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Microwave-assisted alkaline pretreatment of white grape pomace for the production of bioethanol†

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Grape pomace is a suitable substrate for bioethanol production as it contains carbohydrates and various amino, but its high lignin content hinders productivity. Microwave-assisted alkaline pretreatment was performed in this study to selectively remove the lignin and the pretreatment conditions (alkaline concentration, temperature, and reaction time) were optimized by the response surface method (RSM). NaOH concentration and temperature were determined to be factors that significantly impact the subsequent bioethanol production, while reaction time showed less impact. The highest delignification of 83.69% was achieved with 3% NaOH at 90 °C for 45 minutes, and the corresponding ethanol concentration reached 20.98 g L^{-1} with a yield of 90.61%. This study developed an efficient pretreatment process for the biological conversion of grape pomace, providing support for the sustainable utilization of agricultural waste.

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1. Introduction

Fossil fuels are gradually being exhausted as non-renewable energy sources, and the carbon emissions generated when they are burned exacerbate climate warming, environmental pollution, and other problems. Green and renewable biofuel is regarded as one of the most important ways to achieve global sustainable development. Lignocellulosic biomass can be used to produce biofuels and replace fossil fuels. Compared with biofuels produced from starch as substrates, the production of bioethanol from lignocellulosic biomass, such as agricultural and forestry waste, does not compete for food and land.[1]

Grape pomace is the waste produced after collecting the juice during the wine-making process which accounts for approximately 25% to 30% of the fresh grape's weight. Over 9 million tons of grape pomace are generated globally each year.[2] As lignocellulosic biomass, grape pomace contains carbohydrates, lipids, and amino acids, making it a suitable substrate for microbial fermentation to produce ethanol.[3] The carbohydrate contents in grape pomace vary depending on the variety and the winemaking process. The carbohydrates in grape pomace after pressing mainly consist of water-soluble monosaccharides, oligosaccharides, and polysaccharides, as well as water-insoluble structural polysaccharides. Fresh white grape pomace has a high content of soluble sugars, accounting for about one-third (37.6% w/w) of the dry weight and 70% of the total carbohydrate content, while the percentage of soluble carbohydrates in red grape pomace is much lower, at 4.6% w/w.[4] This difference is caused by the different production routes: the red grape pomace is collected after alcoholic fermentation while the white grape pomace is separated before winemaking and has a higher sugar content.[5] So, white grape pomace was selected as the substrate for bioethanol production.

In lignocellulosic materials, cellulose is closely associated with hemicellulose and lignin, forming a dense structure. The complexity of this structure poses significant challenges to ethanol production, as it restricts the accessibility of cellulase enzymes to carbohydrates, thereby reducing the yield of monosaccharides and hindering the overall conversion of biomass.[6] To enhance the conversion efficiency, pretreatment has become a necessary step to break down this natural structure and reduce its recalcitrance. The most common pretreatment methods used in the bioconversion of lignocellulosic biomass include dilute acid, ball milling, hydrothermal, and alkaline.[7] Alkaline pretreatment is one of the most effective methods for delignification. Pretreatment of lignocellulosic biomass with alkali can reduce the lignin content by breaking the ester bond that forms the crosslinking of xylan and lignin[8] and altering the structure of lignocellulosic biomass. Alkaline pretreatment not only improves the lignin depolymerization and the digestibility, but also offers distinct advantages including wide availability of raw materials, low cost, and recyclability.[9] These characteristics confer significant economic benefits for industrial-scale applications. Alkaline pretreatments were generally carried out at a relatively mild temperature but prolonged reaction time with the traditional heating method, such as oven or incubator. Microwave heating has unique advantages which can greatly reduce the pretreatment duration. Microwave radiation utilizes

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the interaction between the applied electromagnetic field and the molecules of the heated object to generate thermal effects.[10] Unlike conventional heating methods, microwave irradiation has the advantage of higher rate, high efficiency, and uniform heating.[11,12] Using microwave-assisted alkaline pretreatment can shorten the reaction time and improve the pretreatment efficiency. Singh *et al.* reported that lignin removal from biomass was only 69.7% under traditional heating, but it could be improved to 82.3% with microwave-assisted alkaline pretreatment.[13] Zhu *et al.* found that the lignin content in biomass was 7.2% after traditional alkaline pretreatment, while the lignin content could be reduced to 5.7% by microwave-assisted alkaline pretreatment under the same conditions.[14] The use of microwave-assisted alkaline pretreatment can achieve significant lignin removal in a shorter pretreatment time, which can be attributed to the difference in their heating mechanisms.[15]

In this study, microwave-assisted alkaline pretreatment was applied to selectively remove the lignin content in white grape pomace for the production of bioethanol *via* simultaneous saccharification and fermentation (SSF). The pretreatment conditions, including alkaline concentration (1–3%), temperature (50–90 °C), and pretreatment time (30–60 min) were investigated and optimized by the response surface method (RSM). This study aimed to develop an efficient pretreatment method for grape pomace and promote the recycling of this valuable solid waste.

