Agilent Cary 60 UV-Vis spectrophotometer (USA). High-performance liquid chromatography (HPLC) was used to determine the lactic acid concentration in the fermentation broth. The resulting lactic acid was acidified with 2% sulfuric acid, centrifuged at 10000 rpm, adjusted to a pH of 2–3, and filtered for HPLC analysis. Analysis was performed using a Coregel 87H3 column (Shimadzu LC-2030C 3D Plus, Japan) paired with a refractive index detector (Shimadzu RID-20A, Japan). The mobile phase for the analysis was 0.01 M sulfuric acid, maintained at a flow rate of 1.0 mL/min, with the column temperature stabilized at 65°C.

RESULTS AND DISCUSSION

This study investigated the effects of inoculum concentration (microorganism quantity), substrate concentration (nutrient availability), and fermentation time (process duration) on key response variables. The results demonstrated that variations in inoculum and substrate concentrations significantly influenced the levels of reducing sugars and lactic acid produced. However, fermentation time did not significantly impact all response variables. These findings suggest that the concentration of microorganisms and nutrients is more critical in determining outcomes. At the same time, the importance of fermentation time may vary depending on the specific response measured. Consequently, optimizing inoculum and substrate concentrations is essential to achieve the desired results in the fermentation process. Table-2 presents the design and experimental results for reducing sugar and lactic acid, including both the predicted and actual values based on the experiment conducted using the RSM BBD approach.

Table-2: Design and Experiment Results: Reducing Sugar and Lactic Acid

Standard Order	Experimental Range of Actual Variables			Predicted and Actual Value of the Response			
	Time (h)	Inoculum Concentration (% v/v)	Substrate Concentration (mg/mL)	RS (mg/mL)		LA (mg/mL)	
				Predicted	Actual	Predicted	Actual
1	12	10	5	1.44	1.48	7.79	7.73
2	42	5	5	-0.08	0.24	7.92	7.70
3	72	15	10	0.43	0.79	8.24	7.96
4	42	15	15	1.52	1.20	9.38	9.60
5	42	10	10	0.86	0.96	6.52	6.15
6	12	10	15	3.94	4.47	6.90	6.49
7	42	15	5	0.78	0.94	9.31	9.17
8	72	5	10	0.24	0.45	6.69	6.50
9	12	5	10	1.79	1.43	6.06	6.33
10	72	10	5	0.99	0.46	7.62	8.04
11	42	10	10	0.86	0.95	6.52	7.27
12	12	15	10	2.74	2.53	6.92	7.12

13	42	10	10	0.86	0.77	6.52	7.21
14	72	10	15	0.53	0.49	9.02	9.09
15	42	10	10	0.86	0.80	6.52	6.16
16	42	5	15	1.24	1.07	8.35	8.48
17	42	10	10	0.86	0.80	6.52	5.79

The optimization process with a BBD aims to examine the relationships between three independent variables and the response variables. RSM-BBD was employed to develop the model equations using Design-Expert software. Reducing sugar is represented through the mathematical model shown in Equation (1), and the model for lactic acid is presented in Equation (2).

$$\text{Reducing Sugar (mg/mL)} = 1.266-0.031A+0.334B+0.194C-0.001AB-0.004AC-0.005BC+0.001A^2-0.008B^2+0.009C^2 \quad (1)$$

$$\text{Lactic Acid (mg/mL)} = 14.545-0.013A+0.437B+1.330C-0.001AB-0.004AC-0.004BC+0.000A^2-0.027B^2+0.062C^2 \quad (2)$$

The Equation comprises linear terms (A, B, C), interaction effects (AB, BC, AC), and quadratic components (A^2, B^2, C^2). ANOVA analysis produced p-values smaller than 0.05, signifying that the respective coefficients in this investigation were statistically significant.

Analysis of Variance for Reducing and Lactic Acid

BBD was used to assess the impact of different factors on acid production during fermentation, with *L. casei* B75 as the lactic acid-producing bacterium and glucose obtained from bagasse as the carbon source. The fermentation time was carefully controlled to maintain consistency throughout the experiments.

