



HAL
open science

Statistical Optimization of Dilute Acid Hydrolysis of Wood Sawdust for Lactic Acid Production

Aicha Nancib, Naàssa Diboune, Nabil Nancib, Joseph Boudrant

► **To cite this version:**

Aicha Nancib, Naàssa Diboune, Nabil Nancib, Joseph Boudrant. Statistical Optimization of Dilute Acid Hydrolysis of Wood Sawdust for Lactic Acid Production. *Journal of Applied Biotechnology & Bioengineering*, 2017, 4 (1), 10.15406/jabb.2017.04.00093 . hal-01865707

HAL Id: hal-01865707

<https://hal.science/hal-01865707>

Submitted on 21 Apr 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Statistical optimization of dilute acid hydrolysis of wood sawdust for lactic acid production

Abstract

Sawdust wood (SW) is a promising lignocellulosic biomass for lactic acid production due to its abundant availability, low cost and high carbohydrates content that can be converted to fermentable sugars. The dilute acid hydrolysis of SW was investigated in this study and *Lactococcus lactis* was used to determine the feasibility of utilizing WS as feedstock for the production of lactic acid. Central composite design (CCD) was employed to optimize sulfuric acid, substrate loading and hydrolysis time for maximum sugar recovery. The results obtained were interpreted by analysis of variance and response surface methodology. The optimal acid hydrolysis conditions were as follows: sulfuric acid concentration of 8% (v/v) with substrate loading of 16% (w/v) and hydrolysis time of 2h. Under the optimal conditions, a glucose concentration of 8.78g/L was achieved. A regression model was generated according to the experimental data. Upon statistical analysis, coefficient of determination (R^2) obtained was 0.96. *Lactococcus lactis* was found to be a potent lactic acid bacteria (LAB) strain for the production of lactic acid utilizing acid hydrolysate of WS and yeast extract as carbon and nitrogen sources, respectively. The maximum lactic acid concentration of 9.87g/L was obtained with a yield of 1.1g/g.

Keywords: wood sawdust, acid hydrolysis, lignocellulosic biomass, central composite design, fermentation, lactic acid

Volume 4 Issue 1 - 2017

Aicha Nancib,¹ Naassa Diboune,¹ Nabil NanciS,¹ Joseph Boudrant²

¹Department of Applied Microbiology, Ferhat Abbas University, Algeria

²Laboratory Reactions and Process Engineering (LRPE), University of Lorraine, France

Correspondence: Aicha Nancib, Laboratory of Applied Microbiology, Ferhat Abbas University, 19000 Setif, Algeria, Fax 00 213 031 81 90 89, Tel 00-213-798-870-283, Email nancibaicha@yahoo.fr

Received: April 28, 2017 | **Published:** October 16, 2017

Abbreviations: SW, sawdust wood; CCD, central composite design; LAB, lactic acid bacteria; L-LDH, L-lactate dehydrogenase; RSM, response surface methodology; 3D, 3-dimensional; MSW, meranti wood sawdust; OFI, *Opuntia ficus indica*

Introduction

Lactic acid has a wide application in food, pharmaceutical, leather, cosmetic applications, textile, and polylactic acid industries.¹⁻³ Lactic acid can be produced either by fermentation or by chemical synthesis. But the biotechnological fermentation process has received significant importance due to environmental concerns, use of renewable resources instead of petrochemicals, low production temperature, low energy requirements, and high purity. Mammalian cells only contain L-lactate dehydrogenase (L-LDH), so L(+) form of lactic acid is used for food and drug industry, because the human body is only adapted to assimilate this form by the action of the enzyme L-LDH whereas D-lactic acid is often harmful to human metabolism, his accumulation in the blood can cause acidosis.^{4,5} Lactic acid can be synthesized by microbial fermentation of the various raw materials such as molasses,⁶ whey,⁷ and date juice.⁸ As in any industrial organic acid or solvent fermentation, the main economic factors are the choice and cost of the substrate and the cost of recovery in downstream processing. Feedstock that would reduce the processing cost would greatly enhance the competitiveness of fermentative lactate production. Many efforts have been made to develop biotechnologies for lactate generation from such a cheap, abundant and renewable substrate. Recently, fermentation of non-food biomass has also gained considerable attention due to the forthcoming scarcity of fossil fuels and the increased lack of food and feed supplies over the world. Biomass of lignocelluloses is a low-cost and extensively available renewable carbon source as an alternative to the conventional feed stocks.⁹⁻¹¹ Lignocellulosic biomass is primarily

composed of cellulose, hemicellulose, and lignin.^{12,13} A cellulosic feedstock (such as wood) has been widely used to replace refined sugar, and been utilized for the production of ethanol,^{14,15} single cell protein,¹⁶ xylitol¹⁷ and organic acid such as lactic acid.¹⁸