2. Materials and methods

2.1 Grape pomace characterization

White grape pomace was kindly provided by the Sino-French Joint Venture Dynasty Winery Ltd in Tianjin, China. After drying to moisture below 10%, the grape pomace was ground to a size of 40–80 mesh, packed into sealed bags, and stored at 4 °C for further use. The carbohydrates, lignin, and ash content in the pomace were determined following the standard procedure of the National Renewable Energy Laboratory (NREL, USA).[16] Elemental content (C, H, N, O, and S) was analyzed using an elemental analyzer (Elementar UNICUBE, Germany). Phosphorus analysis was performed with an Inductively Coupled Plasma-Optical Emission Spectrometer (Agilent ICP-OES 730, United States). The detailed procedure of elemental analysis has been described earlier.[17] The amino acids were measured by an HPLC system (Shimadzu 20AD) equipped with a C18 column. The method of amino acids analysis was the same as Baranenko's work.[18] All chemicals were purchased from Sinopharm Chemical Reagent Company (Shanghai, China).

2.2 Microwave-assisted alkaline pretreatment of grape pomace

Due to the high level of lignin content present in the grape pomace, alkaline pretreatment was chosen to achieve effective delignification. The pretreatment was carried out in a microwave reactor (Synth WAVE, Italy) with a fixed solid-to-liquid ratio of 1 : 15. Three factors that mostly affect the pretreatment efficiency were investigated, *i.e.*, NaOH concentration (%), temperature (°C), and pretreatment time (min). The levels of each factor were chosen based on literature reports and our preliminary single-factor experiments are shown in Table 1. The response surface method (RSM) was employed to design and optimize this multi-factor and multi-level experiment. A total of 17 runs of experiments, including 13 different combinations and 4 additional repeats of the central point for lack of fit analysis, were designed with Design-Expert 8.0.6 software (Stat-Ease Inc., USA), and the detailed conditions for these runs are provided in Table 1. The coded factors enable experimental variables (temperature, time, and NaOH concentration) with different units and ranges to be comparable, thereby improving the accuracy of the regression model. All experiments were performed in duplicate.

Table 1 Factors and levels in the experimental design of microwave-assisted alkaline pretreatment

		Coded factor levels		
Factors	Symbol	−1	0	1
Temperature (°C)	X_1	50	70	90
Time (min)	X_2	30	45	60
NaOH concentration (%)	X_3	1	2	3

Run	X_1 (°C)	X_2 (min)	X_3 (%)
1	70	45	2
2	50	45	3
3	90	30	2
4	90	45	3
5	90	45	1
6	70	45	2
7	90	60	2
8	70	30	3
9	70	45	2
10	70	60	1
11	50	60	2
12	50	30	2
13	70	30	1
14	70	45	2
15	70	60	3
16	50	45	1
17	70	45	2

After the pretreatment, the grape pomace solids were separated from the liquid and washed with tap water until the pH was close to neutral. The pretreated pomace solids were then dried to constant weight for subsequent study. The cellulose, hemicellulose, lignin, and ash content of the pretreated grape pomace were determined. The lignin removal efficiency (delignification) is calculated as follows:

$$\text{Delignification}(\%) = \frac{\text{the weight of lignin removed by pretreatment(g)}}{\text{initial lignin mass in grape pomace(g)}} \times 100\%$$

2.3 Simultaneous saccharification and fermentation (SSF) of grape pomace

Novozymes CTec3 cellulase and *Escherichia coli* (ATCC 55124) were used for the SSF of grape pomace. Cellulase enzyme

(Novozymes Cellic CTec3, 152 FPU per mL) was purchased from Sigma-Aldrich (Shanghai, China). *E. coli* (ATCC 55124) was purchased from the Shanghai Bioresource Collection Center (Shanghai, China). MRS cultural medium, glucose, and yeast extracts were purchased from Sigma-Aldrich (China). SSF was conducted in 50 mL serum bottles with a total working volume of 30 mL, with a solid loading level of 10% (w/v). The fermentation system was provided by 15 g per L yeast extract as the nutrient source. After loading all fermentation materials, the serum bottles were crimp-sealed with a rubber stopper to maintain anaerobic conditions during the fermentation. Before inoculation, the bottles were steam-sterilized at 121 °C for 15 min in an autoclave (Sanyo MLS3750, Japan). After cooling to room temperature, the sterilized medium was inoculated with 10% (v/v) *E. coli* seed culture, followed by the addition of cellulase solution at the loading of 15 FPU per g-solids. SSF was carried out in a shaker incubator at 37 °C and 200 rpm. All experiments were performed in duplicate. The samples were collected at 0, 6, 12, 24, 36, 48, 72, and 96 h. Immediately after each sampling, the samples were centrifuged at 10 000 rpm for 5 minutes, and the supernatants were collected and analyzed for glucose and ethanol concentrations.