Table-3: ANOVA Analysis for Reducing Sugar

Source	Sum of Squares	Df	Mean square	F-value	p-value	
Model	14.79	9	1.64	9.63	0.0035	Significant
A	7.45	1	7.45	43.63	0.0003	
B	0.6441	1	0.6441	3.77	0.0932	
C	2.11	1	2.11	12.37	0.0098	
AB	0.1444	1	0.1444	0.8457	0.3884	
AC	2.19	1	2.19	12.83	0.0090	
BC	0.0812	1	0.0812	0.4757	0.5126	
A^2	1.80	1	1.80	10.52	0.0142	
B^2	0.1844	1	0.1844	1.08	0.3333	
C^2	0.1960	1	0.1960	1.15	0.3195	
Lack of Fit	1.16	3	0.3873	46.50	0.0014	
Pure Error	0.0333	4	0.0083			
Cor Total	15.99	16				

Table-3 presents the results of the ANOVA analysis for reducing sugars. It shows that both fermentation time and substrate concentration significantly impact reducing sugar levels. The analysis highlights that variations in these factors lead to measurable changes in reducing sugar production, with fermentation time playing a critical role in the process's overall effectiveness. Microbes metabolize the substrate, producing organic acids, enzymes, and secondary metabolites essential for their metabolic functions. Over time, the accumulation of these acids, primarily acetic and lactic acids, creates increasingly acidic conditions. These conditions influence fermentation dynamics and enzyme activity, ultimately reducing sugar production.[20]

Table-4 presents the ANOVA analysis for lactic acid production, showing that variations in substrate concentration significantly influence lactic acid. The data highlights that changes in substrate concentration lead to notable shifts in lactic acid production, implying its critical role in the biochemical processes governing fermentation. Understanding this relationship allows for precise adjustments to substrate concentration, enabling optimization or control of lactic acid production in fermentation processes. The ANOVA analysis provided p-values, which indicate statistical significance when below 0.05. This shows a 95% confidence level, supporting the model's validity under the given parameters. In this study, the

selected model produced p-values of 0.0035 for reducing sugars and 0.0153 for lactic acid, both statistically significant. The high coefficients of determination (R^2) further validate the model's accuracy, with R^2 values of 92.52% for reducing sugars and 88.13% for lactic acid. A model is considered reliable when R^2 exceeds 70%.[13] The predicted values closely aligned with experimental results, explaining 92.52% of the variation in reducing sugars and 82.51% in lactic acid production.[17]

Table-4: ANOVA Analysis for Lactic Acid Production

Source	Sum of Squares	Df	Mean square	F-value	p-value	
Model	18.90	9	2.10	5.78	0.0153	Significant
A	1.91	1	1.91	5.26	0.0556	
B	2.92	1	2.92	8.04	0.0252	
C	0.1290	1	0.1290	0.3548	0.5702	
AB	0.1156	1	0.1156	0.3180	0.5904	
AC	1.31	1	1.31	3.61	0.0993	
BC	0.0320	1	0.0320	0.0879	0.7754	
A^2	0.2071	1	0.2071	0.5696	0.4751	
B^2	1.97	1	1.97	5.41	0.0530	
C^2	9.97	1	9.97	27.42	0.0012	
Lack of Fit	0.7086	3	0.2362	0.5144	0.6941	
Pure Error	1.84	4	0.4591			
Cor Total	21.45	16				

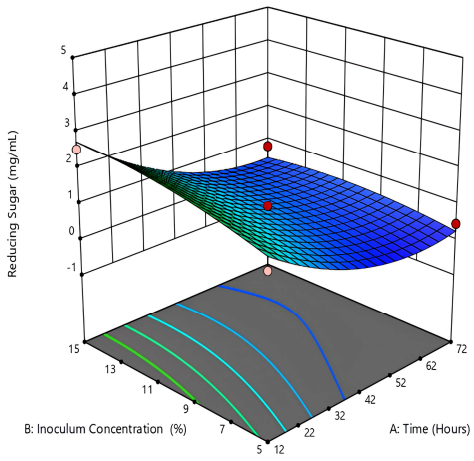
The Effect of Time Fermentation, Inoculum Concentration, and Substrate Concentration on Reducing Sugar Response

The optimal operating conditions determined using RSM are visualized through three-dimensional plots, including surface and contour plots. The x- and y-axes represent the tested variables in these plots, while the z-axis indicates the response or result value derived from their interaction. The contour plot in this study displays an ascending ridge pattern for reducing sugar, with the contour area illustrating variations in reducing sugar yield. Each color on the plot corresponds to a specific yield level, with the darkest blue area representing the lowest reducing sugar yield.