The conversion of wood to lactic acid is more challenging due to the complex structure of the lignocellulose. The three polymers of Lignocellulosic biomass are arranged to form a highly recalcitrant structure,¹⁹ hindering the availability of carbohydrates for fermentation processes, representing a high barrier for the bioconversion of lignocellulosic material. In fact it is necessary to pretreat the wood to alter its structural and chemical composition to facilitate hydrolysis of carbohydrates to fermentable sugars.^{20,21} Pretreatment uses various techniques, including ammonia fiber explosion, chemical treatment, biological treatment, and steam explosion, to alter the structure of cellulosic biomass to make cellulose more accessible. Conversion of wood lignocellulosic biomass to glucose and other monomeric sugars can be achieved by acids or enzymes hydrolysis.²²⁻²⁵ Due to its low cost, pretreatment of lignocellulosic biomass through sulfuric acid is a conventional method. As the main component of lignocelluloses materials, cellulose is a biopolymer consisting of many glucose units connected through β -1,4-glycosidic bonds. The breakage of the β -1,4-glycosidic bonds by acids leads to the hydrolysis of cellulose polymers, resulting in the sugar molecule glucose or oligosaccharides. While depolymerization of hemicelluloses yields a mixture of different sugars that may contain xylose, glucose, mannose, arabinose, galactose and traces of other sugars, depending on the kind of the wood used. The hydrolysis reactions using dilute acid are very complex, mainly because the substrate is in a solid phase and the catalyst in a liquid phase. The reaction rate of hydrolysis depends on a number of variables, such as: temperature, acid concentration, time, substrate concentration and substrate composition.²⁶ Few studies have reported

the dilute-acid hydrolysis of lignocellulosic biomass from wood and the use of microorganism with ability to produce lactic acid in these hydrolysates.^{18,27–29}

Optimization of hydrolysis conditions is necessary for improving the efficiency of biomass conversion processes. Rafiqul et al.³⁰ pointed out that xylose release via acid bioconversion of *Meranti* wood sawdust depended on a number of process factors like temperature, H₂SO₄ concentration, and residence time. The present study was undertaken to evaluate the ability of a strain of *Lactococcus lactis* to grow on wood sawdust hydrolysate, prepared by dilute acid hydrolysis, for lactic acid production. Response surface methodology (RSM) based on central composite design (CCD) was applied to identify the optimum hydrolysis conditions for maximum sugar production. A mathematical correlation between acid concentration, substrate loading, and hydrolysis time was developed to obtain a maximum fermentable sugar concentration. The batch fermentative mode was studied to evaluate the feasibility of utilizing hydrolysate of sawdust as feedstock for the production of lactic acid.

Materials and methods

Substrate

The sawdust of different wood species such as pine, beech, spruce was provided from the joinery (woodworking Setif/Algeria). The sawdust was dried at 60°C for 72h. The dried sawdust was milled to increase its surface area and make the cellulose readily available for hydrolysis. It was then stored at room temperature for subsequent use.

Dilute acid hydrolysis

In this step, pretreated sawdust was hydrolysed at 92°C with dilute sulfuric acid (Sigma-Aldrich, Deisenhofen, Germany). The sulfuric acid, substrate loading and the hydrolysis time were varied according to the experimental design. After acid hydrolysis, the solid residue was separated by centrifugation and the pH of the resulting supernatant was adjusted to 10 using 2N Ca(OH)₂ (Sigma-Aldrich, Deisenhofen, Germany), filtering, acidifying to pH 5.5, adding sodium sulfite

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3 \dots (2)$$

The fitness of the polynomial model equation to the response was evaluated by the coefficient of R² as well as the lack of fit, using F-test.

Table 1 Experimental range and levels of variables

Variables	Codes	Coded levels				
		-α	-1	0	1	α
H ₂ SO ₄ % (v/v)	X ₁	4.63	6	8	10	11.36
Substrate loading % (w/v)	X ₂	1.59	5	10	15	18.41
Hydrolysis time (h)	X ₃	0.63	2	4	6	7.36

α (axial distance)=(N)^{1/4}, Where N_i is the number of experiments of the factorial design. In this case, 1.682.

Media and growth conditions

Lactococcus lactis subsp. *lactis* (*Lactococcus lactis*) was used. Stock cultures were stored in Elliker medium with 20% (v/v) glycerol at -20°C. The inoculum was prepared by transferring glycerol stock culture to an Erlenmeyer flask containing 100ml of Elliker medium³¹ for pre-culture. The flask was subsequently incubated at 33.5°C for 12h. Then, the culture containing the production medium (wood

(Merck, Darmstadt, Germany) (1g/L). The resulting precipitate was centrifuged off and the pH was adjusted to 6. All experiment runs were performed in duplicate

Design of experiment

A three variable central composite design for response surface methodology was used to study the combined effect of sulfuric acid concentration (4.63-11.36%), substrate loading (1.59-18.41%) and hydrolysis time (0.63-7.36h) on glucose concentration over five level combinations (-α, -1, 0, +1, +α) shown in Table 1. Based on CCD, the experimental runs comprises of 20 trials (8 factorial points, 6 axial points and 6 central points). The coded values for independent variable were -1 (lowest level), 0 (middle level) and +1 (highest level). The factors (-α, +α) that ran along the axes drawn from the middle of the cube through the center of each face cube is coded as -1.682 and +1.682, respectively. The complete experimental design and results consisting of coded levels, actual variables, and responses (experimental and predicted values) are given in Table 2. To determine if there exist a relationship between the independent variables (sulfuric acid concentration, substrate loading and hydrolysis time) and the dependent variable (glucose concentration), the data collected were subjected to regression analysis using response surface regression procedure of MINITAB 16 (Minitab Inc, State College, PA). Second-order polynomial Eq (1) including main effects and interaction effects of each variable was used to calculate the predicted response:

$$Y_i = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i=1}^k \beta_{ij} x_i x_j \dots (1)$$

Where Y_i is the predicted response used to relate to the independent variable, x_i and x_j are the variables, k is the number of studied factors; while β₀ is the constant coefficient and β_i, β_{ij} and β_{ii} the coefficient of linear, interaction and square terms respectively. Multivariate regression analysis with model equation (1) was carried out on the data to yield equation (2) which was used to optimize the product responses.

