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Effect of dilute acid pretreatment on the chemical properties of Paulownia wood (*Paulownia fortunei*) for bioethanol production

Farbod Hadinezhad[1], Ahmad Abbaszadeh-Mayvan[1*], Ahmad Taghizadeh-Alisaraei[1],
Mohammad hadi Aryaie Monfared[2], Ali Moayedi[3], Aliasghar Tatari[2]

[1] *Department of Biosystems Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*
[2] *Department of Paper Science and Engineering, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*
[3] *Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran*

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*** Corresponding author**
abbaszadeh@gau.ac.ir

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ABSTRACT

Dilute acid pretreatment is a widely used method for lignocellulosic biomass breakdown and enhancing the accessibility of cellulose. Despite the growing interest in bioethanol production, the effects of dilute acid pretreatment on the chemical properties of Paulownia wood remain insufficiently understood, limiting the optimization of biomass conversion processes. This study explores the effect of dilute acid pretreatment on the chemical composition of Paulownia wood, aiming to optimize cellulose, holocellulose, and lignin content using response surface methodology. The factors investigated in this work included acid concentration (0.25-1.75% w/w), pretreatment temperature (30-210 °C), and pretreatment time (7.5-142.5 min). The results demonstrated that temperature was the most significant factor affecting cellulose, holocellulose, and lignin content ($p < 0.0001$), followed by acid concentration, while pretreatment time showed no significant influence. The optimal pretreatment condition involved an acid concentration of 1.5%, temperature of 180 °C, and time duration of 30 min, resulting in the lowest lignin and highest cellulose and holocellulose content. Fermentation of this sample yielded a bioethanol concentration of 2.8 g L^{-1}. This study provides a foundation for refining pretreatment strategies to maximize biomass valorization, highlighting the importance of temperature and acid concentration as key variables in optimizing dilute acid pretreatment of Paulownia wood.

1. Introduction

The increasing demand for renewable and sustainable resources has led to extensive research on lignocellulosic biomass as a promising feedstock for biofuels and bioproducts (Shahnouri et al., 2024). Among various lignocellulosic materials, Paulownia wood has gained attention due to its rapid growth, high biomass yield, and favorable chemical composition (Del Río et al., 2020; Rodríguez-Seoane et al., 2020). As one of the fastest-growing deciduous trees, Paulownia can attain harvestable maturity within five to seven years, far surpassing the rotation periods of conventional hardwoods such as oak or poplar. This rapid biomass accumulation translates directly into higher productivity per unit area and shorter cycles of resource renewal, thereby reducing land use pressures and enabling scalable bioethanol production. From a compositional standpoint, Paulownia wood is characterized by a high holocellulose content comprising both cellulose and hemicellulose, which provides a rich source of fermentable sugars essential for ethanol yield. Simultaneously, it possesses comparatively low lignin content, which minimizes the recalcitrance typically associated with lignocellulosic feedstocks and facilitates milder, less energy-intensive pretreatment processes. Economically, these traits reduce both capital and operational costs associated with enzymatic hydrolysis and fermentation steps. Furthermore, Paulownia's ability to thrive on marginal or degraded lands, its resistance to pests and drought, and its low input requirements enhance its attractiveness as a sustainable energy crop, particularly in regions where agricultural resources are limited.

The complex and recalcitrant nature of lignocellulosic structures poses a significant challenge to efficient biomass conversion (Petridis and Smith, 2018; Singhvi and Gokhale, 2019). The presence of lignin and hemicellulose hinders the accessibility of cellulose to enzymatic hydrolysis, necessitating an effective pretreatment strategy to enhance process efficiency (Meng and Ragauskas, 2014). Various pretreatment methods have been developed to break down the rigid lignocellulosic structure and improve biomass processability. These approaches can be broadly classified into physical, chemical, physicochemical, biological, and emerging advanced methods. Physical methods, such as milling and irradiation, reduce particle size and increase surface area, but they require high energy inputs (Liu et al., 2017; Sitotaw et al., 2023), chemical pretreatments, including alkali (Shafaei et al., 2024), acid (Saulnier et al., 2022), and organosolv methods (Tatari et al., 2017a; 2017b), alter the chemical composition by removing lignin and hemicellulose. Physicochemical approaches, such as steam explosion (Muzamal et al., 2015) and liquid hot water treatment (Chen et al., 2022), utilize heat and pressure to disrupt biomass structure. Biological pretreatment involves the use of lignin-degrading microorganisms, though it generally requires long processing times (Sharma et al., 2019). Recently, novel and more sustainable pretreatment strategies, such as SO2-alcohol-

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water fractionation (SAW) (Dehghani Firouzabadi and Tatari, 2024; Tatari et al., 2024), deep eutectic solvents (Yu et al., 2024), ionic liquids (Brandt et al., 2010), ultrasound-assisted (Qu et al., 2024), and microwave-assisted techniques (Shahnouri et al., 2024), have gained attention due to their ability to enhance biomass fractionation with reduced environmental impact. Among these methods, dilute acid pretreatment has gained prominence due to its efficiency in hydrolyzing hemicellulose and enhancing cellulose accessibility for enzymatic hydrolysis (Chu et al., 2021; du Pasquier et al., 2024; Saulnier et al., 2022; Zhang et al., 2013).

