第 49 卷 第 3 期
2022 年 3 月

湖南大学学报（自然科学版）
Journal of Hunan University (Natural Sciences)

Vol. 49 No. 3
March 2022

Open Access Article https://doi.org/10.55463/issn.1674-2974.49.3.15

Optimization of Acid-Catalyst Hydrolysis Process in Lactic Acid Production from Rice Husk by Using *Lactobacillus bacteria*

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Abstract: Lactic acid is an important component of manufacturing polylactic acid (PLA), which can be produced with high-lignocellulosic biomass, still few of them have utilized that. The production process starts with Alkaline Hydrogen Peroxide (AHP) pretreatment, acid-catalyzed (H_2SO_4) hydrolysis followed by post-hydrolysis, and fermentation using a mixture of *Lactobacillus brevis* + *Lactobacillus rhamnosus* and single *Lactobacillus delbrueckii bacteria.* The effectiveness of pretreatment results was analyzed by using Thermal Gravity Analysis (TGA), which resulted in the removal of lignin and cellulose are 32.60% and 43.18%, respectively. Lignin reduction results are supported by Scanning Electron Microscope (SEM) for morphology analysis; it is shown the surface of rice husk becomes rough and cracks after pretreatment. Cellulose reduction was analyzed by using X-Ray Diffraction (XRD) and shown the Crystallinity Index (CrI) decrease after chemical pretreatment. The optimum operating condition of hydrolysis was also studied using Face Centered Composite Design (FCCD) by Response Surface Methodology (RSM), and get the optimum conditions at a 4 M H_2SO_4 for 30.7 minutes 63.1 ℃. Followed by post-hydrolysis for 10 minutes at 121 ℃, it resulted in sugar concentration of 15.3056 g/L, which was analyzed by using dinitrosalicylic-acid (DNS) with Root Mean Square Error of 0.78009. The last process is fermentation which is carried out at 125 rpm 37 ℃, for 48 hours. Through High-Performance Liquid Chromatography (HPLC) analysis, the lactic acid concentration of a single bacterium and a mixture was 24.595 and 24.975%, respectively. Similar research has been previously carried out using palm waste raw materials with the enzymatic hydrolysis method without optimization of RSM. This study investigates the effect of AHP pretreatment in the lignin removal process, the effectiveness of acid-catalyzed hydrolysis in the optimized sugar reduction through FCCD, and compares lactic acid concentrations obtained from the fermentation process with *L. delbrueckii* and culture *L. rhamnosus* + *L. brevis*.

Keywords: acid-catalyst hydrolysis, alkaline hydrogen peroxide, lactic acid, *Lactobacillus bacteria*, response surface methodology.

乳酸菌优化稻壳生产乳酸的酸催化水解工艺

摘要：乳酸是制造聚乳酸的重要组成部分，可以用高木质纤维素生物质生产，但很少有人利用它。生产过程开始于碱性过氧化氢预处理、酸催化 (H2SO4) 水解，然后是后水解，以及使用短乳杆菌 + 鼠李糖乳杆菌和单一德氏乳杆菌的混合物进行发酵。采用热重分析法对预处理结果的有效性进行了分析，结果木质素和纤维素的去除率分别为 32.60%和 43.18%。扫描电子显微镜支持木质素还原结果进行形态分析；经预处理后，稻壳表面出现粗糙和裂纹。通过使用 X 射线衍射分析纤维素还原，并显示化学预处理后结晶度指数降低。还采用响应面法采用面心复合设计研究了水解的最佳操作条件，得到了在 4 M H2SO4 30.7 分钟 63.1 ℃下的最佳水解条件。随后在 121 ℃下水解 10 分钟，得到的糖浓度为 15.3056 g/L，用二硝基水杨酸分析，均方根误差为 0.78009。最后一个过程是发酵，在 125 转数 37 ℃下进行 48 小时。经高效液相色谱分析，单一细菌和混合物的乳酸浓度分别为 24.595 和 24.975%。类似的研究

Received: 21 December, 2021 / Revised: 09 January, 2022 / Accepted: 22 February, 2022 / Published: 28 March, 2022
Fund Project: contract number 913/PKS/ITS/2021, Technology/National Research and Innovation Agency of the Republic of Indonesia
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已经在没有优化响应面方法的情况下使用酶水解法使用棕榈废料原料进行。**本研究通**过面心复合设计研究了碱性过氧化氢预处理在木质素去除过程中的效果，**酸催化水解在**优化糖还原中的效果，**并将**发酵过程中获得的乳酸浓度与德氏乳杆菌和培养物进行了比较鼠李糖乳杆菌 + **短乳杆菌**。