sawdust hydrolysate) was inoculated. A 10% (v/v) inoculum was used in the fermentation. The pH of the final culture medium was adjusted to 6. The agitation speed was controlled at 200rpm. Flask cultures were conducted in 250ml Erlenmeyer flasks containing 100ml of production medium. Wood sawdust hydrolysate was used essentially as the carbon source in the fermentation medium. Table 3 summarizes the characteristics of wood sawdust hydrolysate used in fermentation experiments. The medium was sterilized at 121°C for 20min. After cooling, the hydrolysate was supplemented with extract yeast, 5g/L; KH₂PO₄ (Sigma-Aldrich, Deisenhofen, Germany), 0.5g/L; K₂HPO₄ (Sigma-Aldrich, Deisenhofen, Germany) 0.5g/L; MnSO₄·H₂O (Merck, Darmstadt, Germany) 0.07g/L; MgSO₄·7H₂O (Merck, Darmstadt, Germany) 0.5g/L; CH₃COONa·3H₂O (Sigma-Aldrich, Deisenhofen, Germany) 6g/L. The solutions of nutrients were sterilized separately.

Analytical methods

The concentration of lactic acid was measured based on colorimetric determination by Taylor method.³² Glucose was measured using an enzymatic kit (Glucose PAP SL[®] *Elitech*). The total reducing sugars content of the final hydrolysate was determined by the colorimetric

method using the UV-Vis spectrophotometer, (Spectronic Genesis 20) at 540nm using 3, 5-dinitrosalicylic acid (DNS reagent) with glucose as standard.³³ Total phenolic content in sawdust extracts was estimated by the Folin-Ciocalteu Method.³⁴

Results and discussion

The results of CDD experiments for studying the effects of three independent variables: sulfuric acid concentration, substrate loading and hydrolysis time are presented in the Table 2 along with the mean

predicted and observed response. The coefficient of determination (R^2) of the model was 0.96, this value means that 96% of the variability was explained by the model and indicated that the model adequately represented the real relationship between the variables under consideration. The adjusted determination coefficient ($R^2_{adj}=92.40\%$) was also able to confirm the significance of the model.

The mathematical model which represents a second-order polynomial is given by the following equation:

$$Y\left(\frac{\text{glucose g}}{\text{l}}\right) = -5.07 + 1.86X_1 - 0.07X_2 + 0.25X_3 - 0.12X_1^2 + 0.02X_2^2 + 0.08X_3^2 + 0.03X_1X_2 - 0.05X_1X_3 - 0.05X_2X_3 \quad (3)$$

Where Y is the glucose concentration and positive sign in front of the terms indicates synergetic effect, whereas negative sign indicates antagonistic effect.

Table 2 Experimental and predicted values of glucose concentration recorded in the experimental set up of RSM

Run	Coded factor		Actual factor			Glucose (g/L)		
	X ₁	X ₂	X ₃	X ₁ (%)	X ₂ (%)	X ₃ (h)	Observed response	Predicted response
1	-1	-1	-1	6	5	2	2.84	2.72
2	1	-1	-1	10	5	2	2.94	2.91
3	-1	1	-1	6	15	2	7.28	7.01
4	1	1	-1	10	15	2	8.65	8.23
5	-1	-1	1	6	5	6	3.83	4.18
6	1	-1	1	10	5	6	3.42	3.63
7	-1	1	1	6	15	6	6.71	6.68
8	1	1	1	10	15	6	7.1	7.16
9	0	0	0	8	10	0	5.46	4.93
10	0	0	0	8	10	0	5.55	4.93
11	0	0	0	8	10	0	4.29	4.93
12	0	0	0	8	10	0	4.33	4.93
13	0	0	0	8	10	0	5.32	4.93
14	0	0	0	8	10	0	4.65	4.93
15	-α	0	0	4.63	10	0	3.33	3.34
16	+α	0	0	11.36	10	0	3.83	3.9
17	0	-α	0	8	1.59	0	3.44	3.16
18	0	+α	0	8	18.41	0	9.37	9.73
19	0	0	-α	8	10	0.63	5.18	5.65
20	0	0	+α	8	10	7.36	6.36	5.98
21a	0	1.2	-1	8	16	2	8.78	8.39

^a, Optimum conditions of acid hydrolysis.

Table 3 Characteristics of wood sawdust hydrolysate used in fermentation experiments

Characteristics	TPC ^a mg eq. GA/g	Glucose (g/L)	Reducing sugars (g/L)	Moisture (%)	pH (units)
Wood sawdust hydrolysate	50.8	8.7	34	7.5	6

TPC^a, total phenolic content mg eq. gallic acid/g extract.

Pareto chart, which is very useful in design of experiments, is used in this work, to make it much easier to visualize the main and interactions effects of the factors to the response variable, that is, glucose concentration (Figure 1). The model identified that within the studied range of experiments the sulfuric acid (X_1) has the highest positive impact on the hydrolysis process followed by the hydrolysis time (X_3), quadratic effect of hydrolysis time (X_3^2), interactive effect of sulfuric acid and substrate loading (X_1X_2), quadratic effect of substrate loading (X_2^2) in a decreasing order ($X_1 > X_3 > X_3^2 > X_1X_2 > X_2^2$). While the sulfuric acid has a slight positive impact on the glucose concentration, its quadratic effect has the highest negative impact on the hydrolysis process, followed by the negative effect of substrate loading (X_2), interactive effect of sulfuric acid and hydrolysis time (X_1X_3), interactive effect of substrate loading and time (X_2X_3) in a decreasing order.