2.4 Analytical methods

Sugar concentrations of samples were determined by HPLC (Waters, United States), which was equipped with an RI detector and an HPX-87P column (Bio-Rad, USA). The column temperature was 80 °C, and ultra-pure water was used as the mobile phase at a flow rate of 0.6 mL min^{-1}. The ethanol concentration was determined by HPLC (Waters, United States) equipped with an HPX-87H chromatographic column (Bio-Rad, USA). The column temperature was 55 °C, and the mobile phase was 5 mM sulfuric acid solution at a flow rate of 0.6 mL min^{-1}. The ethanol yield is calculated as follows:

$$\text{Ethanol yield}(\%) = \frac{\text{ethanol produced(g)}}{\text{available glucose(g)in substrate} \times 0.511} \times 100\%$$

3. Results and discussion

3.1 Characteristics of white grape pomace

The white grape pomace used in this study contains various amino acids as shown in Fig. 1. A total of 17 different amino acids were identified in this white grape pomace. The rich amino acids are suitable for the growth of various microorganisms, making it an ideal substrate for ethanol fermentation. The results of elemental analysis indicated that carbon (49.88%) is the highest element in this white grape pomace, followed by oxygen (38.65%) and hydrogen (5.38%). The nitrogen content is 2.03%, and trace amounts of phosphorus (0.70%) and sulfur (0.16%) were also detected. Compositional analysis revealed that the white grape pomace used in this study consisted of 9.51% cellulose, 6.50% hemicellulose, and 49.22% lignin. These values differed from the reported composition of

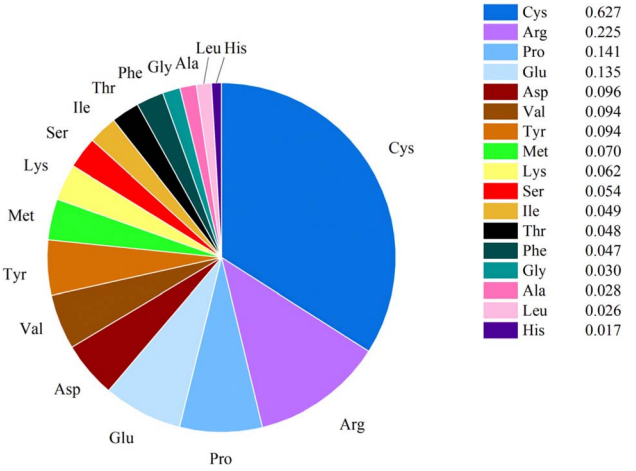

Fig. 1 Amino acids (wt%) in the white grape pomace sample.

red grape pomace (30.3% cellulose, 21.0% hemicellulose, and 17.4% lignin) by Prozil *et al.*[19] Compared with red grape pomace, this white grape pomace exhibited a relatively low cellulose content but a significantly higher lignin content. Cellulose, hemicellulose, and lignin are intricately intertwined through physicochemical interactions, forming a complex and stubborn structure.[20,21] The high lignin content will hinder the subsequent SSF for ethanol production. To mitigate this limitation, microwave-assisted alkaline pretreatment was adopted in this study to selectively remove the lignin content in the substrate. This pretreatment facilitated lignin depolymerization, resulting in enhanced delignification and increased fermentable sugar availability.

3.2 The compositional change of grape pomace after microwave-assisted alkaline pretreatment

Pretreatment can change the physicochemical properties of grape pomace, reduce their recalcitrance, and thereby improve the subsequent ethanol production. The conditions of microwave-assisted alkaline pretreatment were systematically investigated in this study, including NaOH concentrations (1%, 2%, 3%), pretreatment temperatures (50 °C, 70 °C, 90 °C), and pretreatment times (30 min, 45 min, 60 min). The compositional changes (cellulose, hemicellulose, and lignin) of grape pomace after pretreatments were investigated, and the lignin removal efficiency (delignification) was used as the key parameter to compare the efficiency of different pretreatment conditions. The composition and delignification results from 13 sets of different experimental conditions are summarized in Table 2. Microwave-assisted alkaline pretreatment showed overall satisfactory delignification for the substrate of white pomace, ranging from 59.77% to 83.69%. A clear trend can be seen from Table 2 that the delignification increased with the increase in pretreatment intensity. As the concentration of NaOH solution, pretreatment temperature, and pretreatment time increased, the process of lignin removal from grape pomace proceeded more deeply which resulted in higher delignification. On the other hand, the alkaline solution at a mild temperature and