关键词：**酸催化水解**, **碱性**过氧化氢, **乳酸**, **乳酸菌**, **响**应面法。

1. Introduction

One of the world's biggest unresolved problems is plastic waste, and Indonesia gets second ranks in the world's largest plastic producer, which reaches 64 million tons per year [1]. Synthetic plastic will degrade in tens or even hundreds of years. Carbon emission and dioxin compounds will also be produced and released if burned, which can cause health problems [2].

Polylactic acid (PLA) is a polymer composed of lactic acid with good thermal stability used in the food packaging industry [3]. PLA is made from lignocellulosic biomass, such as rice husk which has a cellulose content of 43.046%. An important in the manufacture of PLA is the production of lactic acid, which has a minimum concentration requirement of 85% to obtain plastic according to standards. The process of manufacturing lactic acid is started from pretreatment, hydrolysis, fermentation, and purification to get products that comply with industrial standards. The pretreatment process is important because rice husks contain 26.16% lignin, which can interfere with the concentration of the resulting product [4]. The pretreatment process is carried out by chemical pretreatment using Alkaline Hydrogen Peroxide (AHP). It is considered adequate with natrium hydroxide (NaOH), which functions to hydrolyze chlorolignin and hydrogen peroxide (H_2O_2) to oxidize the lignin structure without damaging the cellulose structure [5].

The following process is hydrolysis, which converts cellulose into reduced sugar through the acid catalysis method for efficiency. It is carried out at a medium temperature and relatively short time which can produce high reducing sugar yields of 62.5% at 90 ℃ [6]. The H_2SO_4 as a catalyst breaks cellulose into glucose and hemicellulose into xylose + arabinose which had C5 sugars, while galactose, mannose, and glucose had C6 sugar [7]. Meanwhile, in previous studies, the hydrolysis process used an enzymatic hydrolysis process from a mixture of *Aspergillus niger* and *Trichoderma reesei* bacteria, which produced reducing sugars with a yield of 33.9 % [8].

In the fermentation process [8] have also conducted research using co-culture bacteria *Lactobacillus rhamnosus*+ *Lactobacillus brevis* in the fermentation process, which only produces a yield of 6.83 % g/g with a lactic acid concentration of 33 %. Thus, it is necessary to develop the use of other bacteria, namely the type of *L. delbrueckii*, which is a homolactic bacteria and can convert reduced sugars into lactic acid without any by-products with a yield of 69-96.2 % [9].

Therefore, this study aims to investigate the effect of AHP pretreatment in the lignin removal process, the effectiveness of acid-catalyzed hydrolysis in the optimized sugar reduction through FCCD, and to determine and comparison of lactic acid concentrations obtained from the fermentation process using single *L. delbrueckii* and culture *L. rhamnosus* + *L. brevis* bacteria. By modifying the process in this study, it is expected to be used as the basis for further PLA production as industrial, biomedical, and food-grade standards.

2. Research Method

2.1. Pretreatment

Pretreatment is started with drying rice husks for 1 day at 60 ℃ then reducing it into 140 mesh. 30 g of rice husks is diluted with 570 ml of 2% NaOH solution and 31.6 ml of 5% H_2O_2 were stirring for 2 hours 30 ℃ [5]. It was neutralized using HCl, then filtered and dried at 60 ℃ for 48 hours. The dried solids were re-sized into 100 – 120 mesh and lignocellulosic content has been analyzed by using TGA [10]. TGA was carried out in a TAG 2400 Setaram thermobalance, which was coupled to a thermostarBalzers quadrupole mass spectrometer with the operation condition of ion source at 70 eV. Analysis processes were performed under argon flow (2 L h^{-1}) using a slow temperature increase (5 ℃ min^{-1}) and samples were heated from room temperature to 700 ℃, where they remained for 30 min [10].

SEM has also been used to observe the differences in sample morphology after the delignification processes. SEM equipment used was a JEOL 840 A. Samples were previously coated with a very thin layer of Au/Pd. The SEM has a magnification range from 50 to 50000 and a resolution of 100 nm [10].