The analysis of variance (ANOVA) for the response surface full quadratic model is given in Table 4. The model was statistically significant as seen from the P -value ($P=0.000$) and Fisher's F -test ($F=26.67$). The lack of fit p value of 0.698 indicates that the model did not show lack of fit. The responses reveal that linear model term

(X_1), interactive model term (X_2X_3) and quadratic term model (X_3^2) are significant ($p < 0.05$). The quadratic model terms (X_1^2 and X_2^2) were more significant than the other terms. The quadratic model terms (X_1^2 and X_2^2) were more significant than the other terms (P -values are less than 0.01). The P -value > 0.05 means that the model terms are insignificant. We can see from Table 4 that interactions between the sulfuric acid and substrate loading (X_1X_2), interactions between the sulfuric acid and hydrolysis time (X_1X_3) and the linear coefficients (X_2 and X_3) are insignificant. Figure 2 is a parity plot showing a comparison between experimental values of the response and those predicted by the statistical model. All points were clustered around the diagonal line, indicating good fitness of the model. The residuals from the least squares are an important tool for judging the model adequacy. Figure 3A shows the plot of residuals vs. the predicted response. The residual plots of the model are randomly distributed without any trends. This result indicates good predictions of maximum response along with constant variance and adequacy of the quadratic models. The standardized residuals versus run plot represented in Figure 3B shows randomly scattered points ranged between ± 0.75 ; the errors were normally distributed and insignificant.

Table 4 ANOVA results for glucose concentration model

Source	Sum of squares	DF	Mean squares	F-value	p-value
Model	64.45	9	7.16	26.67	0
X_1 (H_2SO_4)	0.38	1	2.51	9.36	0.012*
X_2 (Substrate)	52.13	1	0.02	0.25	0.748
X_3 (Time)	0.13	1	0.06	1.99	0.63
X_1X_2	0.53	1	0.53	1.03	0.188
X_1X_3	0.27	1	0.27	6	0.333
X_2X_3	1.61	1	1.61	11.48	0.034*
X_1^2	4.26	1	3.08	15	0.007**
X_2^2	3.71	1	4.14	15.43	0.003**
X_3^2	1.4	1	1.4	5.21	0.046*
Residual	2.68	10	0.26		
Lack of Fit	1.02	5	0.2	0.61	0.698
Pure Error	1.66	5	0.33		
Cor Total	67.13	19			

$R^2=96\%$; $R^2_{adj}=92.40\%$.

** $P < 0.01$ - Significant at 1% level.

* $P < 0.05$ - Significant at 5% level.

Optimization of dilute acid hydrolysis

Three dimensional (3D) response surface plots were generated to determine the optimum levels of the variables that were investigated in this study. The plots were generated by keeping one variable constant at the center point and varying the others within the experimental range. The resulting response surfaces showed the effect of sulfuric acid, substrate loading and time on the glucose concentration. The effects of the sulfuric acid concentration and substrate loading on the glucose concentration are shown in Figure 4A. An increase in the sulfuric acid concentration with substrate loading resulted in an increase in the glucose concentration until an optimum value of about 8.91g/L, i.e., 9%(v/v) acid concentration, 18%(w/v) substrate loading. Generally, dilute acid can dissolve and recover most of the

hemicellulose as dissolved sugars under low process severity.^{35,36} Consequently, the rate at which the glycosidic bonds are broken will increase.^{37,38} The effect of acid concentration and hydrolysis time on the glucose concentration is presented in Figure 4B. Maximum glucose concentration was observed in the zone of acid concentration near the central value of 8% and hydrolysis time range of 6.7 to 7.4h and in the zone of acid concentration of 8.8 to 9.2% and hydrolysis time range of 0.6 to 1.2 h. The concentration of sugar increased with increase in acid concentration. A similar observation was reported by Amenaghawon et al.³⁹ for the dilute acid hydrolysis of cassava bagasse. In another research, Rafiqul et al.³⁰ studied the dilute acid hydrolysis of *Meranti* wood sawdust (MSW) at a temperature ranging from 121 to 125°C, 2-6% sulfuric acid concentration and reaction time of 20-80min. The authors reported that predicted xylose yield reduced with the increase

of acid concentration, when residence time was held at 80 min and the reciprocal result was found with the low residence time (40min). The maximum sugar was obtained at a temperature of 124°C, a time of 80min and an acid concentration about 3.3%.

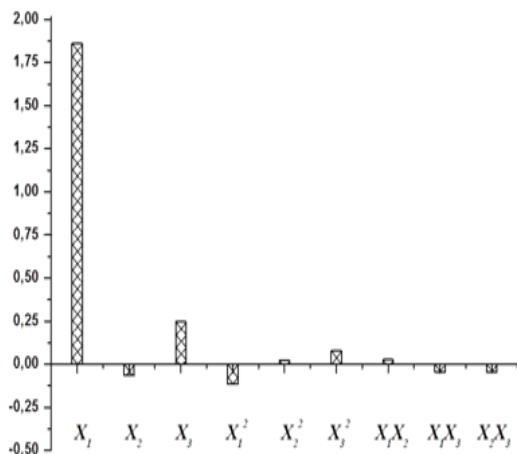


Figure 1 Pareto chart showing the effect of different independent variables on glucose concentration.