Dilute acid pretreatment has developed as a widely used technique to disrupt the lignocellulosic matrix, facilitate the release of fermentable sugars, and improve the overall efficiency of biomass conversion (du Pasquier et al., 2024). This method involves the application of low-concentration acids under controlled conditions to hydrolyze hemicellulose, reduce lignin content, and increase cellulose accessibility (du Pasquier et al., 2023; Shahbazi and Zhang, 2010). However, the effectiveness of dilute acid pretreatment largely depends on various variables, including acid concentration, temperature, and reaction time (du Pasquier et al., 2024).

To achieve an optimal balance between effective lignocellulosic deconstruction and minimal degradation of valuable carbohydrates, response surface methodology (RSM) has been widely employed. RSM is a powerful statistical tool that allows for the systematic investigation of interactions between multiple process variables, leading to the identification of optimal conditions (Nazarpour et al., 2022). Despite significant advancements in biomass pretreatment, there remains a need for a comprehensive understanding of how dilute acid pretreatment influences the chemical composition of Paulownia wood and how these changes impact subsequent processing steps.

This study aims to evaluate the effects of dilute acid pretreatment on the chemical properties of Paulownia wood and optimize the process conditions using RSM to enhance biomass valorization efficiency. This study presents a novel approach by integrating RSM with a detailed chemical characterization to optimize dilute acid pretreatment conditions specifically for Paulownia wood. This biomass remains underexplored for bioethanol production.

Unlike previous studies that have primarily focused on conventional feedstocks or generic pretreatment strategies, our work creates new insights into the compositional transformations of Paulownia wood under varying acid concentrations and residence times, offering a tailored optimization framework that enhances its fermentable sugar yield and overall bioethanol potential. The broader implications of this research pertain to its contribution to advancing sustainable and economically viable strategies for industrial-scale bioethanol production.

By demonstrating the high fermentable sugar yield and favorable chemical profile of Paulownia wood, the study supports its use as a renewable lignocellulosic feedstock that can be cultivated with minimal inputs on marginal lands, thus avoiding competition with food crops and enhancing land-use efficiency. These findings underscore the potential of Paulownia wood-based biorefineries to reduce dependence on fossil fuels, lower greenhouse gas emissions, and contribute to the circular bioeconomy.

Moreover, the compatibility of Paulownia with mild pretreatment conditions not only reduces energy and chemical consumption but also makes downstream processing more cost-effective, thereby addressing critical challenges in scaling up second-generation bioethanol technologies. Ultimately, this study provides a valuable framework for integrating fast-growing, low-input woody biomass into sustainable energy systems, with implications for both environmental resilience and industrial innovation.

2. Material and Methods

2.1. Preparation of raw materials

Three logs of a four-year-old Paulownia tree (Paulownia fortunei) were obtained from the Dr. Bahramnia Forests Plan of Gorgan. After debarking, the wood was cut and ground into chips. The chips were then sieved to attain a relatively uniform size, typically less than 1 mm (mesh No. 20) (Figure 1). Extra pure 98% sulfuric acid was obtained from Merck (Darmstadt, Germany). Distilled water was used in all experimental procedures.

2.2. Acid pretreatment

The effect of sulfuric acid solution in different concentrations on Paulownia wood was investigated based on the methods reported earlier (Chen et al., 2010; Gundupalli and Bhattacharyya, 2023; Sindhu et al., 2011). Combined thermal-acid pretreatment was performed using a laboratory glycerol bath (Figure 2a) using 2.5-L cylinders (Figure 2b). For this purpose, various concentrations of sulfuric acid (0.25-1.75%, w/w) were prepared and transferred into the cylinders at a ratio of 10:1 (%w/w) with 200 g (o.d. basis) of wood chips. Temperature conditions (30-210 °C) and pretreatment times (7.5-142.5 min.) were considered for the pretreatment. After washing and removing chemicals and bringing the pH to neutral (~7), the samples were kept in plastic bags to prevent moisture exchange (Figure 2c,d).

Figure 1. Preparation steps of Paulownia wood for chemical analysis. a) tree logs, b) a wood disc, c) wood chips, and d) wood powder.

Figure 2. a) Electrically heated laboratory glycerol bath, b) 2.5 L steel cylinders, c) Paulownia wood after acid pretreatment, and d) pretreated Paulownia wood after the washing stage.

2.3. Analysis of chemical compositions

The chemical compositions of Paulownia wood were determined based on the following standard methods: cellulose (Kurschner and Hoffer method), holocellulose (Zoia et al., 2017), acid-insoluble lignin (TAPPI T222 om-98), soluble lignin in acid (TAPPI um-250), extractives soluble in alcohol-acetone (TAPPI T204 cm-97), and ash content (TAPPI T211 om-02).