CrI of the sample after delignification was observed by using XRD with specification TTR III X-ray diffractometer (Tokyo) [8]. X-ray diffractometer was set at 40 kV and 200 mA and Cu radiation (λ=1.54 Å) was scanned over diffraction angle (2θ°) of 5-50° with a step of 0.01° [11].

2.2. Hydrolysis

H_2SO_4 according to the variables and operating condition in Table 2 mixed with pretreatment results of 12.5:1 v/w and stirred at 200 rpm. The post-hydrolysis was carried out at 121 ℃, for 10 minutes. The solution was separated using a centrifuge at 4℃, 10,000 rpm, for 10 minutes then neutralized using 25 M NaOH. Reduction sugar concentration was analyzed by using Uv-vis Spectrophotometer with DNS solution as reagent [8, 11].

2.2.1. Hydrolysis Experimental Design and Optimization

The optimization of hydrolysis with FCCD via RSM was performed by Minitab 16 Statistical Software (Minitab Inc., ITS Surabaya, Indonesia). FCCD was performed with three independent variables, X_1 (H_2SO_4 concentration), X_2 (hydrolysis duration), X_3 (hydrolysis temperature), and all as shown in Table 1. Reduction of sugar concentration (Y1) was established as a response to determine the optimal operation condition of acid catalyst hydrolysis as shown in Table 2.

Table 1 Range and experiment level towards the independent variables of the acid catalyst hydrolysis process, 20 full factorials (α = +1)

Independent Variable	Range and Level		
	-1	0	+1
X_1 (H_2SO_4 Concentration, M)	2	3	4
X_2 (Hydrolysis duration, minutes)	15	30	45
X_3 (Hydrolysis temperature, °C)	40	65	90

Table 2 Range and experiment level towards the independent variables of the acid catalyst hydrolysis process, 20 full factorials (α = +1)

Run	Input Variables			Experiment Results
	X_1 Temperature (℃)	X_2 Time (minute)	X_3 H_2SO_4 Concentration (M)	Y_1 (Reducing Sugar Concentration, g/L)
1	90	15	4	13.8857
2	65	15	3	14.2635
3	90	15	2	14.8610
4	65	30	2	15.1753
5	65	45	3	13.2648
6	65	30	3	15.3251
7	65	30	3	14.8171
8	40	15	4	10.9418
9	90	30	3	13.8727
10	40	45	4	14.1341
11	65	30	3	14.6543
12	65	30	3	14.7389
13	65	30	3	14.4806
14	90	45	2	10.9530
15	65	30	3	14.9004
16	90	45	4	11.0480
17	65	30	4	15.6963
18	40	45	2	9.4378
19	40	30	3	13.4602
20	40	15	2	11.2024

2.3. Fermentation

The fermentation process is carried out using two types of bacteria, *L. delbrueckii* for single and mixture with *L. rhamnosus* + *L. brevis* at 125 rpm, 37 ℃ for 48 h under sterilization conditions, respectively. Samples have analyzed the decrease in reducing sugar concentration using DNS solution every 8 hours. The lactic acid concentration was also determined by using HPLC analysis at 48 hours samples fermentation [8]. HPLC equipped with AUV detector SPD-20A, LC-20ADpump,oven column Grace smart RP 18 5µ (length 15 cm and diameter 4,6 mm). Chromatographic separation was carried out at the column oven temperature 40℃ ± 2℃ with a flow rate of 1.0 mL/min of isocratic elution. The sample was injected at 20 µL and the wavelength was set at 200 nm [12].

3. Results and Discussions

3.1. Pretreatment Process using Alkaline Hydrogen Peroxide (AHP)

3.1.1. Effect of AHP Pretreatment on the Rice Husk Lignocellulosic Composition using Thermo Gravimetric Analysis (TGA)

Lignocellulose analysis is shown in Table 2. The lignin content has been reduced after the previous treatment. It happens because AHP can increase the saponification reaction to interrupt the intermolecular foreign bonds between hemicellulose, lignin, and xylan [10]. The breaking of these molecular connections will reduce crystallinity and polymerization to break the

crossed bonds in Lignin. H_2O_2 can be decomposed into hydroxyl radicals and superoxide anions with heat, thus promoting the rupture of the lignin structure.