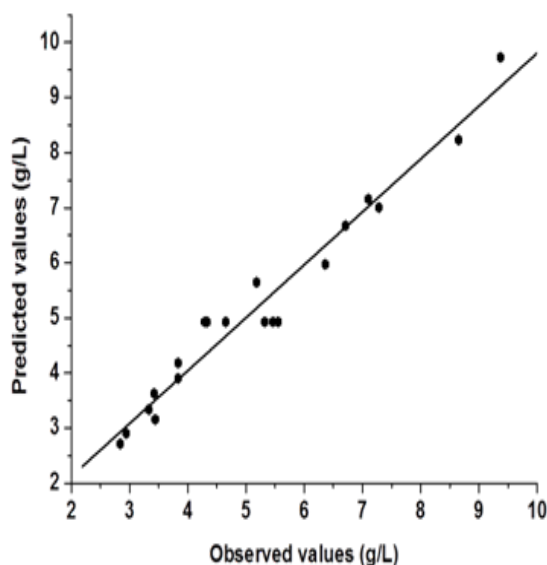


Figure 2 Parity plot showing the distribution of experimental vs. Predicted values of glucose concentration.

The Figure 4C exhibits the interactive effect of substrate loading and hydrolysis time on glucose concentration. As shown in the plot, optimal glucose concentration was observed under increased substrate loading near to 18%, and 1h of hydrolysis time. Any further increase in the hydrolysis time led to no appreciable effect on the production of glucose. The glucose yield was observed to be higher at high substrate loading and low time hydrolysis. Validation of the experimental model was done to check the optimum conditions of acid hydrolysis of sawdust and to check the accuracy of the model. The final optimized hydrolysis conditions obtained with RSM were 8%(v/v) acid concentration, 16%(w/v) substrate loading and 2h hydrolysis time (Table 2). The validity of the results predicted by the regression model was confirmed by carrying out repeated experiments under optimal hydrolysis conditions. The results obtained from two replications demonstrated that the average of the maximum glucose

concentration (8.78g/L) obtained was close to the predicted value (8.39g/L) thus showing validity.

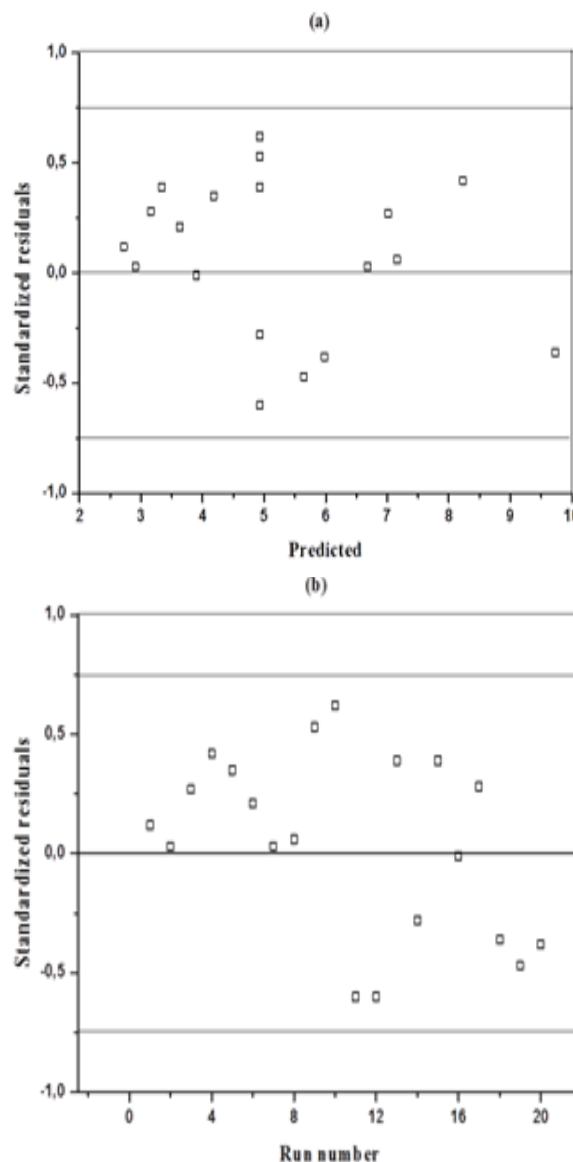


Figure 3 Diagnostic plots for glucose concentration

- A. Residual versus predicted concentration
- B. Residual versus run number.

Lactic acid production from sawdust hydrolysate

To determine the effect of wood sawdust hydrolysate on lactic acid production, batch fermentation was performed in conical flasks for 72h. The production of lactic acid and substrate consumption are shown in Figure 5. Lactic acid production reached 9.87g/L in 72h with a yield of 1.1g/g. Table 5 shows the comparison of lactic acid results of some independent researchers using *Lactococcus lactis* and different processes. The lactic-acid production value obtained is lower than most values that have been reported in the literature. For example, Shi et al.⁴⁰ used immobilized *Lactococcus lactis* to obtain 142g/L lactic acid from Artichoke; Nancib et al.⁴¹ reported 60.3 g/L lactic-acid concentration from date juice using a mixed culture of

Lactobacillus casei and *Lactococcus lactis*. Other research results that have been reported include lactic-acid production using different strains like *L. pentosus* with lactic acid production of 9g/L with a yield of 0.90g/g from wheat-straw hemicellulose hydrolysates and 10g/L with a yield of 0.61g/g using *L. brevis*;⁴² *Bacillus coagulans* MXL-9 with lactic acid production of 33g/L and a yield of 0.73g/g from wood hemicellulose extracts (16); Bustos et al.⁴³ reported 46.0g/L lactic-acid concentration and yield of 0.78g/g from vine-trimming hydrolysate using a *L. pentosus* strain; Buendo et al.¹⁸ reported

43.66g/L lactic-acid concentration with a yield of 0.72g/g from wood extract hydrolysate. Although, lactic acid production by *L. lactis* from this work was lower in terms of concentration, the maximum yield 1.1 is higher than those found by Meziane et al.⁴⁴ and Laopaiboon et al.⁴⁵ which were equal to 0.1 and 0.361 by respectively. The results obtained are in agreement with those reported by Jonglertjunya et al.,⁴⁶ who achieved a production of lactic acid of 10.6g/L with yield of 1.4g/g using sugarcane bagasses hydrolysate.