Table 2 The content of carbohydrates in grape pomace after different pretreatments

	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)	Delignification (%)
Raw	9.51 ± 0.08	6.5 ± 0.01	49.22 ± 0.54	5.09 ± 0.02	—
1	24.62 ± 0.43	4.87 ± 0.39	45.46 ± 0.53	6.15 ± 0.17	75.32 ± 0.43
2	28.17 ± 0.47	4.12 ± 0.02	41.42 ± 0.55	6.14 ± 0.25	77.06 ± 0.31
3	29.29 ± 0.53	4.46 ± 0.20	41.19 ± 0.40	5.74 ± 0.03	78.14 ± 0.23
4	36.80 ± 0.66	7.21 ± 0.32	48.14 ± 0.46	4.99 ± 0.18	83.69 ± 0.16
5	27.33 ± 0.37	11.07 ± 0.09	49.23 ± 0.22	4.90 ± 0.00	72.37 ± 0.12
6	25.75 ± 0.70	5.40 ± 0.39	39.87 ± 0.60	6.35 ± 0.21	79.15 ± 0.46
7	35.27 ± 0.48	5.12 ± 0.54	44.52 ± 0.53	5.26 ± 0.03	83.65 ± 0.28
8	31.65 ± 0.40	4.69 ± 0.15	39.66 ± 0.70	5.97 ± 0.23	80.35 ± 0.35
9	24.83 ± 0.29	5.12 ± 0.27	42.15 ± 0.35	6.25 ± 0.08	76.85 ± 0.33
10	31.80 ± 0.17	14.14 ± 0.75	42.80 ± 0.49	5.48 ± 0.00	76.31 ± 0.55
11	17.43 ± 0.13	3.34 ± 0.36	46.63 ± 0.57	4.87 ± 0.12	61.43 ± 0.47
12	20.53 ± 0.48	4.15 ± 0.15	47.80 ± 0.75	5.31 ± 0.04	59.77 ± 0.47
13	27.02 ± 0.19	10.59 ± 0.12	46.04 ± 0.61	5.41 ± 0.00	70.40 ± 0.39
14	25.41 ± 0.35	4.95 ± 0.13	40.83 ± 0.43	6.29 ± 0.13	77.43 ± 0.23
15	31.49 ± 0.28	5.23 ± 0.04	42.33 ± 0.78	5.86 ± 0.19	80.85 ± 0.35
16	26.59 ± 0.20	10.92 ± 0.46	42.91 ± 0.12	5.78 ± 0.00	73.84 ± 0.08
17	25.19 ± 0.30	5.28 ± 0.22	44.72 ± 0.38	6.18 ± 0.06	78.12 ± 0.19

short reaction time would not affect the cellulose content much. So the percentage of cellulose in the pretreated grape pomace increased dramatically to up to 36.80% compared to 9.51% of the raw substrate. The increase in cellulose content was mainly attributed to the removal of lignin and hemicellulose, which increased the relative content of cellulose.

The delignification process was sensitive to temperature. At a NaOH loading of 2%, the lignin removal increased significantly from 61.43% to 83.65% when the temperature increased from 50 to 90 °C. However, less increment was observed at a higher NaOH loading of 3%, and the delignification rose from 77.06% to 83.69%. Higher lignin removal was achieved at higher temperatures because high temperatures could facilitate the generation of more active hydroxyl radicals (HO*) which directly contributes to the degradation reaction of lignin.[22] The concentration of NaOH was another key factor for lignin removal efficiency. The lignin removal efficiency increased with the increase in the concentration of the NaOH solution. At the pretreatment conditions of 30 min and 70 °C, when the NaOH concentration increased from 1% to 3%, the lignin removal enhanced from 70.40% to 80.35%. Similarly, under more severe conditions of 45 min and 90 °C, elevating the alkali concentration from 1% to 3% improved the lignin removal efficiency, rising from 72.37% to a significant 83.69%. The process of lignin removal occurs through the cleavage of aryl ether bonds, which are the main part related to the total lignin content.[8] With the increase in NaOH concentration, the improvement in lignin removal efficiency can be attributed to the catalysis of hydroxide ions in the cleavage of ether bonds in lignin. The cleavage of these bonds increases the hydrophilicity of lignin in the solution,[23] allowing more lignin to dissolve in the alkali solution and selectively remove lignin from the grape pomace solids, thereby increasing the content of fermentable sugars.