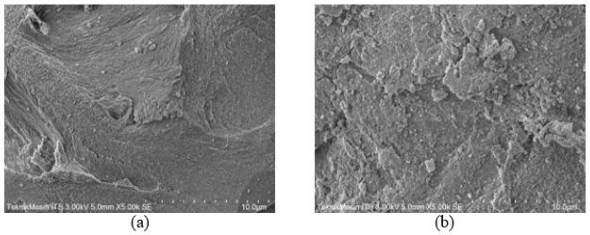
Table 3 shows the percentage of delignification is relatively small, 32.60% because based on the following research [13], rice husks contain high amounts of sodium silicate, which can act as a stabilizer. It can inhibit H_2O_2 decomposition from carrying out the lignin degradation process. Table 2 also showed that the cellulose content after pretreatment decreased, and the hemicellulose content increased, the cause of the decreasing cellulose according to research [14], was due to the regular open structure of cellulose and its molecules were freely dispersed in the solvent (NaOH). With its structure being freely dispersed in the solvent, it is expected that the solvent will carry away cellulose during the filtering process [14].

Cellulose is also oxidized by NaOH and accelerated by H_2O_2 as a catalyst, thus forming a more complex compound, namely hemicellulose. While the cause of increasing hemicellulose, according to research [15], is due to adding NaOH solution, which results in a slower rate of carbohydrate solubility than oxidation or reactions with other compounds, so carbohydrate content in the medium is reduced. When the carbohydrate solubility rate slows down, an oxidation reaction is produced to form more complex compounds and finally accumulates until the hemicellulose content increases. All the case is possible to happen because due to the length of time used, it would destroy hemicellulose and cellulose. After all, alkali hydrolyzes uronic acid and acetic acid esters in the lignocellulosic matrix [16].

Table 3 Lignocellulosic Content of Rice Husk before and after pretreatment by Using TGA

Component	Before Pretreatment		After Pretreatment		Mass Removal (%)
	Content (%)	Mass (gr)	Content (%)	Mass (gr)	
Hemicellulose	2.511	0.753	4.349	1.145	+52.02
Cellulose	43.046	12.915	27.850	7.338	-43.18
Lignin	26.166	7.805	19.967	5.260	-32.60
Others	28.275	8.483	47.832	12.602	+48.55
Total	100	30.004	100	26.34	-12.18

3.1.2 Effect of AHP Pretreatment on The Morphological Changes of Rice Husk using Scanning Electron Microscope (SEM)



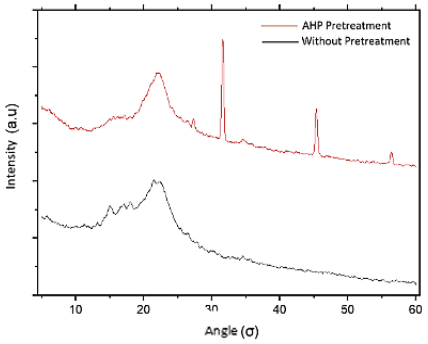
(a) (b)

Fig. 1 SEM results from Rice Husk with (a) without pretreatment, (b) Alkali Hydrogen Peroxide (AHP) pretreatment

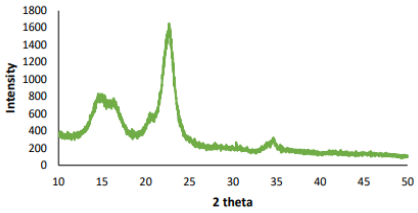
Rice husk without pretreatment produces a close-packed surface and is covered with a continuous and smooth surface as shown in Fig. 1 (a). Compared with rice husk after pretreatment, the surface has a rough texture, the surface is cracked, and some of the outer surfaces are missing as shown in Fig. 1 (b). It indicated that pretreatment with AHP was damaged the pith cells and removed the external fibers. It has indicated that AHP pretreatment has been damaged marrow cells and eliminated external fibers. This External fiber has an essential function in increasing the surface area of the material. If it is damaged, it will make the inner surface of cellulose more open and allow for easy access by the enzyme. This result looks the same as research of [14]; the surface of cacao waste was rough and cracked after getting an AHP chemical pretreatment.

3.1.3. Effect of AHP Pretreatment on The Cellulose CristalinityIndec (CrI) of Rice Husk using X-Ray Diffraction (XRD)

XRD results as shown in Fig. 2 (a), shows the total crystallinity of all components contained in rice husk (cellulose, hemicellulose, lignin, and others). The XRD graph looks like the XRD results of pure cellulose (microcrystalline cellulose) which was reported by research [17] as shown in Fig. 2 (b), this indicates that cellulose is the main component in the rice husk.