Table 5 Representative results reported in the literature for lactic acid production using *Lactococcus lactis*

Microorganisms	Substrates	Lactic acid (g/L)	Yield (Yp/s) ^a (g/g)	References
Immobilized <i>Lactococcus lactis</i>	Artichoke	142	-	40
Mixed culture: (<i>Lactobacillus casei</i> and <i>Lactococcus lactis</i>)	Date juice	60.3	-	41
<i>Lactococcus lactis</i>	OFl fruit juice	32.5	-	47
<i>Lactococcus lactis</i> (10-1 JCM 7638)	Xylose	33	0.6	48
<i>Lactococcus lactis</i>	Molasses	40	-	49
<i>Lactococcus lactis</i> (10-1)	Sugarcane bagasse	10.9	0.36	45
<i>Lactococcus lactis</i>	Glucose and xylose	10.6	1.4	46
Immobilized cells (<i>Lactococcus lactis</i>)	Molasses and whey	15.8	0.1	44
<i>Lactococcus lactis</i>	Sawdust	9.87	1.1	This work

^a:Yield of lactic acid produced (g) to substrate consumed (g).

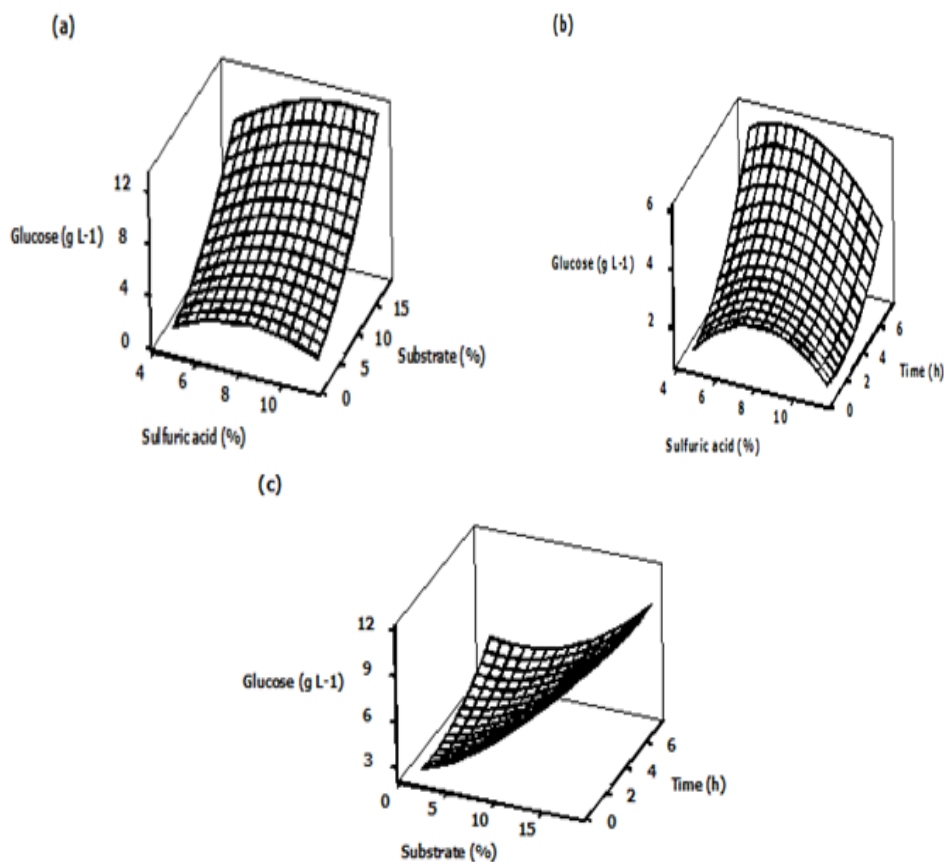


Figure 4 Three-dimensional surface plots showing the effect of different variables on glucose concentration

- Effect of sulfuric acid and substrate loading
- Effect of sulfuric acid and time
- Effect of substrate loading and time.

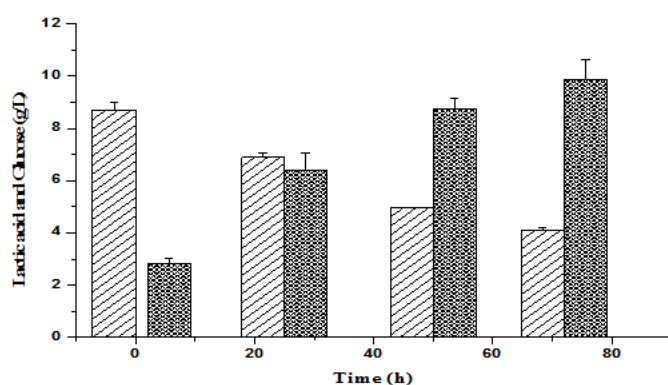


Figure 5 Glucose conversion to lactic acid by *Lactococcus lactis*. (Glucose: ▨; Lactic acid: ▩).