Hemicellulose and lignin are barriers that prevent enzymes from recognizing cellulose.[24] They cover the surface of cellulose, irreversibly adsorbing cellulase and hindering the accessibility between cellulose and cellulase, thereby affecting bioconversion.[25] During the microwave-assisted alkaline pretreatment process, NaOH dissociates into hydroxide ions, which break the ester-carbohydrate bonds between cellulose and hemicellulose and lignin in grape pomace, as well as the acetyl groups in hemicellulose.[26] Increasing the concentration of the alkali solution also facilitates the solvation and swelling of grape pomace, which in turn increases the surface area and disrupts the complexity of its structure. This structural disruption is conducive to the selective removal of lignin and partial hemicellulose, which can prevent hemicellulose and lignin from non-productively adsorbing on the surface of cellulose.[27] Therefore, the accessibility of cellulose is significantly enhanced, enabling cellulose to be enzymatically hydrolyzed and polymerized into fermentable sugars more effectively, and effectively improving the overall conversion efficiency of grape pomace. Although NaOH solution demonstrates high efficiency in lignin removal during biomass pretreatment, its application is associated with significant drawbacks, including severe equipment corrosion and environmental pollution due to the generation of alkaline wastewater. In contrast, deep eutectic solvents (DESs) have emerged as a promising green alternative, offering distinct advantages such as tunability, low toxicity, and recyclability.[28] DESs disrupt hydrogen bonds to solubilize lignin and enhance cellulose accessibility, thereby breaking the recalcitrant structure of lignocellulosic biomass and improving subsequent bioconversion efficiency.[22,29] Similarly, as an environmentally friendly green solvent, ionic liquids are widely employed in the pretreatment of lignocellulosic biomass due to their advantages such as low volatility, high thermal stability, and recyclability. Through the delignification effect of ionic liquids, the enzymatic hydrolysis efficiency of biomass can be significantly improved, thereby increasing the yield of fermentable sugars.[30] Future research will concentrate on the application of DESs and ionic liquids as sustainable solvents for grape pomace pretreatment, aiming to facilitate the value-

added and environmentally friendly utilization of grape pomace resources.

3.3 Simultaneous saccharification and fermentation of grape pomace

3.3.1 Ethanol concentration and yield. The SSF process couples saccharification with the fermentation of sugars in a single reactor, eliminating the potential substrate inhibition and simplifying the operation process.[31] The delignification after pretreatment and the final yield of ethanol after 96 hours SSF are shown in Fig. 2, and changes in ethanol concentration with time during SSF are provided in the ESI (Fig. S1).† The ethanol concentration increased rapidly in the first 24 hours, and the rate of increase decreased after 72 hours and gradually stabilized. At 96 hours of SSF, the pretreatment condition with a 3% NaOH concentration and heating at 90 °C for 45 minutes was the most effective. The concentration and yield of ethanol were 20.98 g L^{-1} and 90.61%, respectively, and the removal efficiency of lignin at this condition is also the highest at 83.69%.

The efficiency of SSF is closely related to the intensity of pretreatment. The concentration and yield of ethanol increase with the rise of pretreatment temperature and the concentration of the NaOH solution. Under the conditions of lower temperature (50 °C) and lower concentration of NaOH (1%), the concentration and yield of ethanol are relatively low (≤11.03 g L^{-1}, ≤65.93%). It can be observed that when the pretreatment temperature and time were both 50 °C and 45 min when the concentration of NaOH increased from 1% to 3%, the ethanol concentration only increased by 1.97 g L^{-1}, and the ethanol yield only increased by 7.47%. This might be due to the relatively low pre-treatment temperature, which resulted in insufficient removal of lignin during the pre-treatment process and incomplete destruction of the structure of the grape pomace.[32] However, when the pretreatment temperature and time were both 90 °C and 45 min, and the NaOH concentration increased from 1% to 3%, the ethanol concentration increased by 9.43 g L^{-1}, and the ethanol yield increased by 25.19%. It is indicated that the increase in pretreatment temperature has a strong positive correlation with the increase in ethanol concentration and yield. This improvement suggests that higher pretreatment intensity significantly improves the ethanol concentration and yield. High temperature and high NaOH concentration pretreatment could effectively disrupt the lignin matrix and enhance cellulose accessibility, thereby facilitating glucose release from grape pomace. During the subsequent 96-hour SSF process, the liberated glucose was efficiently converted into ethanol. The microwave-assisted alkaline pretreatment developed in this study demonstrated superior performance in both delignification and ethanol production compared to conventional pretreatment methods reported in the literature (Table 3).