(a)



(b)

Fig. 2 (a) Rice Husk XRD Analysis Result Before and After Pretreatment; (b) XRD Analysis Graph of Pure Cellulose (Micro Crystalline Cellulose) [15]

CrI value before and after pretreatment is obtained in Table 4, below.

Table 4 Lignocellulosic Content of Rice Husk before and after pretreatment

Sample	Crystallinity Index (CrI)
Before Pretreatment	60,58%
After Pretreatment	54,48%

Table 4 shows that the CrI value of rice husks decreased after pretreatment by less than 10%. The decrease happened because cellulose dissolved during the pretreatment process. The occurrence of cellulose removal also evidences this at the time after pretreatment of -43.18%. The effect of decreasing the CrI value causes the level of degradability of rice husk to increase. It is due to the reduced crystals in rice husks after pretreatment.

Similarly, in rice straw research, the results also showed a decrease in the CrI value of 51 – 46% [10]. In addition, the slight difference in cellulose changes after pretreatment was also supported by the research of [18], which showed the XRD pattern of cellulose crystals in rice straw before and after pretreatment did not change. It indicated that pretreatment with NaOH only reached the crystal surface and amorphous and swelling occurs between the crystal areas.

3.2. Optimization of Acid Catalyst Hydrolysis using Face Centered Composite Design (FCCD) by Response Surface Methodology

This stage is desired to produce a high concentration of reducing sugar with the optimum operating conditions. The result of hydrolysis optimization was shown in Table 2.

In a previous study, regarding the hydrolysis of lignocellulose using H_2SO_4 at low temperatures (35; 65; and 97.5 ℃) and concentrations of 20 – 70% wt for 4 hours. It gave results of low concentration reducing sugar with 4.2 g/L for maximum concentration at 65 ℃, 4.5% wt H_2SO_4 [19].

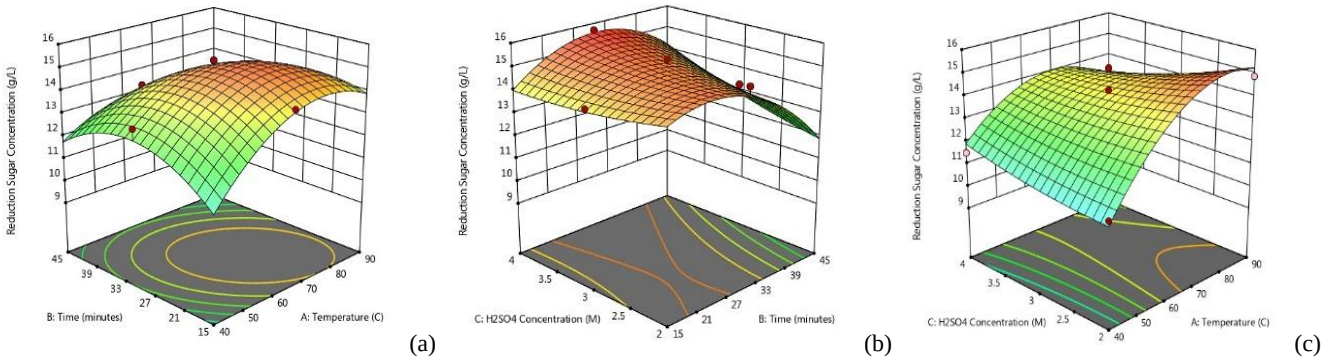
These results study as presented in Table 2 is used almost the same concentration of sulfuric acid with a much shorter time than research by [19], but the maximum reducing sugar concentrations produced were much different, which are 15.6963 and 4.2 g/L. It is because of the different processes of using saturated steam at 121 ℃ for 10 minutes. The use of saturated steam made the crystalline structure of cellulose can be degraded, so the contact area between cellulose, water, and the H^+ of H_2SO_4 becomes larger, resulting in better hydrolysis performance. The advantage is reducing the concentration of H_2SO_4 used so it is not made too corrosive for the operating unit. In addition, using low temperature and pressure is expected to suppress the formation of furfural and other by-products such as 5-hydroxymethylfurfural (5-HMF), acetic acid, formic acid, and phenolic compounds due to further degradation reactions of reducing sugars [20]. This by-product formed can be an inhibitor of the growth of microorganisms in the fermentation process [21].