2.4. Statistical analysis

The RSM, through three factors and five levels of central composite design (CCD), was used for the optimization of acid pretreatment utilizing Design-Expert® 11 software. The acid pretreatment variables, i.e. acid concentration (A), temperature (B), and time (C) were investigated for acid pretreatment optimization.

The coded and actual levels of the CCD independent variables used to optimize acid pretreatment variables for Paulownia wood are presented in Table 1. The experimental design of the RSM-CCD matrix is shown in Table 2, comprising 20 runs, including six center points to ensure reliable estimation of experimental error and model adequacy. The experimental data obtained from the RSM-CCD were modeled using the RSM regression, by a linear equation (Eq. 1)

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i<j}\sum^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} {X_i}^2 + \varepsilon \qquad (1)$$

where Y is the predicted response factor (cellulose, holocellulose, and lignin content), β_0 is the intercept, β_i are the linear coefficient of the main factors, β_{ii} values represent the coefficient of the quadratic terms, β_{ij} are the interaction coefficients and, ε is the random model error.

The generated second-order polynomial model' statistical assessment was performed through the analysis of variance (ANOVA). The model's performance was assessed using various evaluation criteria such as lack of fit (LOF), coefficient of determination (R^2), coefficient of variation (CV), Adequate precision, and the *F* value from the ANOVA. Furthermore, optimal conditions for combined acid-hydrothermal (CAH) pretreatment were identified using numerical optimization, and under these conditions, bioethanol yield was measured through the fermentation process.

2.5. Hydrolysis and fermentation process

The pretreated sample (containing the highest cellulose and lowest lignin) was enzymatically hydrolyzed with cellulase enzyme (30 FPU/800 μl) at pH 4.5 and 45 °C for 72 h in a shaker incubator to produce simpler sugars. After sterilization by autoclaving and centrifugation, the liquid phase was used for fermentation with Saccharomyces cerevisiae. The culture medium (20 g glucose, 4 g yeast extract, 8 g peptone, and 0.18 g ammonium chloride per 200 mL distilled water) was inoculated with 3.8 g of yeast and incubated at 30 °C for 24 h in a shaker incubator.

Finally, 5 mL of the culture and 5 mL of the hydrolysate were combined in a 20 mL Falcon tube and incubated at 36 °C for 72 h in a shaker incubator before measuring bioethanol concentration.

Table 1. Variables and their levels for RSM-CCD design.

Factors	Symbol	Experimental level				
		-α	Low (-1)	Medium (0)	High (+1)	+α
X_1: Acid concentration (% w/w)	A	0.25	0.5	1	1.5	1.75
X_2: Pretreatment temperature (°C)	B	30	60	120	180	210
X_3: Pretreatment time (min.)	C	7.5	30	75	120	142.5

Table 2. RSM-CCD design of experiment at different operating conditions.

Run	Factors			Responses		
	A: (% w/w)	B: (°C)	C: (min)	Cellulose content (%)	Holocellulose content (%)	Lignin content (%)
1	1.5	180	30	56.48	78.31	21.15
2	1	120	75	54.13	75.99	23.5
3	1	120	75	54.53	76.4	23.1
4	1	120	142.5	54.23	75.95	23.4
5	1	120	7.5	54.13	75.92	23.5
6	1	120	75	54.63	76.49	23
7	1.5	60	120	50.48	72.24	27.15
8	1	210	75	58.42	80.2	19.21
9	1	120	75	54.13	75.98	23.5
10	0.5	180	30	55.76	77.69	21.87
11	1	120	75	54.73	76.59	22.9
12	1.75	120	75	56.45	78.21	21.18
13	0.5	60	30	49.57	71.34	28.06
14	1	120	75	54.33	76.22	23.3
15	0.5	180	120	57.49	79.41	20.14
16	0.25	120	75	50.97	73.03	26.66
17	1.5	60	30	49.86	71.62	27.77
18	1.5	180	120	58.08	79.89	19.55
19	0.5	60	120	49.94	71.77	27.69
20	1	30	75	49.62	71.34	28.01

A: Acid concentration, Pretreatment temperature, C: Pretreatment time

2.6. Measuring bioethanol concentration

To measure the amount of bioethanol yield from the optimized pretreated sample after the hydrolysis and fermentation process, the potassium dichromate method was employed. 0.75 g of potassium dichromate powder was dissolved in distilled water to a final volume of 1000 mL, and a 70% sulfuric acid solution was also prepared. A mixture was then formed by combining 5 mL of the potassium dichromate solution, 2 mL of the sulfuric acid, and 1.0 mL of the distillate obtained from the bioethanol-containing sample. This mixture was then heated in a 60 °C water bath for 20 min until it developed a dark coloration. After cooling, the optical density of the solution was measured at a wavelength of 578 nm using a spectrophotometer, and the bioethanol concentration (g L^{-1}) was subsequently calculated using a standard bioethanol calibration curve.