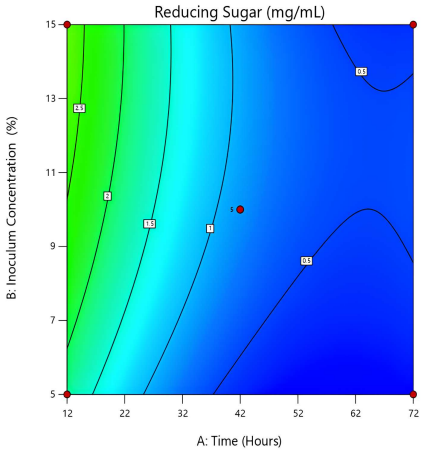
Fig. -1 shows the three-dimensional response surface and contour plots, which illustrate three factors' impact on reducing sugar content. The optimal reducing sugar concentration (4.47 mg/mL) was achieved after 12 hours of fermentation, with a 10% inoculum concentration and a 15 mg/mL substrate concentration. Graphs (a) and (b) show a nonlinear relationship between fermentation time and inoculum concentration on reducing sugar content. Higher inoculum concentrations required shorter fermentation times to reduce sugar levels than lower concentrations, likely due to inhibition and reduced process efficiency. Graphs (c) and (d) reveal a similar nonlinear relationship between fermentation time and substrate concentration. Higher substrate concentrations required longer fermentation times to overcome end-product inhibition, while lower substrate concentrations facilitated faster fermentation but resulted in lower reducing sugar content (0.46 mg/mL after 72 hours). Graphs (e) and (f) depict the relationship between inoculum concentration and substrate concentration in reducing sugar content. Higher substrate concentrations generally enhanced the conversion of reducing sugars into lactic acid, although this effect was influenced by inoculum concentration. The optimal conditions for reducing sugar conversion occurred at high inoculum and substrate concentrations, while medium and low levels had a less significant impact on lactic acid production.

The Effect of Time Fermentation, Inoculum Concentration, and Substrate Concentration on Lactic Acid Response

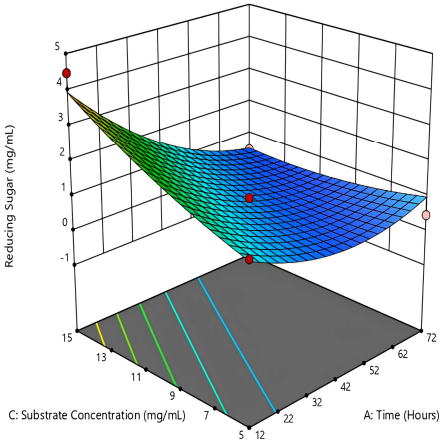
The contour plot displays an ascending ridge pattern for lactic acid production, with yield variations represented by different colors. The darkest blue area indicates the lowest lactic acid yield, while lighter colors represent higher yields. These plots are instrumental in identifying the optimal fermentation time, inoculum concentration, and substrate concentration needed to maximize lactic acid production under the tested conditions.



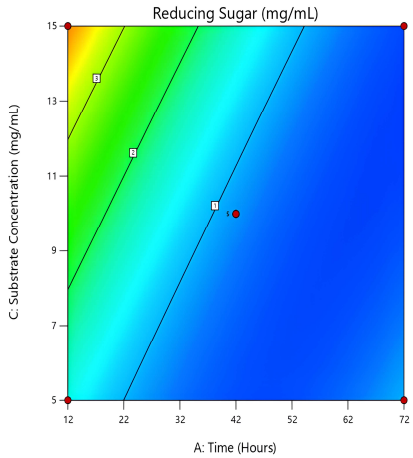
(a)