Conclusion

The hydrolysis of wood sawdust was carried out using dilute sulfuric acid according to a three variable central composite design. The optimum hydrolysis conditions are a sulfuric acid concentration of 8%(v/v), substrate loading of 16%(w/v), and hydrolysis time of 2h. Under these conditions, the maximum concentration of glucose obtained (8.78g/L) was close to the predicted value (8.39g/L). So wood sawdust hydrolysate supplemented with yeast extract, was found to be a potential substrate for lactic acid production by *Lactococcus lactis*. The maximum lactic acid concentration obtained was 9.87g/L, which is equivalent to a conversion yield of about 1.1g/g.

Acknowledgements

None.

Conflict of interest

The author declares no conflict of interest.

References

- Mohamed Ali Abdel-Rahman, Yukihiro Tashiro, Kenji Sonomoto. Recent advances in lactic acid production by microbial fermentation processes. *Biotechnology Advances*. 2013;31(6):877–902.
- Maeda T, Yoshimura T, Shimazu T, et al. Enhanced production of lactic acid with reducing excess sludge by lactate fermentation. *Journal of Hazardous Materials*. 2009;168(2-3):656–663.
- Wang P, Li J, Wang L, et al. L(+)-Lactic acid production by co-fermentation of glucose and xylose with *Rhizopus oryzae* obtained by low-energy ion beam irradiation. *J Ind Microbiol Biotechnol*. 2009;36(11):1363–1368.
- Mohd Adnan AF, Tan IK. Isolation of lactic acid bacteria from Malaysian foods and assessment of the isolates for industrial potential. *Biore-sour Technol*. 2007;98(7):1380–1385.
- Md Altaf, Naveena BJ, Venkateshwar M, et al. Single step fermentation of starch to L(+) lactic acid by *Lactobacillus amylophilus* GV6 in SSF using inexpensive nitrogen sources to replace peptone and yeast extract-optimization by RSM. *Process Biochemistry*. 2006;41(2):465–472.
- Komesu A, Martins P, Oliveira J, et al. Purification of lactic acid produced from sugarcane molasses. *Chemical Engineering Transactions*. 2014;37:367–372.
- Nancib A, Nancib N, Azzoug A, et al. Valorization of residual yoghurt whey by lactic acid production: An optimized process. *Journal of IAR*. 2016;3(60):13–17.
- Nancib A, Nancib N, Boubendir A, et al. The use of date waste for lactic acid production by a fed-batch culture using *Lactobacillus casei* subsp. *rhamnosus*. *Braz J Microbiol*. 2015;46(3): 893–902.
- Zhang Y, Kumar A, Hardwidge PR, et al. D-lactic acid production from renewable lignocellulosic biomass via genetically modified *Lactobacillus plantarum*. *Biotechnol Prog*. 2016;32(2):271–278.
- Ge XY, Qian H, Zhang WG. Improvement of L-lactic acid production from Jerusalem artichoke tubers by mixed culture of *Aspergillus niger* and *Lactobacillus* sp. *Bioresour Technol*. 2009;100(5):1872–1874.
- Pang X, Zhuang X, Tang Z, et al. Polylactic acid (PLA): research, development and industrialization. *Biotechnol J*. 2010;5(11):1125–1136.
- Rafiqul SM, Mimi Sakinah AM. Processes for the production of xylo-ol-a review. *Food Reviews International*. 2013;29(2):127–156.
- Kumar Saini J, Reetu Saini, Lakshmi Tewari. Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. *3 Biotech*. 2015;5(4):337–353.
- Kim TH, Choi CH, Oh KK. Bioconversion of sawdust into ethanol using dilute sulfuric acid-assisted continuous twin screw-driven reactor pretreatment and fed-batch simultaneous saccharification and fermentation. *Bioresour Technol*. 2013;130:306–313.
- Nwakaire JN, Ezeoha SL, Ugwuishiwu BO. Production of cellulosic ethanol from wood sawdust. *Agric Eng Int: CIGR Journal*. 2013;15(3):136–140.
- Hsu CC, Thayer DW. Single-cell protein production in alkaline mesquite wood extracts. *Appl Environ Microbiol*. 1982;44(2):339–345.
- Rafiqul ISM, Mimi Sakinah AM. Design of process parameters for the production of xylose from wood sawdust. *Chemical Engineering Research and Design*. 2012;90(9):1307–1312.
- John P Buyondo, Shijie Liu. Lactic acid production by *Lactobacillus pentosus* from wood extract hydrolysates. *Journal of Science & Technology for Forest Products and Processes*. 2011;1:38–47.
- Chundawat SP, Beckham GT, Himmel ME, et al. Deconstruction of lignocellulosic biomass to fuels and chemicals. *Annu Rev Chem Biomol Eng*. 2011;2:121–145.
- Walton SL, Bischoff KM, Van Heiningen AR, et al. Production of lactic acid from hemicellulose extracts by bacillus coagulansMXL-9. *J Ind Microbiol Biotechnol*. 2010;37(8):823–830.
- Ahmad Khattak W, Ul-Islam M, Kon Park J. Prospects of reusable endogenous hydrolyzing enzymes in bioethanol production by simultaneous saccharification and fermentation. *Korean Journal of Chemical Engineering*. 2012;29(11):1467–1482.
- Álvarez C, Reyes-Sosa FM, Diez B. Enzymatic hydrolysis of biomass from wood. *Microb Biotechnol*. 2016;9(2):149–156.
- Ruofei Hu, Lu Lin, Tingjun Liu, et al. Dilute sulfuric acid hydrolysis of sugar maple wood extract at atmospheric pressure. *Bioresour Technol*. 2010;101(10):3586–3594.
- Iranmahboob J, Nadim F, Monemi S. Optimizing acid- hydrolysis: a critical step for production of ethanol from mixed wood chips. *Biomass and Bioenergy*. 2002;22(5):401–404.
- Suharti MI, Mimi Sakinah AM. Comparison of acid hydrolysis and combination system method for production of xylose from meranti wood sawdust (MWS). *Journal of Applied Science and Agriculture*. 2015;10(5):210–214.