Response Surface Method analysis was carried out using statistical software, obtained the Analysis of Variance (ANOVA) results are shown in Fig. 4 and Response Surface graphs as shown in Fig. 6. Based on the ANOVA results as shown in Fig. 4, it was found the factors that most influence the concentration of reducing sugar resulting from hydrolysis are variations in temperature and time. It can be seen from the 'p-value <0.05 because the smaller p-value indicates that the parameter affects the results of reducing sugar concentration. P-value <0.05 is also considered as a parameter that affects the results of reducing sugar concentrations [22].

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	57.5109	6.3901	28.82	0.000
Linear	3	7.6339	2.5446	11.48	0.001
Temperature (C)	1	1.8976	1.8976	8.56	0.015
Time (minutes)	1	4.0414	4.0414	18.23	0.002
H2SO4 Concentration (M)	1	1.6949	1.6949	7.65	0.020
Square	3	33.9951	11.3317	51.12	0.000
Temperature (C)*Temperature (C)	1	6.8341	6.8341	30.83	0.000
Time (minutes)*Time (minutes)	1	6.0132	6.0132	27.12	0.000
H2SO4 Concentration (M)*H2SO4 Concentration (M)	1	0.1024	0.1024	0.46	0.512
2-Way Interaction	3	15.8819	5.2940	23.88	0.000
Temperature (C)*Time (minutes)	1	6.2755	6.2755	28.31	0.000
Temperature (C)*H2SO4 Concentration (M)	1	5.1262	5.1262	23.12	0.001
Time (minutes)*H2SO4 Concentration (M)	1	4.4801	4.4801	20.21	0.001
Error	10	2.2169	0.2217		
Lack-of-Fit	5	1.8061	0.3612	4.40	0.065
Pure Error	5	0.4108	0.0822		
Total	19	59.7277			

Fig. 3 Analysis of Variance (ANOVA) of reducing sugar concentration



(a) (b) (c)

Fig. 4 Response Surface graph of reducing sugar concentration as a function of (a) time and temperature, (b) H_2SO_4 concentration and time, and (c) H_2SO_4 concentration and temperature

From this p-value, as shown in Fig. 3, it is found that temperature, time, and concentration of H_2SO_4 show linear interactions, they are 0.015, 0.002, and 0.020, for temperature, time, and H_2SO_4 concentration parameters, respectively. Quadratic interactions are shown only by temperature and time, is shown with 0.000, 0.000, and 0.512 for temperature, time, and H_2SO_4 concentration parameters, respectively, while

bidirectional interactions are shown by all combinations of parameters. It is supported with the response surface graph in Fig. 4, which is shown that the best surface formed is known by temperature and time variables. This matches research by [6, 23]. Based on the research of [23], it was found that the time parameters and H_2SO_4 concentration showed a linear interaction, the quadratic interaction was only indicated by the time parameter, the H_2SO_4 concentration parameter did not show a quadratic parameter because it had a p-value of 0.1678. Meanwhile, based on research of [6], linear interactions are also shown by the parameters of temperature, time, and concentration of H_2SO_4. It results also supported by the response surface graph as shown in Fig. 4, which is the best surface graph is known in parameter variables of time and temperature.

From the calculations in Minitab based on the result as shown in Table 3, the empirical model generated from processing data on reducing sugar concentration in the acid hydrolysis process as a function of temperature, time, and H_2SO_4 concentration at the optimum condition is shown as Equation 1.

$$Y_1 = -7.3 + 0.5122\ x\ X_1 + 0.3558\ x\ X_2 - 0.16\ x\ X_3 - 0.002522\ x\ {X_1}^2 - 0.00657\ x\ {X_2}^2 + 0.193\ x\ {X_3}^2 - 0.002362\ x\ X_1\ x\ X_2 - 0.03202\ x\ X_1\ x\ X_3 + 0.0499\ x\ X_2\ x\ X_3 \quad (1)$$