3. Results and Discussion

3.1. Compositional analysis of raw materials

The raw materials used in this study were subjected to chemical analysis and the results are summarized in Table 3. These characteristics have been reported to vary depending on geographical conditions, climate, species, and age of the tree. However, they are in good agreement with the results of this part of the study (Ates et al., 2008; López et al., 2012; Zamudio et al., 2015). The cellulose and lignin contents of the Paulownia wood used were 48.8 and 28.83%, respectively. These values were similar to some lignocellulosic materials such as softwoods, hardwoods, and non-woody biomass, which typically contain 35-55% cellulose, 20-40% hemicelluloses, and 10-25% lignin (Biswas et al., 2015; Boutaieb et al., 2020; Gulsoy and Ozturk, 2015). The total solids and volatiles of the Paulownia wood used were 94.34 and 93.78%, respectively.

Table 3. Chemical composition of Paulownia wood.

Component	Value (%)
Cellulose	48.80
Holocellulose (Cellulose + hemicellulose)	70.44
Acid-insoluble lignin (AIL)	25.70
Acid-soluble lignin (ASL)	3.13
Total lignin	28.83
Alcohol-acetone soluble extractives	6.67
Ash content	0.73
Total solids (TS)	94.34
Volatile solids (VS*)	93.78

* % of TS, VS= TS - ash content

3.2. Effect of acid pretreatment on cellulose content

The results of the ANOVA of the fitted response surface model for the cellulose content of the pretreated samples are presented in Table 4. These results indicate that the relationship between the proposed regression model in predicting the cellulose content of the pretreated samples and the independent variables is significant at the 1% probability level. The results also show that the single effect of the independent variables of acid concentration (A) and temperature (B) has a significant effect at the 1% level on the cellulose content variable of the pretreated samples. All the variables related to pretreatment, except for the pretreatment time (C), showed a significant effect on the cellulose content of the pretreated samples. Also, it can be seen that the pretreatment temperature variable (B) had a greater impact on the changes in the dependent variable of cellulose content of pretreated samples, due to its higher sum of squares (SS) value. The evaluation criteria of the developed model, such as the coefficient of determination (R2), adjusted coefficient of determination (adjusted R^2), standard error (Std. Dev.), and coefficient of variation (C.V.%) were 0.92, 0.90, 0.91, and 1.68%, respectively. The predicted model in terms of coded value is shown in Eq. (2).

$$\text{Cellulose content (\%)} = 53.898 + 0.8288 \times A + 3.2928 \times B + 0.3576 \times C \quad (2)$$

Table 4. ANOVA for linear model (response: cellulose content).

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	145.72	3	48.57	59.25	< 0.0001
Acid concentration (A)	8.59	1	8.59	10.47	0.0052
Temperature (B)	135.53	1	135.53	165.34	< 0.0001
Time (C)	1.60	1	1.60	1.95	0.1817
Residual	13.12	16	0.8197		
Lack of fit	12.79	11	1.16	17.70	0.0026
Pure error	0.3283	5	0.0657		
Cor Total	158.83	19			

Figure 3 presents a comprehensive statistical analysis of the effect of process variables on cellulose content using RSM. Figure 3(a) shows the predicted versus actual cellulose content, indicating a strong correlation between experimental and predicted values along the diagonal reference line, suggesting the model's reliability. Figure 3(b) illustrates a three-dimensional (3D) response surface plot depicting the interaction between acid concentration (%) and temperature (°C) on cellulose content, demonstrating that increasing these variables positively affects cellulose content. Figure 3(c) provides another 3D surface plot, showing the interaction between acid concentration and time (min), where time appears to have a negligible effect on cellulose yield, as indicated by the nearly flat surface. Figure 3(d) presents the perturbation plots for all studied factors highlighting that temperature and acid concentration significantly impact cellulose content, while time has a minor effect. The dashed lines in these plots represent confidence intervals, with the red cross indicating the central point of the experimental design.

In acid pretreatment, particularly with dilute sulfuric acid, the primary mechanism involves the partial hydrolysis of hemicellulose ultimately increasing cellulose accessibility (Liu et al., 2022; Zhao et al., 2012). Sulfuric acid breaks glycosidic and ether bonds within hemicellulose, leading to its significant removal from the cell wall (Carvalheiro et al., 2008; Wyman et al., 2005). This process also disrupts the linkages between lignin and carbohydrates, reducing the structural integrity of lignin and creating more porosity within the lignocellulosic matrix. Additionally, sulfuric acid enhances the solubilization of hemicelluloses and alters lignin into more soluble forms, thereby increasing the exposed surface area of cellulose for subsequent processes such as enzymatic hydrolysis. Lim et al. (2019) reported that the highest cellulose content (59.7%) was obtained at 140 °C and the cellulose content decreased significantly with increasing temperature due to peeling reactions. They also reported the effect of pulping time, and that the highest cellulose content was obtained at 100 minutes, but the cellulose content decreased with increasing time. Song et al. (2016) also reported a decrease in pulp yield with increasing pulping temperature from 140 °C onwards. This indicates that high temperature, due to a decrease in the degree of polymerization (DP), causes cellulose degradation and ultimately reduces pulp yield.