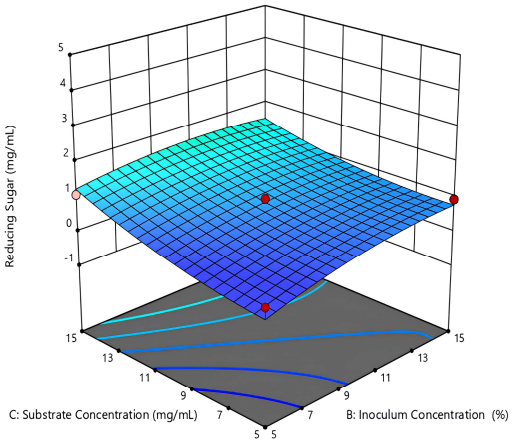
(b)



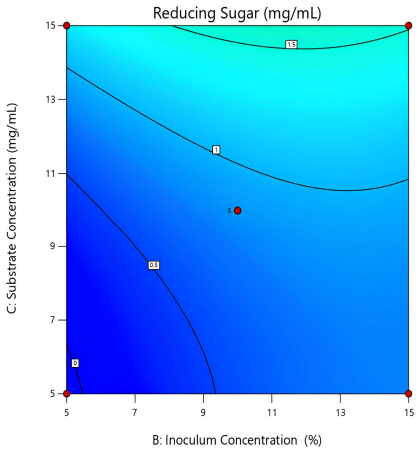
(c)



(d)



(e)



(f)

Fig.-1: 3D Response Surface and Contour Plots Illustrating the Impact of (a, b) Inoculum Concentration and Fermentation Time, (c, d) Substrate Concentration and Fermentation Time, and (e, f) Substrate and Inoculum Concentrations on RS (mg/mL).