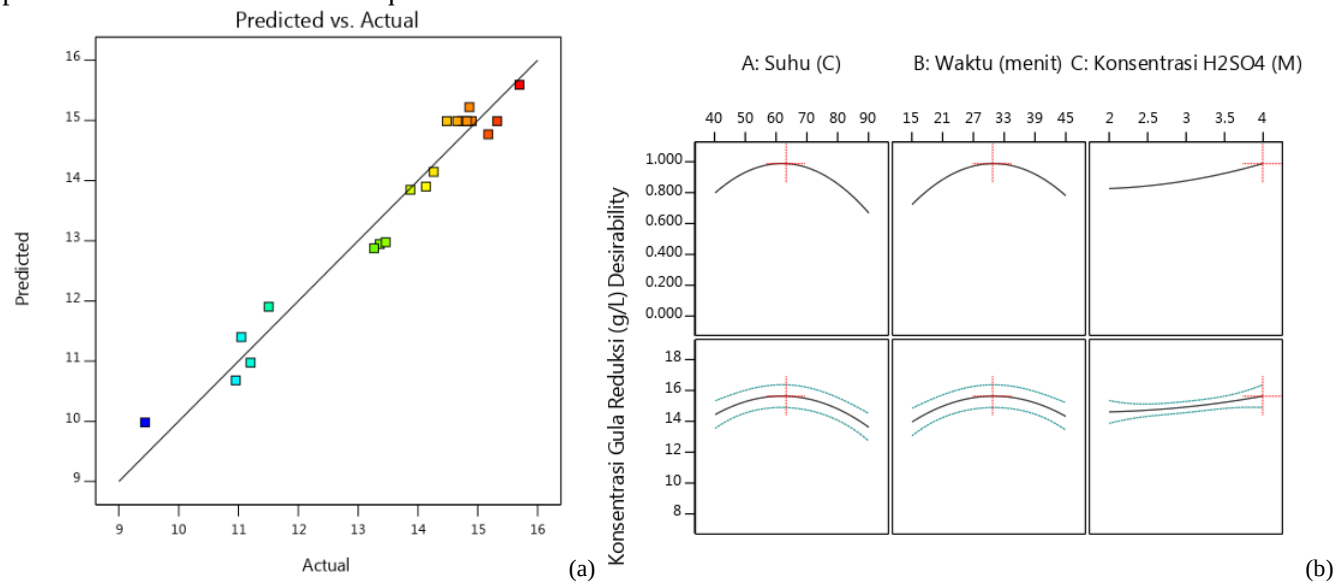
Based on Equation 1, R^2 was obtained of 96.29% as shown in Fig. 5. The high value of R^2 which is close to 100%, indicated that analyzed data through RSM is closed to the true value (experimental data is appropriate).

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.470837	96.29%	92.95%	53.60%

Fig. 5 Summary Model of R^2 Value

This is also supported by the normal probability graph in Fig. 6 (a). The graph shows that experimental data are close to the existing straight line, so it can be said that the experimental data obtained are following the existing theory.



(a) (b)

Fig. 6 (a) Comparative data of reducing sugar concentration from experimental with theoretical; (b) Desirability profile for parameters Temperature (℃), Time (minutes) and H_2SO_4 Concentration (M)

The optimization results of the three experimental data parameters are determined from the desirability plot as shown in Fig. 6 (b). From the optimization results, the optimum point for the hydrolysis process was obtained at 63.1 ℃, 30.7 minutes, and using a 4 M H_2SO_4. It is produced a lactic acid concentration of 15.617 g/L. The optimum operating conditions results are obtained from the Minitab software based on the desirability value which has a value range of 0 – 1. In the optimization, it is desired that the maximum reducing sugar concentration results so that the desirability value of '1' indicates a condition where the concentration is maximum while it is value '0' if the concentration is the smallest in that condition.

Based on the optimization results as shown in Fig. 8, the concentration of H_2SO_4 4 M for 30.7, 63.5 ℃ became the optimum condition with a desirability value of 0.984. As a comparison, the same study conducted by Adeoye et al in 2019 using H_2SO_4 with a concentration of 1 – 3.5 M at a temperature of 60 – 90 ℃ for 0 – 140 minutes resulted in optimum hydrolysis conditions using H_2SO_4 with a concentration of 1 M for 60 minutes at a temperature of 60 ℃. If the hydrolysis reaction time is shortened, the sulfuric acid concentration will increase [20], following the results of the study where the hydrolysis time was shorter, namely 30.7 minutes with a higher sulfuric acid concentration of 4 M.

In the validation process of the optimization results, the data is tested again between the theoretical data from the optimization results and the experimental data, then the results are obtained as shown in Table 5.