3.3. Effect of acid pretreatment on holocellulose content

The ANOVA results for the linear model predicting holocellulose content, as shown in Table 5, indicate that the model is highly significant ($p < 0.0001$), demonstrating its suitability for explaining variations in the response variable. Among the independent variables, temperature (B) exhibits the most substantial effect, with the highest sum of squares (138.58) and an extremely significant impact ($p < 0.0001$). Acid concentration (A) also significantly influences holocellulose content ($p = 0.0077$), though to a lesser extent. However, reaction time (C) does not significantly affect the response ($p = 0.1830$). The residual sum of squares (12.76) and the mean square error (0.7975) indicate an adequate fit, but the significant lack of fit ($p = 0.0029$) suggests that the linear model may not fully capture the response variation, implying the potential need for higher-order terms or interactions to improve the model's predictive accuracy. The R2, adjusted R^2, Std. Dev., and C.V.% were 0.92, 0.91, 0.89, and 1.18%, respectively. The predicted model in terms of the coded value is shown in Eq. (3)

$$\text{Holocellulose content (\%)} = 75.7295 + 0.7696 \times A + 3.3296 \times B + 0.3516 \times C \quad (3)$$

Figure 4 presents the statistical analysis of the effects of acid concentration, temperature, and time on the holocellulose content. Figure 4(a) shows the correlation between predicted and actual values, where data points closely follow the diagonal reference line, confirming the robustness and accuracy of the developed model. Figure 4(b) shows a 3D response surface plot depicting the interactive influence of acid concentration and temperature on holocellulose content, revealing that an increase in both variables enhances the yield. Figure 4(c) shows the combined effect of acid concentration and reaction time, where the nearly planar surface suggests that time has an insignificant role in altering the holocellulose content. Figure 4(d) presents perturbation plots for the three studied variables, showing that acid concentration and temperature exert a noticeable impact on holocellulose content, whereas reaction time has a minimal effect. The holocellulose content represents the total carbohydrate (cellulose + hemicelluloses). This factor is mainly affected by the chemical reactions of the pretreatment and pulping processes. With increasing pretreatment time, due to the removal of lignin during the delignification phases, the amount of remaining holocellulose (relative to total TS) increases. In other words, the purity of holocellulose also increases with increasing lignin solubility. These results are consistent with earlier reports (du Pasquier et al., 2024; Mirmohamadsadeghi et al., 2014; Saulnier et al., 2022).

Table 5. ANOVA for linear model (response: holocellulose content).

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	147.53	3	49.18	61.67	< 0.0001
Acid concentration (A)	7.40	1	7.40	9.28	0.0077
Temperature (B)	138.58	1	138.58	173.77	< 0.0001
Time (C)	1.55	1	1.55	1.94	0.1830
Residual	12.76	16	0.7975		
Lack of fit	12.43	11	1.13	17.00	0.0029
Pure error	0.3323	5	0.0665		
Cor Total	160.29	19			