26. Lenihan P, Orozco A, O'Neill E, et al. Dilute acid hydrolysis of lignocellulosic biomass. *Chemical Engineering Journal*. 2010;156(2):395–403.
27. Neureiter M, Danner H, Madzingaidzo L, et al. Lignocellulose feedstocks for the production of lactic acid. *Chem Biochem Eng*. 2004;18(1):55–63.
28. Wee YJ, Yun JS, Park DH, et al. Biotechnological production of L(+)-lactic acid from wood hydrolyzate by batch fermentation of *Enterococcus faecalis*. *Biotechnol Lett*. 2004;26(1):71–74.
29. Pushparani Mudaliyar, Chandrashekar Kulkarni. Screening of novel substrates for lactic acid production by *Rhizopus Oryzae*. *International Journal of Life Science and Pharma Research*. 2012;2(1):122–127.
30. Rafiqul IS, Sakinah AM, Karim MR. Production of xylose from meranti wood sawdust by dilute acid hydrolysis. *Appl Biochem Biotechnol*. 2014;174(2):542–555.
31. Elliker PR, Anderson AW, and Hannesson G. An agar medium for lactic acid streptococci and lactobacilli. *J Dairy Sci*. 1965;39(31):1611–1612.
32. Kimberley ACC Taylor. A simple colorimetric assay for muramic acid and lactic acid. *Applied Biochemistry and Biotechnology*. 1996;56(1):49–58.
33. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Anal Chem*. 1959;31(3):426–428.
34. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic*. 1965;16:144–153.
35. Li C, Knierim B, Manisseri C, et al. Comparison of dilute acid and ionic liquid pretreatment of switchgrass: biomass recalcitrance, delignification and enzymatic saccharification. *Bioresour Technol*. 2010;101(13):4900–4906.
36. Störker T Moe, Kando K Janga, Terje Hertzberg, et al. Saccharification of lignocellulosic biomass for biofuel and biorefinery applications—a renaissance for the concentrated acid hydrolysis. *Energy Procedia*. 2012;20:50–58.
37. Mosier NS, Ladisch CM, Ladisch MR. Characterization of acid catalytic domains for cellulose hydrolysis and glucose degradation. *Biotechnol Bioeng*. 2002;9(6):610–618.
38. Parveen Kumar, Diane M Barrett, Michael J Delwiche, et al. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind Eng Chem Res*. 2009;48(8):3713–3729.
39. Amenaghawon NA, Enoroghoe Ogbeide S, Ovbromoka Okieimen C. Application of statistical experimental design for the optimisation of dilute sulphuric acid hydrolysis of cassava bagasse. *Acta Polytechnica Hungarica*. 2014;11(9):239–250.
40. Shi Z, Wei P, Zhu X, et al. Efficient production of L-lactic acid from hydrolysate of Jerusalem artichoke with immobilized cells of *Lactococcus lactis* in fibrous bed bioreactors. *Enzyme Microb Technol*. 2012;51(5):263–268.
41. Nancib A, Nancib N, Boudrant J. Production of lactic acid from date juice extract with free cells of single and mixed cultures of *Lactobacillus casei* and *Lactococcus lactis*. *World Journal of Microbiology and Biotechnology*. 2009;25(8):1423–1429.
42. Arvid Garde, Gunnar Jonsson, Anette S Schmidt, et al. Lactic acid production from wheat straw hemicellulose hydrolysate by *Lactobacillus pentosus* and *Lactobacillus brevis*. *Bioresour Technol*. 2002;81(3):217–223.
43. Bustos G, Moldes AB, Cruz JM, et al. Influence of the metabolism pathway on lactic acid production from hemicellulosic trimming vine shoots hydrolyzates using *Lactobacillus pentosus*. *Biotechnol Prog*. 2005;21(3):793–798.
44. Meziane M, Dilmi Bouras A, El Hameur H. Lactic acid fermentation of a diluted molasses medium by two strains of *Lactococcus lactis* ssp. Immobilized on pouzzolane and bone bovine. ISITES Conference, Sakkarya, Turkey; 2013. p. 774–782.
45. Laopaiboon P, Thani A, Leelavatcharamas V, et al. Acid hydrolysis of sugarcane bagasse for lactic acid production. *Bioresour Technol*. 2010;101(3):1036–1043.
46. Jonglertjunya W, Pranrawang N, Phookongka N, et al. Utilization of sugarcane bagasse for lactic acid production by acid hydrolysis and fermentation using *Lactobacillus* sp. *World Acad Sci Eng Technol*. 2013;66:173–178.
47. Tamine M, Nancib A, Nancib N, et al. Prickly pear cactus as a raw material for lactic acid production by *Lactococcus lactis* subsp. *lactis*. *Malays J Microbiol*. 2017;13(3):1–9.
48. Tanaka K, Komiyama A, Sonomoto K, et al. Two different pathways for D-xylose metabolism and the effect of xylose concentration on the yield coefficient of L-lactate in mixed-acid fermentation by the lactic acid bacterium *Lactococcus lactis* IO-1. *Appl Microbiol Biotechnol*. 2002;60(1-2):160–167.
49. Ohashi R, Yamamoto T, Suzuki T. Continuous production of lactic acid from molasses by perfusion culture of *Lactococcus lactis* using a stirred ceramic membrane reactor. *J Biosci Bioeng*. 1999;87(5):647–654.