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Optimizing the Conditions of Pretreatment and Enzymatic Hydrolysis of Sugarcane Bagasse for Bioethanol Production

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Cite This: *ACS Omega* 2024, 9, 29566−29575

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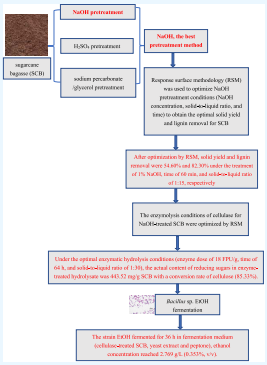
ABSTRACT: The agricultural waste sugarcane bagasse (SCB) is a kind of plentiful biomass resource. In this study, different pretreatment methods (NaOH, H_2SO_4, and sodium percarbonate/glycerol) were utilized and compared. Among the three pretreatment methods, NaOH pretreatment was the most optimal method. Response surface methodology (RSM) was utilized to optimize NaOH pretreatment conditions. After optimization by RSM, the solid yield and lignin removal were 54.60 and 82.30% under the treatment of 1% NaOH, a time of 60 min, and a solid-to-liquid ratio of 1:15, respectively. Then, the enzymolysis conditions of cellulase for NaOH-treated SCB were optimized by RSM. Under the optimal enzymatic hydrolysis conditions (an enzyme dose of 18 FPU/g, a time of 64 h, and a solid-to-liquid ratio of 1:30), the actual yield of reducing sugar in the enzyme-treated hydrolysate was 443.52 mg/g SCB with a cellulose conversion rate of 85.33%. A bacterium, namely, *Bacillus* sp. EtOH, which produced ethanol and Baijiu aroma substances, was isolated from the high-temperature Daqu of Danquan Baijiu in our previous study. At last, when the strain EtOH was cultured for 36 h in a fermentation medium (reducing sugar from cellulase-treated SCB hydrolysate, yeast extract, and peptone), ethanol concentration reached 2.769 g/L (0.353%, v/v). The sugar-to-ethanol and SCB-to-ethanol yields were 13.85 and 11.81% in this study, respectively. In brief, after NaOH pretreatment, 1 g of original SCB produced 0.5460 g of NaOH-treated SCB. Then, after the enzymatic hydrolysis, reducing sugar yield (443.52 mg/g SCB) was obtained. Our study provided a suitable method for bioethanol production from SCB, which achieved efficient resource utilization of agricultural waste SCB.



1. INTRODUCTION

With the rapid depletion of fossil energy, such as coal and petroleum, research studies focus on renewable energy.[1] So far, ethanol is the most successful biofuel, which can be produced from sugarcane and grain crops, such as sorghum and corn (first-generation), and nongrain crops, such as lignocellulose (second-generation) and microalgal biomass (third-generation).[2−4] At present, as a raw material of biofuel production, lignocellulose has attracted widespread attention because of its rich reserve and renewable characteristics.[5] Lignocellulose is primarily composed of cellulose, hemicellulose, and lignin.[6] These components form a compact structure by chemical bonds, which seriously obstructs the lignocellulosic conversion into value-added products.[7] Lignocellulose can be divided into four categories: hardwood, softwood, agricultural wastes, and grasses.[8] The agricultural waste SCB, originating from the sugar industry, is a kind of plentiful biomass resource. In recent years, China's sugarcane production has accounted for about 5% of global production. Sugarcane is an important economic crop in China, especially in southern China. The annual sugarcane yields reached about 100 million tons from 2019 to 2021. In 2021, Guangxi's sugarcane production reached 73.651 million tons, accounting for 69.05% of the total sugarcane production in China. Besides, SCB is rich in sugars such as cellulose and hemicellulose, which is suitable for microbial fermentation to turn agricultural waste into high-value-added products such as ethanol.[9,10]

Pretreatment of lignocellulose biomass can destroy the stable structure of lignocellulose by removing hemicellulose and lignin and enhance the porosity and decrease the crystallinity and polymerization degree of cellulose, which is favorable to converting lignocellulose into high-value-added products.[11,12] At present, many pretreatment methods have been developed, which include physical processes (such as size reduction, ultrasonication, steaming/boiling, and popping), chemical methods (such as acid, base, solvent, and salt), physicochemical methods (such as ammonia fiber explosion and liquid hot water), and biological methods (such as white-rot fungi and brown-rot fungi) to fractionate lignocellulose into its components.[13−15] However, most pretreatment methods have their disadvantages, such as the appearance of inhibitors and high cost, which severely hamper the subsequent utilization.[16,17] For example, liquid hot water pretreatment of lignocellulosic biomass is suitable for bioethanol production, but this method requires a high temperature, which is costly.[18] There have been some advanced research studies. Anugwom et al.[19] obtained

Received: March 15, 2024
Revised: June 19, 2024
Accepted: June 20, 2024
Published: June 26, 2024

optimal process conditions for wood fractionation with switchable ionic liquids based on monoethanol amine and 1,8-diazabicyclo-[5.4.0]-undec-7-ene. About 95 wt % wood lignin was extracted in a short time. Jogi et al.[20] dealt with the fractionation of birch wood powder, which was liquefied under supercritical ethanol over acidic or nonacidic catalysts (5 wt % Fe-β-H-150 and 5 wt % Fe−SiO_2), respectively. Different types of lignocellulose biomass and production technologies will influence the effect of pretreatment.