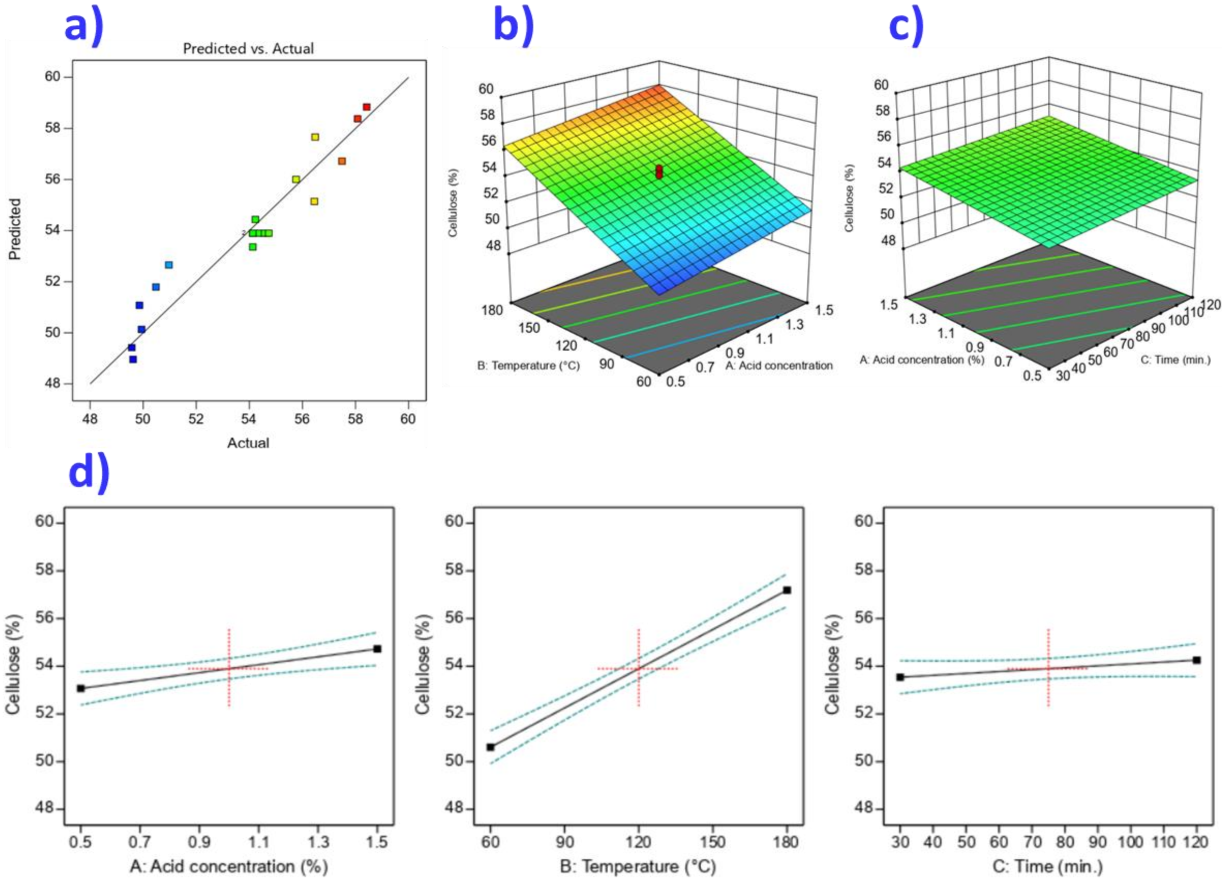

Figure 3. a) Predicted data vs. actual data plot, b) 3D response surface plot (interaction of acid concentration and temperature), c) 3D response surface plot (interaction of acid concentration and time), and d) independent effects of variable factors on cellulose content.

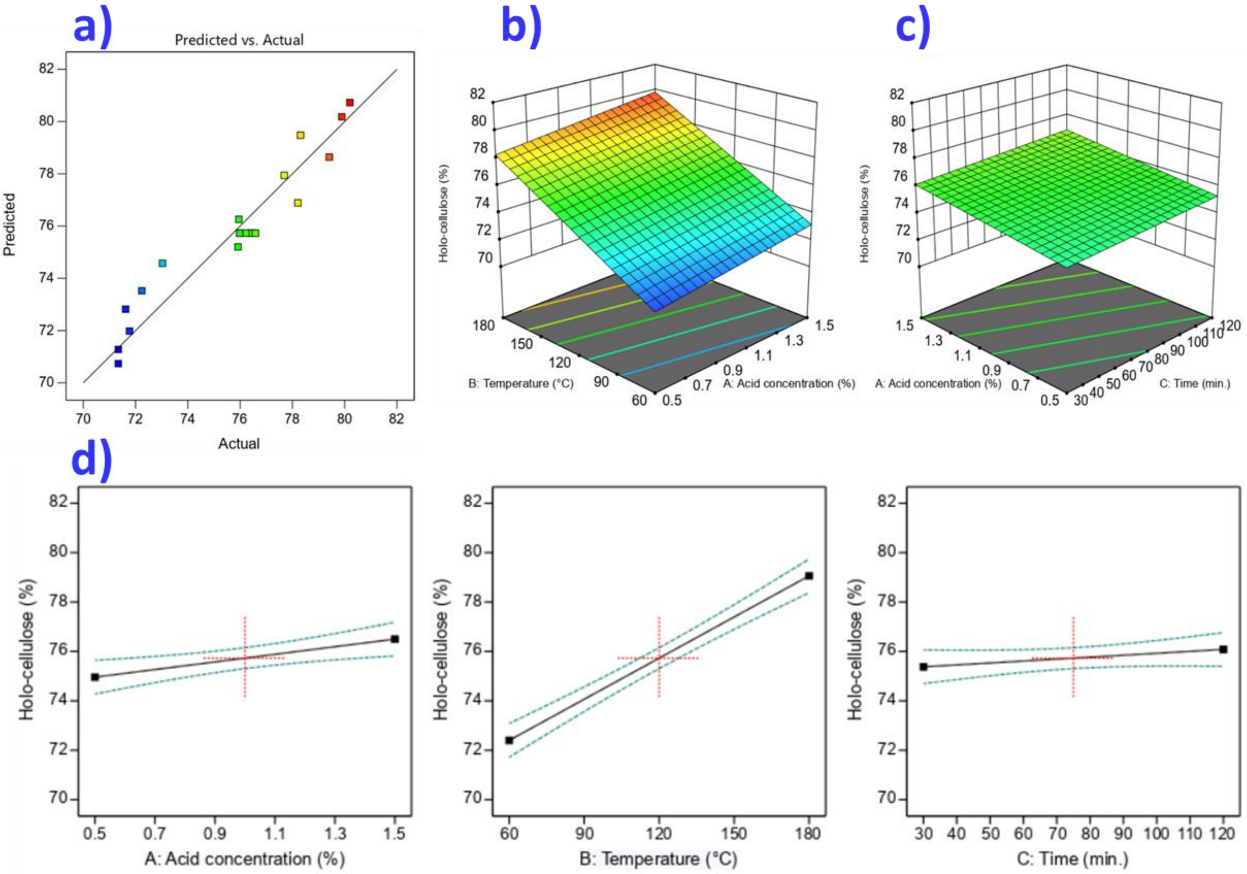

Figure 4. a) Predicted data vs. actual data plot, b) 3D response surface plot (interaction of acid concentration and temperature), c) 3D response surface plot (interaction of acid concentration and time), and d) independent effects of variable factors on holocellulose content.