Table 5 Optimization result data validation

Temp (^{0}C)	Time (minutes)	[H_2SO_4] (M)	Reducing Sugar Concentration (g/L)				RMSe
			I	II	Avarage	Teoritic	
63.1	30.7	4	14.3286	16.2825	15.3506	15.617	0.78009

The RMS error result is 0.78009, where this is less than 5% which is the error limit for experiment repetition, thus indicating that the experimental results at the optimum points carried out are correct.

3.3. Fermentation of Reducing Sugar using Single Bacteria L. delbrueckii and Mixture Bacteria L. rhamnosus + L. brevis

The fermentation process is done at 37 ℃, pH 6, for 48 h. A study conducted by [24], stated that the operating temperature of 37 °C is the optimum temperature for lactic acid production using *Lactobacillus* culture. During the fermentation process, DNS analysis was carried out to test reducing sugar by taking samples every 8 hours as shown in Fig. 8. Single bacteria *L. delbrueckii* decreased by 175 g/L while the mixture of *L. rhamnosus + L. brevis* is 171 g/L. It indicates that the activity of microorganisms forms a product in the form of lactic acid. Based on the study results, the most negligible reduction in reducing sugar was found in using a mixture of bacterium *L. rhamnosus*+ *L delbrueckii*. Following the research of [25], using a mixture of microorganisms can convert reducing sugars from the substrate more efficiently when compared to using a single microorganism.

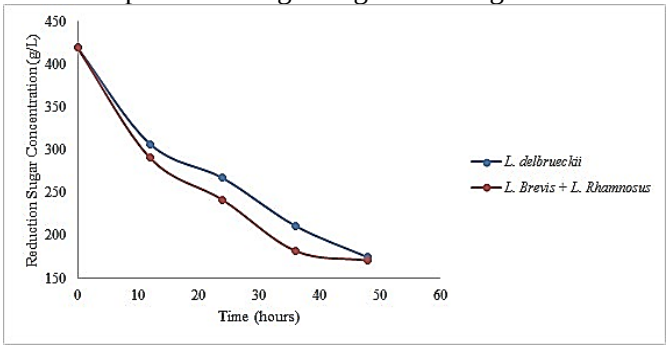


Fig. 8 Reducing Sugar Every 8 hours

This result is supported by an Analysis of lactic acid concentration using HPLC as shown in Table 6. The best results are the mixed culture of *L. rhamnosus + L. brevis* with a lactic acid concentration of 24.975%. It is in line with research conducted by [25], that the production of lactic acid from corn cobs using mixed culture *L. rhamnosus + L. brevis* and comparing it with single culture obtained the highest concentration using mixed culture *L. rhamnosus*+ *L. brevis*.

Table 6 Optimization result data validation

Bacteria Type	Acid Lactic Concentration (%)
L. delbrueckii	24.595
L. rhamnosus + L. brevis	24.975

4. Conclusions

Lactic acid synthesis from rice husk waste was carried out through 3 main processes, namely AHP pretreatment at a temperature of 30 ℃ for 2 hours, resulting in a delignification of 32.99%. The hydrolysis process using the optimization results of RSM is H_2SO_4 4 M for 30.7 minutes, 63.5 ℃, followed by post hydrolysis at a temperature of 121 ℃ for 10 minutes. It produces a reducing sugar of 15.3056 g/L with an RMS error of -1.856% of the theoretical concentration. The fermentation process uses a mixture of *L. brevis + L. rhamnosus* bacteria at a speed of 125 rpm at 37 ℃ for 48 hours and produces 24.975% and *L. delbrueckii* had 24.595% lactic acid. In previous research, similar research has been carried out using palm waste raw materials with the enzymatic hydrolysis method without optimization of RSM. This study aims to investigate the effect of AHP pretreatment in the lignin removal process, the effectiveness of acid-catalyzed hydrolysis in the optimized sugar reduction through FCCD, and to determine and comparison of lactic acid concentrations obtained from the fermentation process using single *L. delbrueckii* and culture *L. rhamnosus + L. brevis* bacteria. In future research, the authors hope that research can be developed in the pilot project plan, so this research is more applicable.

4.1. Credentials

The author would like to thank the Ministry of Research and Technology/National Research and Innovation Agency of the Republic of Indonesia for the financial support of this research with derivative contract number 913/PKS/ITS/2021. The authors also thank the Directorate of Research and Community Service of Institut Teknologi Sepuluh Nopember (DRPM-ITS) and for the contribution of Prof. Ir. Ali Altway, M.Sc., so this research can be completed properly.

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