Cellulosic bioethanol is produced using lignocellulose through three primary steps: pretreatment, hydrolysis, and microbial fermentation.[21] Chemical hydrolysis can produce a variety of inhibitory substances and byproducts. Enzymatic hydrolysis is more popular at present, which has a lot of advantages such as more specific hydrolysis, mild conditions, and higher yields.[22] Hemicelluloses and celluloses are degraded to sugar monomers by enzymatic hydrolysis. The enzymolysis effects are related to enzyme dosage, time, and solid-to-liquid ratio.[23,24]

Ethanol production through yeast (especially *Saccharomyces cerevisiae*) is popular at present.[25] Thermophilic bacteria can also ferment lignocellulosic hydrolysates.[26] An adapted xylanolytic bacterium, namely, *Thermoanaerobacter mathranii*, could ferment xylose from wheat straw to ethanol.[27,28] In addition, thermophilic bacteria such as *Bacillus* played an important role in liquor making.[29]

In our previous study, a bacterium named *Bacillus* sp. EtOH, which produced ethanol and Baijiu aroma substances, was isolated from HTD of Danquan Baijiu.[30] First, different pretreatment methods (NaOH, H_2SO_4, and sodium percarbonate/glycerol) were utilized to determine the optimal method in this study. Second, RSM was utilized to optimize NaOH pretreatment conditions (NaOH concentration, solid-to-liquid ratio, and time) and elaborate on the effect of different pretreatment conditions on solid yield and lignin removal. Third, enzymolysis conditions of cellulase including enzyme dosage, time, and solid-to-liquid ratio, were optimized by RSM to obtain the optimal enzymolysis effect. At last, *Bacillus* sp. EtOH effectively utilized the enzymolysis product to produce bioethanol.

2. MATERIALS AND METHODS

2.1. Experimental Materials and Strain. Sugarcane bagasse was obtained from Hainan Guzun Technology Co., Ltd. (19°31′32″−20°04′52″ north latitude, 110°07′22″−110°42′32″ east longitude). *Bacillus* sp. EtOH was isolated from HTD of Danquan Baijiu Co., Ltd. (24°42′−25°37′ north latitude, 107°1′−107°55′ east longitude) in our previous study.[30]

2.2. Selection of SCB Pretreatment Methods. SCB was pretreated by acid, alkali, and sodium percarbonate/glycerol based on the previous studies.[31,32] Four compounds of analytical grade (NaOH, H_2SO_4, sodium percarbonate, and glycerol) were purchased from Sangon Biotec Co., Ltd. (Shanghai, China). SCB was dried to constant weight and then crushed with a grinder. 1 g of SCB through a 100-mesh screen (diameter, 0.15 mm) was treated with 2% NaOH, 2% H_2SO_4, and 12% sodium percarbonate/glycerol at 121 °C for 40 min. A solid-to-liquid ratio of 1:10 (g/mL) was used. After centrifugation (Centrifuge 5702R, Eppendorf, Germany) at 8000 rpm for 5 min, the solid part was washed with deionized water to neutral pH. Then, the solid part was filtered, dried to constant weight, and crushed. The solid yield and lignin removal of each treatment method were measured. Three replicates were conducted for each group. The solid yield = dry weight after pretreatment/dry weight before pretreatment. The acid detergent lignin method was used to calculate lignin removal.[33]

2.3. Optimization of NaOH Pretreatment Conditions for SCB by RSM. After the determination of the best method (NaOH pretreatment), three factors including NaOH concentration (A), time (B), and solid-to-liquid ratio (C) were optimized to obtain the optimal lignin removal and solid yield.[34] Different combinations of the three factors were designed by the Box−Behnken design (BBD) of RSM (Tables S1 and S2 in the Supporting Information). All tests were conducted in triplicate to obtain the mean of lignin removal and solid yield. The NaOH pretreatment conditions for SCB were optimized through response surface analysis of Design-Expert 10.0.4 software.