3.4. Effect of acid pretreatment on lignin content

The ANOVA results for the linear model predicting lignin content, as presented in Table 6, indicate that the model is highly significant ($p<0.0001$), confirming its effectiveness in explaining the response variability. Among the independent variables, temperature (B) has the most pronounced effect, with the highest sum of squares (135.53) and an extremely significant impact ($p<0.0001$). Acid concentration (A) also plays a significant role ($p = 0.0052$), although its influence is notably lower than that of temperature. In contrast, reaction time (C) does not have a statistically significant effect on lignin content ($p = 0.1817$). The residual sum of squares (13.12) and mean square error (0.8197) suggest an acceptable model fit; however, the significant lack of fit ($p = 0.0026$) indicates that the linear model may not fully capture the response variation, suggesting the potential need for interaction terms or a more complex model to enhance predictive accuracy. The R2, adjusted R^2, Std. Dev., and C.V.% are 0.92, 0.90, 0.91, and 1.18%, respectively. The predicted model in terms of coded value is shown in Eq. (4)

$$\text{Lignin content (\%)} = 23.732 - 0.8288 \times A - 0.2928 \times B - 0.3576 \times C \tag{4}$$

Figure 5 illustrates the effects of process variables, including acid concentration, temperature, and time, on lignin content. Figure 5(a) presents the correlation between predicted and actual values, confirming the model's accuracy. The 3D surface plots in Figure 5(b) and c indicate that increasing temperature and acid concentration lead to a reduction in lignin content, whereas time has a negligible effect. The perturbation plots in Figure 5(d) further demonstrate that temperature and acid concentration significantly influence lignin removal, while time plays a minor role. Overall, higher acid concentration and temperature enhance lignin removal efficiency. Lignin is a complex and aromatic polymer that provides structural rigidity to the plant cell wall and acts as a barrier, limiting enzyme access to cellulose fibers (Barhoum et al., 2020; Li et al., 2019). During dilute acid pretreatment, lignin undergoes partial depolymerization and loss of β-O-4 bonds (Pu et al., 2015). The acid can break ether and carbon-carbon bonds within lignin, leading to its partial solubilization or redistribution within the cell wall (Liu et al., 2022; Luo et al., 2024). Additionally, acid pretreatment may cause lignin to condense, altering its physical structure and reducing its interference with cellulose accessibility (Zhao et al., 2012). Temperature plays a significant role in lignin behavior during the pretreatment process (Duan et al., 2018). Higher temperatures enhance the cleavage of lignin bonds, increasing lignin removal or altering its structure to make cellulose more accessible (Tatari et al., 2024). However, excessive temperatures may lead to lignin condensation reactions, where lignin fragments recombine into more complex, recalcitrant structures, further hindering enzymatic hydrolysis (Vázquez et al., 1997). Generally, alkaline delignification processes of lignocellulosic materials are carried out in three phases: initial, bulk, and residual delignification (Lindgren and Lindstrom, 1997), while acidic delignification processes are carried out in two phases (bulk and residual) (Dehghani Firouzabadi and Tatari, 2024; Tatari et al., 2024).

Table 6. ANOVA for linear model (response: lignin content).

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	145.72	3	48.57	59.25	< 0.0001
Acid concentration (A)	8.59	1	8.59	10.47	0.0052
Temperature (B)	135.53	1	135.53	165.34	< 0.0001
Time (C)	1.60	1	1.60	1.95	0.1817
Residual	13.12	16	0.8197		
Lack of fit	12.79	11	1.16	17.70	0.0026
Pure error	0.3283	5	0.0657		
Cor Total	158.83	19			