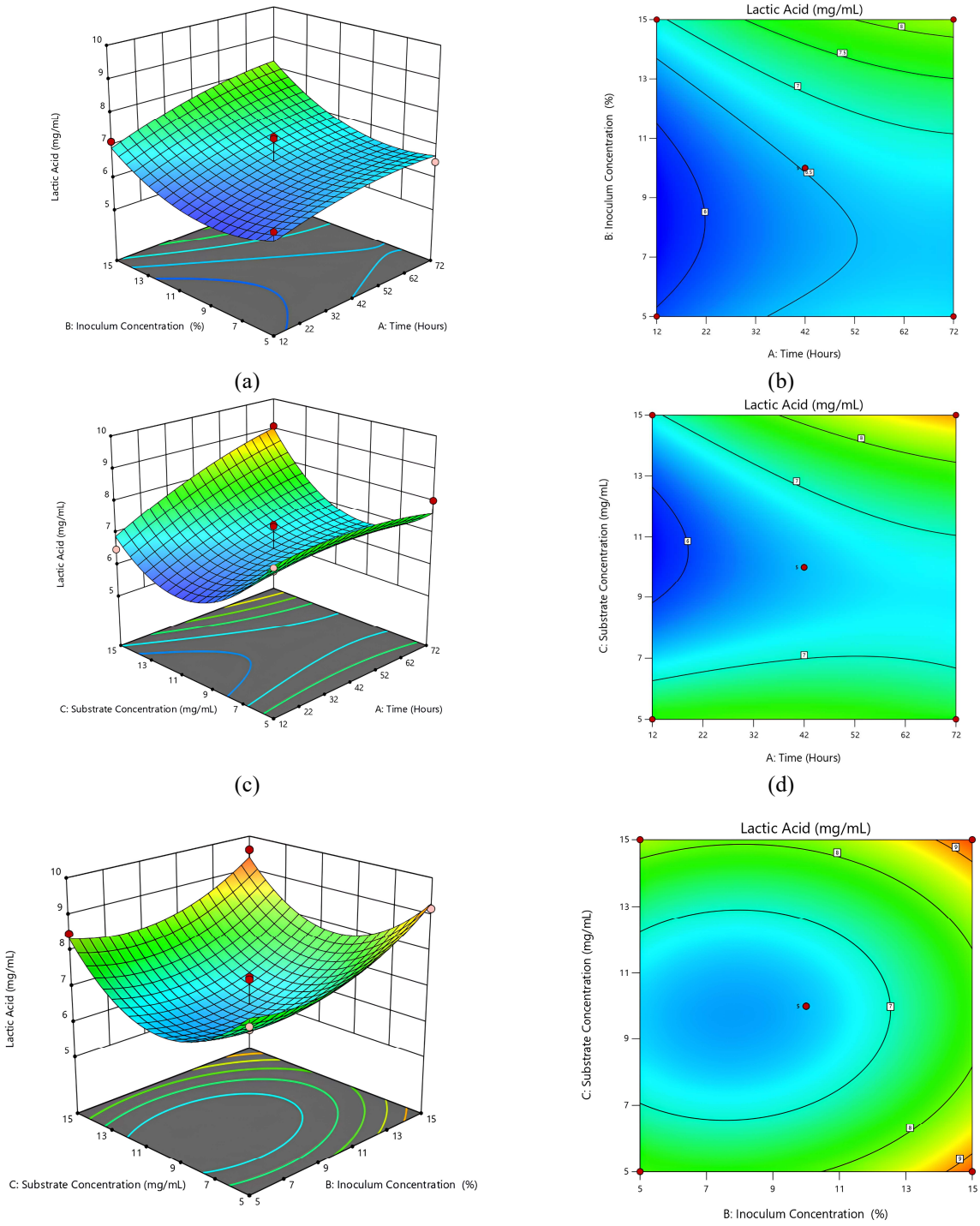


Fig.-2: 3D Response Surface and Contour Plots Illustrating the Impact of (a, b) Inoculum Concentration and Fermentation Time, (c, d) Substrate Concentration and Fermentation Time, and (e, f) Substrate and Inoculum Concentrations on LA Yield (mg/mL)

Figure-2 shows the three-dimensional response surface and contour plots, which demonstrate the influence of three factors on lactic acid production. Optimal lactic acid production, yielding 9.60 mg/mL, was observed after 42 hours of fermentation at a 15% inoculum concentration and a substrate concentration of

15 mg/mL. Graphs (a) and (b) show the nonlinear relationship between inoculum concentration and fermentation time on lactic acid production. Generally, increasing inoculum concentration and fermentation time enhanced lactic acid yield. However, shorter fermentation times were sufficient at higher inoculum concentrations due to inhibition and reduced efficiency. When the substrate concentration exceeds a critical level, high consumption is required. The nonlinear effects of fermentation time and substrate concentration on lactic acid production are represented in graphs (c) and (d). High substrate concentrations caused substrate inhibition, reducing the microorganisms' ability to utilize the substrate.[21] Higher substrate concentrations necessitated extended fermentation durations to mitigate the effects of end-product inhibition. Reducing the substrate concentration to 10% for a 72-hour fermentation decreased lactic acid production to 7.96 mg/mL High inoculum and substrate concentrations achieved the highest lactic acid yield (9.60 mg/mL). The differences in lactic acid yield were insignificant at medium and low concentrations.

Optimal Lactic Acid Fermentation Conditions Suggested by RSM

Subsequently, mathematical models were developed to reduce sugar and lactic acid responses. These models were employed to optimize formulas, determining the optimal combination of inoculum concentrations, substrate concentrations, and fermentation times. This optimization procedure is considered relative, as outlined in Table-5.

Table-5: Optimization Reducing Sugar and Lactic Acid of Fermentation Lactic Acid

Name	Goal	Lower Limit	Upper Limit	Low Weight	Upper Weight	Importance
A: Time	Maximize	12	72	1	1	5
B: Inoculum Concentration	Maximize	5	15	1	1	5
C: Substrate Concentration	Maximize	5	15	1	1	5
Reducing Sugar	Minimize	0.24	4.47	1	1	5
Lactic Acid	Maximize	5.7876	9.5964	1	1	5

Table-6: Validation of Reducing Sugar and Lactic Acid Values from Optimization Results

Response	Predicted	Actual				Validation
		1	2	3	Average	
Reducing sugar (mg/mL)	0.27	0.48	0.36	0.27	0.37	72%
Lactic acid (mg/mL)	10.40	9.00	9.36	10.85	9.72	93%