2.4. Optimization of the Enzymolysis Conditions of Cellulase for Pretreated SCB by RSM. Cellulase (10 000 U/g) with analytical grade was purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Three factors including enzyme dose (A), time (B) and solid-to-liquid ratio (C) were optimized to obtain the optimal reducing sugar yield.[35] Different combinations of the three factors were designed by the BBD of RSM (Tables S3 and S4 in the Supporting Information). All tests were conducted in triplicate to obtain the mean of the reducing sugar yield. The enzymolysis conditions of cellulase for SCB were optimized through response surface analysis of Design-Expert 10.0.4 software after pretreatment.

2.5. Configuration and Inoculation of Sugarcane Bagasse Fermentation Medium. After enzymatic hydrolysis, the hydrolysate was centrifuged at 5000 rpm for 10 min. The supernatant was collected to determine the yield of the reducing sugar. A fermentation medium was prepared with reducing sugar from the hydrolysate (20 g/L), peptone (20 g/L), yeast extract (10 g/L), and pH (7.0−7.2) and then sterilized at 115 °C for 20 min. *Bacillus* sp. EtOH was cultured at 37 °C and 200 rpm for 84 h in the fermentation medium. The ethanol production was measured at 12, 24, 36, 48, 60, 72, and 84 h.

3. RESULTS

3.1 Effects of Different Pretreatment Methods on Solid Yield and Lignin Removal of SCB. Solid yield and lignin removal of SCB under different pretreatment methods are shown in Figure 1. The results showed that solid yield (51.15%) and lignin removal (75.52%) were the highest under NaOH pretreatment. Therefore, the subsequent RSM was designed for NaOH concentration, solid-to-liquid ratio, and time.

3.2. Optimization of NaOH Pretreatment Conditions for SCB using RSM. Solid yield and lignin removal of SCB are shown in Table S2in the Supporting Information. The regression equation reflected the relationship among solid yield (Y) or lignin removal (Z) and NaOH concentration (A), time (B), and solid-to-liquid ratio (C).

$$Y = 0.51 - 0.044A + 0.031B - 0.0022C - 0.013AB + 0.013AC - 0.004BC - 0.029A^2 - 0.019B^2 + 0.016C^2$$

$$Z = 0.84 + 0.020A + 0.030B + 0.005C - 0.027AB + 0.062AC + 0.001BC - 0.031A^2 + 0.006B^2 + 0.003C^2$$

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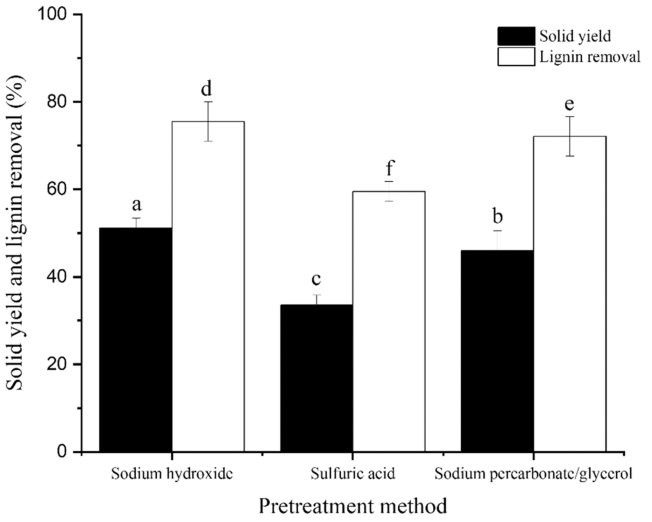

Figure 1. Effects of different pretreatment methods on the solid yield and lignin removal.

Tables S5 and S6 (Supporting Information) show the analysis of variance (ANOVA) of two models (solid yield and lignin removal). The F value (7.150, P = 0.0083), the lack of fit (P = 0.1777), and the coefficient of determination R^2 (0.9019) indicated the reliability of the model for solid yield. The F value (10.214, P = 0.0029), the lack of fit (P = 0.1999), and R^2 (0.9292) indicated the reliability of the model for lignin removal. As shown in Figure 2, there were very small differences in the actual and predicted values for solid yield and lignin removal. As shown in Figure 3, most of the points reflecting the relationship between the predicted response values and the residuals were in an irregular distribution. The results of Figures 2 and 3 showed that the two models (for solid yield and lignin removal) were reliable.

The interaction effects of the three factors on solid yield and lignin removal were shown in 3D response surface plots (Figures 4 and 5). The steeper slope indicates a more significant interaction between the two variables. In addition, a preliminary determination can be made from the color of the response surface plot, which tends to deepen when the trend of change is sharp.