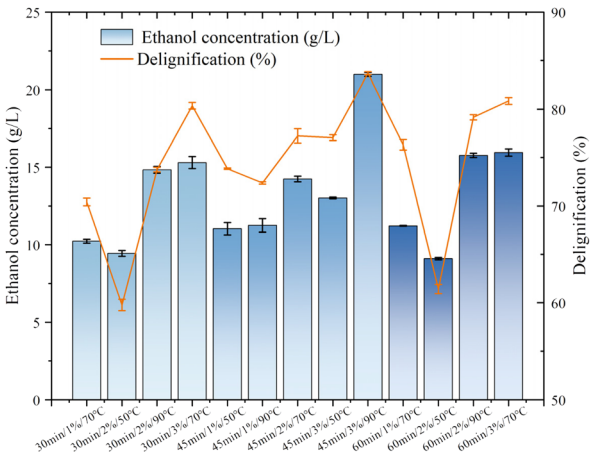

Fig. 2 The delignification and corresponding ethanol production at different pretreatment conditions.

The relationship between the concentration of ethanol and three pretreatment variables was explored through Pearson's correlation coefficient (Fig. 3). Statistical analysis revealed a significant moderate positive correlation between pretreatment temperature and delignification efficiency ($r = 0.62$). While a weak positive correlation was observed between NaOH concentration and delignification ($r = 0.39$). Time duration demonstrated the weakest correlation with delignification ($r = 0.18$). Notably, NaOH concentration exhibited a strong statistically significant positive correlation with ethanol concentration ($r = 0.65$). Similarly, temperature showed a significant correlation with ethanol yield ($r = 0.63$). In contrast, pretreatment time displayed a negligible correlation with ethanol production ($r = 0.07$). Therefore, increasing the pretreatment temperature concurrently enhances both delignification efficiency and ethanol yield. Moderately elevating NaOH concentration during pretreatment facilitates improved ethanol productivity in subsequent SSF. The pretreatment time can be moderately

Table 3 The delignification and ethanol yield of different lignocellulosic biomass under different pretreatment

Lignocellulosic biomass	Pretreatment	Delignification (%)	Ethanol yield (%)	References
Oil palm trunk	Alkaline peroxide with autoclave	83.26	66.14	33
Poplar wood chips	Hydrotrope pretreatment	63.70	82.90	34
Sugarcane bagasse	Soaking in concentrated aqueous ammonia	41.51	75.88	35
Rise husk	Diluted acid	90.10	57.63	36
Corn stover	Soaking in aqueous ammonia	74.00	77.00	37
Grape pomaces	Microwave-assisted alkaline pretreatment	83.69	90.61	This study

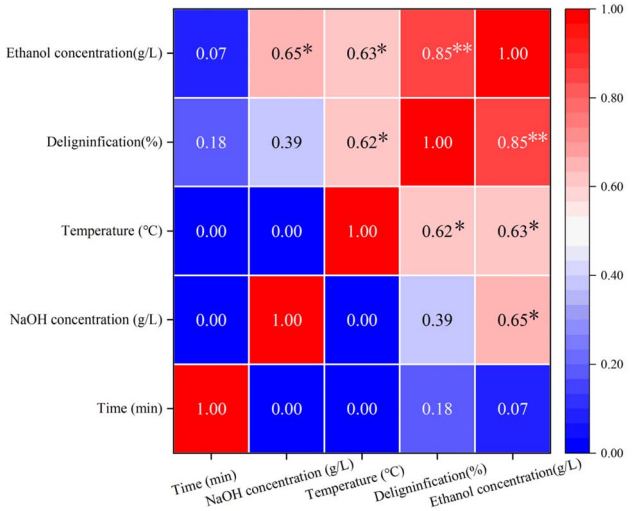

Fig. 3 Pearson's correlation heat map.

reduced with minimal impact on both delignification and ethanol production.

3.3.2 Optimization by RSM. Response surface optimization was carried out using the Box–Behnken design principle in Design-Expert 8.0.6 software. In this study, the ethanol concentration after SSF was taken as the experimental index. Three factors of temperature (X_1), time (X_2), and NaOH concentration (X_3) in pretreatment, were selected as independent variables, and a 3-factor and 3-level response surface orthogonal experiment was designed. The experimental groups were divided into 13-factor analysis experimental groups and 4 error estimation experimental groups. The experimental factor results are presented in Table 4.