Experiments were conducted following the optimal parameters suggested by the RSM-BBD model, detailed in Table-6. The model estimated reducing sugar and lactic acid concentrations to be 0.27 mg/mL and 10.4 mg/mL, respectively. In the actual experiment, the concentrations measured were 0.37 mg/mL for reducing sugar and 9.72 mg/mL for lactic acid. The alignment of predicted and observed values validates the robustness and reliability of the model. This study converted bagasse hydrolysate to lactic acid under optimized conditions, yielding a maximum concentration of 9.72 mg/mL during static fermentation at 37°C, with a $Y_{(P/S)}$ of 0.648 mg/mg. The optimal parameters included an inoculum concentration of 15% (v/v), a substrate concentration of 15 mg/mL, and a fermentation duration of 72 hours. This result is slightly better than other studies of lactic acid fermentation with *L.casei*. Thakur *et al.* (2019) utilized L. casei MTCC 1423 for lactic acid fermentation, employing corn-steep liquor as a carbon source. The process was conducted at 37°C for 72 hours without agitation, yielding a $Y_{(P/S)}$ value of 0.629 mg/mg.[22] This study also shows a better result than the dynamic fermentation study. Similarly, Oonkhanond et al. (2017) obtained a lactic acid yield of 21.3 g/L from bagasse hydrolysate under conditions of 150 rpm agitation for 120 hours, starting with an initial reducing sugar concentration of 33.7 g/L and achieving a yield ($Y_{p/s}$) of 0.632 mg/mg.[23] However, long agitation duration requires higher energy, which prevents production efficiency. This study shows that producing lactic acid with static bagasse fermentation offers benefits such as process simplification and lower energy requirements with a slightly better yield. This approach may be a viable strategy for efficiently converting biomass into lactic acid.

CONCLUSION

This study optimized fermentation conditions for lactic acid production from bagasse acid hydrolysate using *L. casei* B75 under static conditions. Optimal parameters, including a 15% inoculum concentration, 15 mg/mL substrate concentration, and a 72-hour fermentation time, were identified through Response Surface Methodology (RSM) - Box-Behnken Design (BBD). Under these parameters, lactic acid production reached 9.72 mg/mL with $Y_{(P/S)}$ 0.648 mg/mg, demonstrating the efficiency of *L. casei* B75 in converting agricultural residues into valuable biochemicals. The findings emphasize the potential of bagasse as an affordable and sustainable biomass source for lactic acid production while improving energy efficiency in static fermentation processes. Future research should focus on developing efficient purification techniques to enhance lactic acid recovery under acidic conditions. Purified L-(+)-lactic acid can then be utilized as an eco-friendly feedstock for producing poly(lactic acid) (PLA), a biodegradable and sustainable polymer.

ACKNOWLEDGMENTS

The DIPA Research Grant supported this study through the National Research Program of the National Research and Innovation Agency Republic of Indonesia in the Research Organization for Life Sciences and Environment year 2024 (1/III.5/HK/2024). The authors also acknowledge the facility's scientific and technical support provided by Advanced Characterization Laboratories Bandung, National Research and Innovation Agency Republic of Indonesia, through e-Layanan Sains BRIN.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

R.C. Nissa https://orcid.org/0000-0002-3202-3579
F.F. Soleha https://orcid.org/0009-0002-9559-1810
A. Junitasari https://orcid.org/0000-0003-3062-3658
M. Irmayanti https://orcid.org/0009-0002-2292-4266
H. Hidawati https://orcid.org/0000-0003-0783-692X
D.T.N. Adi https://orcid.org/0000-0002-7858-2704
Y. Nurhamiyah https://orcid.org/0000-0002-2353-2144
A.H.D. Abdullah https://orcid.org/0000-0002-0613-2405
S. Ishmayana https://orcid.org/0000-0002-9825-4425
D. Sumiarsa https://orcid.org/0000-0003-2483-3915

REFERENCES

1. W.R. Alves, T.A. Silva, A.Z. Filho, and L.P. Ramos, *Fermentation*, **9(9)**, 789(2023), https://doi.org/10.3390/fermentation9090789
2. A. Ahmad, F. Banat, and H. Taher, *Environmental Technology & Innovation*, **20**, 101138(2020), https://doi.org/10.1016/j.eti.2020.101138
3. Y.J. Liu, Y. Zhang, F. Chi, C. Chen , W. Wan, Y. Feng, X. Song, and Q. Cui, *Journal of Environmental Management*, **342**, 118281(2023), https://doi.org/10.1016/j.jenvman.2023.118281
4. H. Zhang, T. Dai, S. Huang, and J. Xie, *Fermentation*, **9(4)**, 371(2023), https://doi.org/10.3390/fermentation9040371
5. Y.Dragomir, *Frontiers in Chemistry*, **10,** (2022), https://doi.org/10.3389/fchem.2022.823005