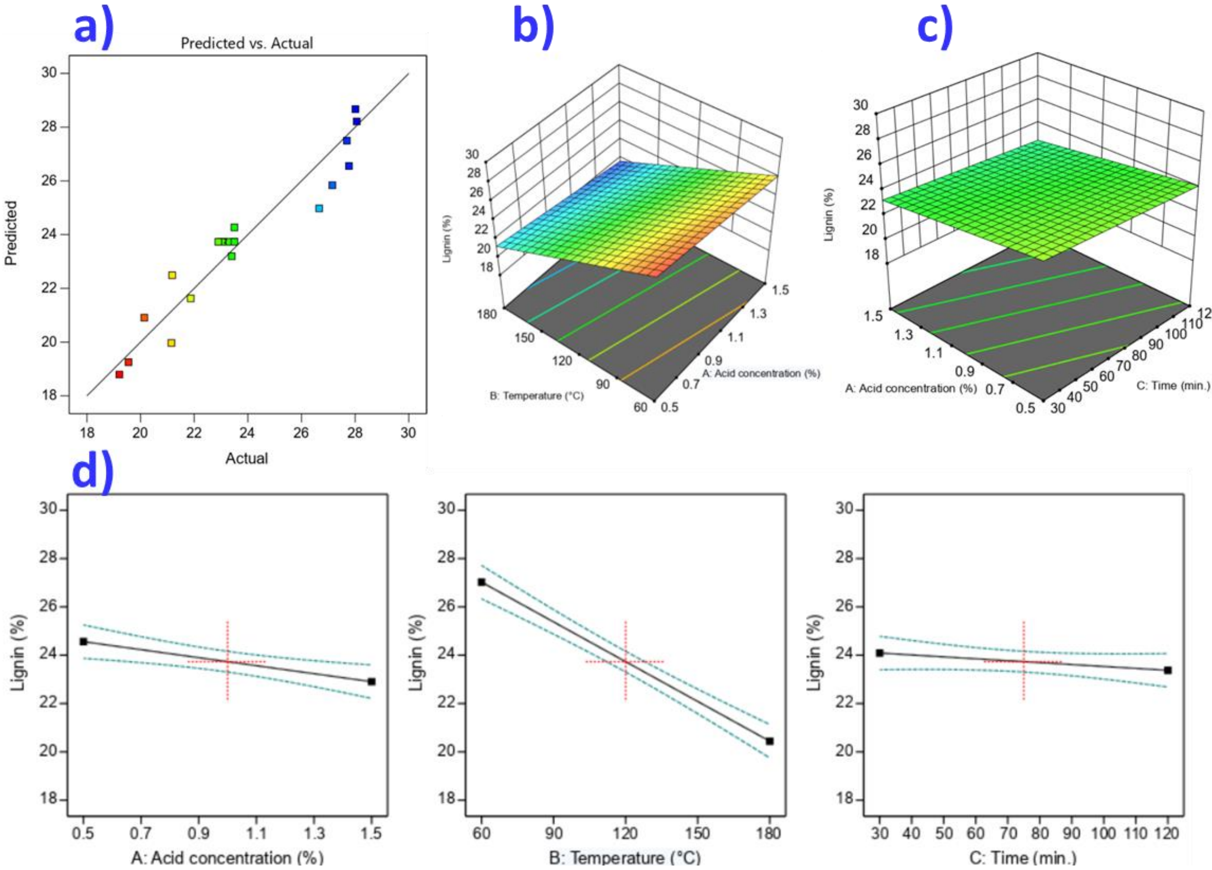

Figure 5. a) Predicted data vs. actual data plot, b) 3D response surface plot (interaction of acid concentration and temperature), c) 3D response surface plot (interaction of acid concentration and time), and d) independent effects of variable factors on lignin content.

Usually, the delignification of softwood is more difficult than that of hardwood due to the specific lignin structure and higher lignin content (Sjostrom, 2013). This means that the process conditions for pretreatment of softwoods wood must be more severe to achieve delignification under similar conditions to hardwood (similar kappa number). In a study by Asghar et al. (2015), the delignification rate of wheat straw increased with increasing temperature in alkaline pretreatment.

3.5. Bioethanol production

The findings revealed that the optimal conditions for dilute acid pretreatment were a temperature of 180 °C, an acid concentration of 1.5%, and a reaction time of 30 min, which resulted in the lowest lignin content (21.91%) and the highest cellulose content (55.71%) and holocellulose content (77.53%) in the samples (desirability = 1). Consequently, fermentation was carried out using the sample treated under these specific conditions. Following fermentation, the bioethanol yield was measured at 2.8 g L^{-1}. The lower ethanol yield compared to other studies on paulownia wood may be attributed to the absence of detoxification, hydrolysis and fermentation methods, yeast type, and the ethanol measurement technique used.

4. Conclusions

This study examined the effect of dilute acid pretreatment on the chemical composition of Paulownia wood, with a particular focus on cellulose, holocellulose, and lignin contents. The ANOVA demonstrated that temperature is the most influential factor in determining both holocellulose and lignin content, followed by acid concentration, while reaction time had no statistically significant effect. Under optimal pretreatment conditions (180 °C, 1.5% acid, 30 min), the sample showed the lowest lignin and highest cellulose and holocellulose content. Fermentation of this sample yielded a bioethanol concentration of 2.8 g L^{-1}. These findings have practical implications for biomass valorization, particularly in biofuel and bioproduct industries, where optimizing pretreatment conditions can enhance cellulose accessibility and reduce lignin content, ultimately improving enzymatic hydrolysis efficiency. By identifying temperature and acid concentration as key variables, this study provides a basis for pretreatment strategies to maximize sugar yield while minimizing degradation byproducts.

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Competing interests

No competing financial interests or personal relationships are known to the authors that could have influenced this study.

Data availability statement

The data supporting the results of this study are available from the corresponding author upon reasonable request.

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