Based on the Box–Behnken principle, experiments were designed and the ethanol concentrations at 96 hours of fermentation were measured. The experimental data were processed and regression fitting was conducted using the Design-Expert 8.0.6 statistical analysis software. The quadratic polynomial regression equation correlating relative ethanol concentration (Y) with pretreatment temperature (X_1), time (X_2), and NaOH concentration (X_3) is derived from the response surface experimental design data as follows:

$$Y = 14.23 + 2.57X_1 + 0.28X_2 + 2.65X_3 + 0.32X_1X_2 + 1.86X_1X_3 - 0.082X_2X_3 - 0.49{X_1}^2 - 1.46{X_2}^2 + 0.40{X_3}^2$$

Based on this equation, the relative impact of independent variables on ethanol concentration is determined as follows: NaOH concentration exhibits the strongest influence ($\beta_{X_3} = 2.65$), followed by temperature ($\beta_{X_1} = 2.57$), while time demonstrates the least direct effect ($\beta_{X_2} = 0.28$). The R^2 value of the regression coefficients of the above-mentioned quadratic regression full model equation is 0.9768, indicating that the correlation between the predicted values and the actual values is relatively high. The established model can well reflect the experimental data and the fitting degree of the regression equation is good, with small experimental errors.

The analysis of variance (ANOVA) results used to fit the response level models are presented in Table 5, and significance test of the coefficients in the regression equation are provided in the ESI (Table S1).† Under the condition of a given significance level of $P < 0.05$, in the ethanol concentration regression model, the first-order terms of pretreatment temperature X_1 ($P < 0.0001$) and NaOH concentration X_3 ($P < 0.0001$) showed significant effects, while the first-order term of pretreatment time X_2 ($P = 0.2826$) was not significant. This indicates that the temperature and NaOH concentration in the pretreatment conditions have a greater impact on the ethanol concentration after SSF, while the time is a less significant factor. The order of significance of the three pretreatment conditions on the change in ethanol concentration after SSF is NaOH concentration X_3 ($F = 123.02$) > temperature X_1 ($F = 115.55$) > time X_2 ($F = 1.35$). Therefore, the NaOH concentration used for pretreatment and the holding time are the main factors for improving the ethanol concentration. The interaction terms X_1X_3 ($P = 0.0009$) showed significant effects, while X_1X_2 ($P = 0.3774$) and X_2X_3 ($P = 0.8148$) did not. This indicates that the interaction between temperature and NaOH concentration in the pretreatment conditions is strong. The quadratic terms showed no significant effects, indicating that the nonlinear effects among the variables are weak. The P value of the lack-of-fit term was 0.2237, which was greater than 0.05, indicating that the lack-of-fit was not significant, and the model structure was stable and reasonable, and could well predict the changes in ethanol concentration after actual synchronous enzymatic hydrolysis fermentation. Therefore, this model can be used to analyze and predict the ethanol concentration.

As shown in Fig. 4, the 3D response surface plots formed by the interaction of treatment temperature (A) and treatment time (B), as well as treatment time (B) and NaOH solution concentration (C) are relatively steep, and have a significant impact on the ethanol concentration. Their interaction is highly

Table 4 Ethanol concentrations and yield from grape pomace after different pretreatments

Run	Ethanol concentration (g L^{-1})	Ethanol yield (%)
1	14.10 ± 0.32	86.23 ± 0.24
2	13.00 ± 0.06	73.40 ± 0.31
3	14.82 ± 0.22	80.46 ± 0.23
4	20.98 ± 0.12	90.61 ± 0.15
5	11.55 ± 0.44	67.22 ± 0.12
6	13.57 ± 0.28	88.57 ± 0.19
7	15.76 ± 0.14	71.05 ± 0.28
8	15.30 ± 0.40	76.85 ± 0.35
9	14.58 ± 0.26	87.92 ± 0.18
10	11.21 ± 0.03	56.04 ± 0.55
11	9.10 ± 0.07	82.98 ± 0.47
12	9.44 ± 0.19	73.08 ± 0.59
13	10.23 ± 0.13	60.20 ± 0.39
14	13.93 ± 0.36	87.31 ± 0.25
15	15.95 ± 0.23	80.51 ± 0.35
16	11.03 ± 0.40	65.93 ± 0.08
17	14.95 ± 0.18	89.22 ± 0.47