6. W. Sriphochanart, and W. Skolpap, *Process Biochemistry*, **113**, 11(2022), https://doi.org/10.1016/j.procbio.2021.12.006
7. M.C. Groff, S.E. Noriega, R.M. Gil, N. Pantano, and G. Scaglia, *Fermentation*, **10(2)**, 101(2024), https://doi.org/10.3390/fermentation10020101
8. S. Haris, A.K. Eldin, M.M. Ayyash, B.V. Bruggen, M.M. Mohamed, and A.H. Al-Marzouqi, **31**, 103151(2023), https://doi.org/10.1016/j.eti.2023.103151
9. S. Costa, D. Summa, M. Radice, S. Vertuani, S. Manfredini, and E. Tamburini, *Frontiers in Bioengineering and Biotechnology*, **12**, (2024), https://doi.org/10.3389/fbioe.2024.1447278
10. P.M. de Oliveira, L.P. Santos, L.F. Coelho, P.M.A. Neto, D.C. Sass, and J. Contiero, *Fermentation*, **7(3)**, 151(2021), https://doi.org/10.3390/fermentation7030151
11. M. Malik, J. Bora, and V. Sharma, **43(11)**, (2019), https://doi.org/10.1111/jfpp.14214
12. J. Kim, Y.M. Kim, V.R. Lebaka, and Y.J. Wee, *Fermentation*, **8(11)**, 609(2022), https://doi.org/10.3390/fermentation8110609
13. J.R. Xavier, I. Nallamuthu, and O.P. Chauhan, *Sustainable Food Technology*, **2**, 741(2024), https://doi.org/10.1039/D3FB00213F
14. C. Anagnostopoulou, K.N. Kontogiannopoulos, M. Gaspari, M.S. Morlino, A.N. Assimopoulou, and P.G. Kougias, *Chemosphere*, **296**, 133871(2022), https://doi.org/10.1016/j.chemosphere.2022.133871
15. L. Jaramillo, D. Santos, E. Borges, D. Dias, and N. Pereira, *Annals of Microbiology*, **68(9)**, 547(2018), https://doi.org/10.1007/s13213-018-1362-y
16. A.A. Idowu, A.S. Enahoro, and A.T. Jolaade, *Bioprocess Engineering*, **7(1)**, (2023), https://doi.org/10.11648/j.be.20230701.11
17. A. Abdullah, I. Winaningsih, and A. Hadiyarto, *IOP Conference Series: Materials Science and Engineering*, **1053(1)**, 012033(2021), https://doi.org/10.1088/1757-899X/1053/1/012032
18. P. Laopaiboon, A. Thani, V. Leelavatcharamas, and L. Laopaiboon, *Bioresource Technology*, **101(3)**, 1036(2010), https://doi.org/10.1016/j.biortech.2009.08.091
19. G.L. Miller, *Analytical Chemistry*, **31**, 426(1959), https://doi.org/10.1021/ac60147a030
20. A. Hanifah, N. Putu, V. Primarista, S. Prasetyawan, A. Safitri, T. Adyati, and A. Srihadyastutie, *Advance Biological Science Research*, **22**, 586(2022), https://doi.org/10.2991/absr.k.220406.084
21. P.P. Krumova, S. Danova, N. Atanasova, and D. Yankov, **12(4),** 739(2024), https://doi.org/10.3390/microorganisms12040739
22. A. Thakur, P.S. Panesar, and M.S. Saini, *Waste and Biomass Valorization*, **10(5)**, 1119(2017), https://doi.org/10.1007/s12649-017-0129-1
23. B. Oonkhanond, W. Jonglertjunya, N. Srimarut, P. Bunpachart, S. Tantinukul, N. Nasongkla, and C. Sakdaronnarong, *Journal of Environmental Chemical Engineering*, **25(3)**, 2533(2017), https://doi.org/10.1016/j.jece.2017.05.004

[RJC-9129/2024]