Table 5 ANOVA results for ethanol concentration

Source	Sum of squares	DF	Mean square	*F*-value	*P*-value
Model	134.63	9	14.96	32.74	<0.0001
X_1-temperature	52.79	1	52.79	115.55	<0.0001
X_2-time	0.62	1	0.62	1.35	0.2826
X_3-NaOH concentration	56.20	1	56.20	123.02	<0.0001
X_1X_2	0.41	1	0.41	0.89	0.3774
X_1X_3	13.90	1	13.90	30.43	0.0009
X_2X_3	0.027	1	0.027	0.059	0.8148
X_1^2	1.01	1	1.01	2.21	0.1810
X_2^2	8.96	1	8.96	19.61	0.0031
X_3^2	0.68	1	0.68	1.50	0.2606
Residual	3.20	7	0.46		
Lack of fit	2.01	3	0.67	2.26	0.2237
Pure error	1.19	4	0.30		
Cor total	134.63	9	14.96	32.74	

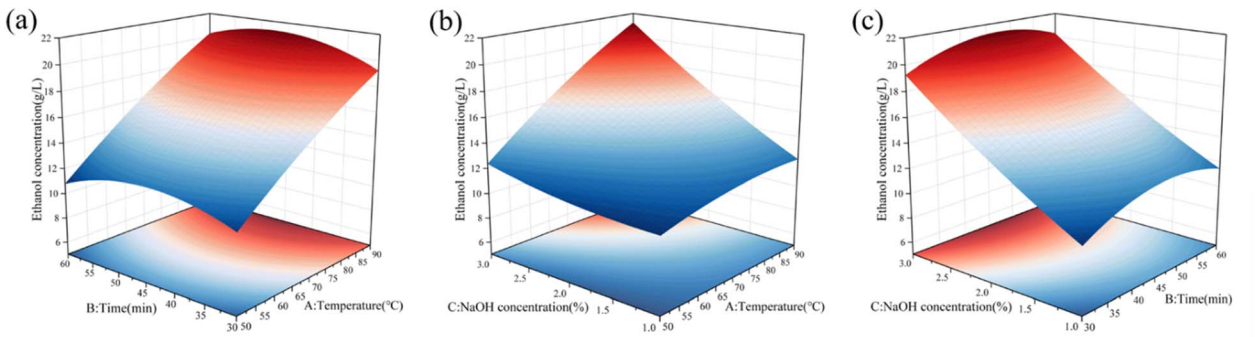

Fig. 4 Interaction of three factors on the ethanol concentration: (a) temperature and time, (b) temperature and NaOH concentration, and (c) time and NaOH concentration.

significant. Any change in any of the variables will affect the magnitude of the response value.

As shown in Fig. 4(a), when the pretreatment temperature is constant, the influence of pretreatment time on ethanol concentration conforms to the form of a quadratic function. The ethanol concentration increases first and then decreases as the pretreatment time increases. When the pretreatment time is 45 minutes, the ethanol concentration reaches its maximum. This may be because the long pretreatment process causes some cellulose to be degraded, resulting in a decrease in the content of degradable sugars and a reduction in ethanol concentration. When the pretreatment time is constant, the ethanol concentration increases with the increase in temperature. From the trend of the response surface changes, it can be seen that the slope of the surface of the pretreatment temperature is steeper than that of the pretreatment time, indicating that the influence of pretreatment temperature on the relative crystallinity change is greater than that of pretreatment time. As shown in Fig. 4(b), with the increase of pretreatment temperature and the concentration of the used NaOH solution, the ethanol concentration continuously increases. The response surface formed by interaction is relatively steep, indicating that the interaction between these two factors has a significant effect on the ethanol concentration and has a relatively significant nonlinear effect. As shown in Fig. 4(c), when the pretreatment time is constant, the response surface is relatively steep. When the NaOH concentration is constant, the ethanol concentration increases first and then decreases as the pretreatment time increases. The curve is relatively flat, indicating that the influence of NaOH concentration on ethanol concentration is greater than that of time. Through the analysis of the response surface model, the optimal pretreatment process conditions for grape pomace are: pretreatment temperature (A) is 90 °C, pretreatment time (B) is 45 min, and NaOH concentration (C) is 3%. Under this process condition, the efficiency of ethanol production is the highest.

4. Conclusion

White grape pomace was proven to be a good substrate for the production of bioethanol. Microwave-assisted alkaline pretreatment could effectively remove up to 83.69% of the lignin content in the white grape pomace, resulting in satisfactory bioethanol in the subsequent simultaneous saccharification and fermentation. The pretreatment condition was optimized by the response surface method to be: temperature is 90 °C, the treatment time is 45 minutes, and the concentration of the NaOH solution is 3%. Under the optimal conditions, the

ethanol concentration and yield after SSF are 20.98 g L^{-1} and 90.61%, respectively. This study developed an efficient pretreatment process for the biological conversion of grape pomace, providing support for the sustainable utilization of agricultural waste.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare that they have no competing interests.

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