Figure 4 shows the effects of NaOH concentration (A), time (B), and solid-to-liquid ratio (C) on the solid yield. As shown in Figure 4a, when time was certain, the solid yield decreased with the increase of NaOH concentration. When NaOH concentration was constant, solid yield increased with the increase of time. Figure 4b shows the effects of NaOH concentration (A) and solid-to-liquid ratio (C) on solid yield. When the NaOH concentration was certain, the solid yield increased with the increase of the solid-to-liquid ratio. When the solid-to-liquid ratio was constant, the solid yield decreased with the increase of NaOH concentration. Figure 4c shows the effects of time (B) and solid-to-liquid ratio (C) on solid yield. When time was certain, the solid yield increased with the increase of the solid-to-liquid ratio. When the solid-to-liquid ratio was certain, the solid yield increased with the increase of time. The above analysis (Figure 4a–4c) indicated that the interactions among NaOH concentration, time, and solid-to-liquid ratio had a certain effect on solid yield.

Figure 5 shows the effects of NaOH concentration (A), time (B), and solid-to-liquid ratio (C) on lignin removal. As shown in Figure 5a, when time was certain, lignin removal increased with the increase in NaOH concentration. When NaOH concentration was certain, lignin removal increased with the increase of time. Figure 5b shows the effects of NaOH concentration (A)

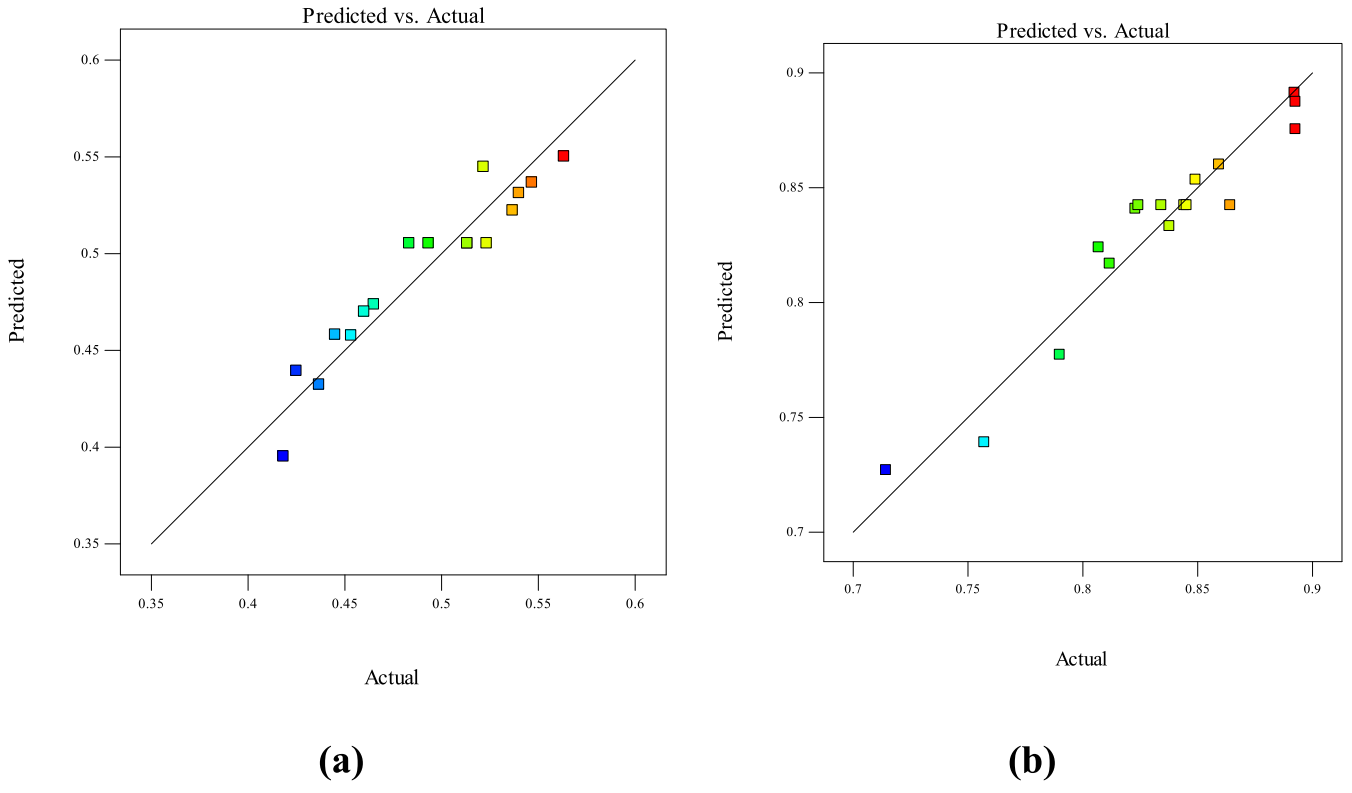

Figure 2. Predicted and actual response values for solid yield (a) and lignin removal (b).

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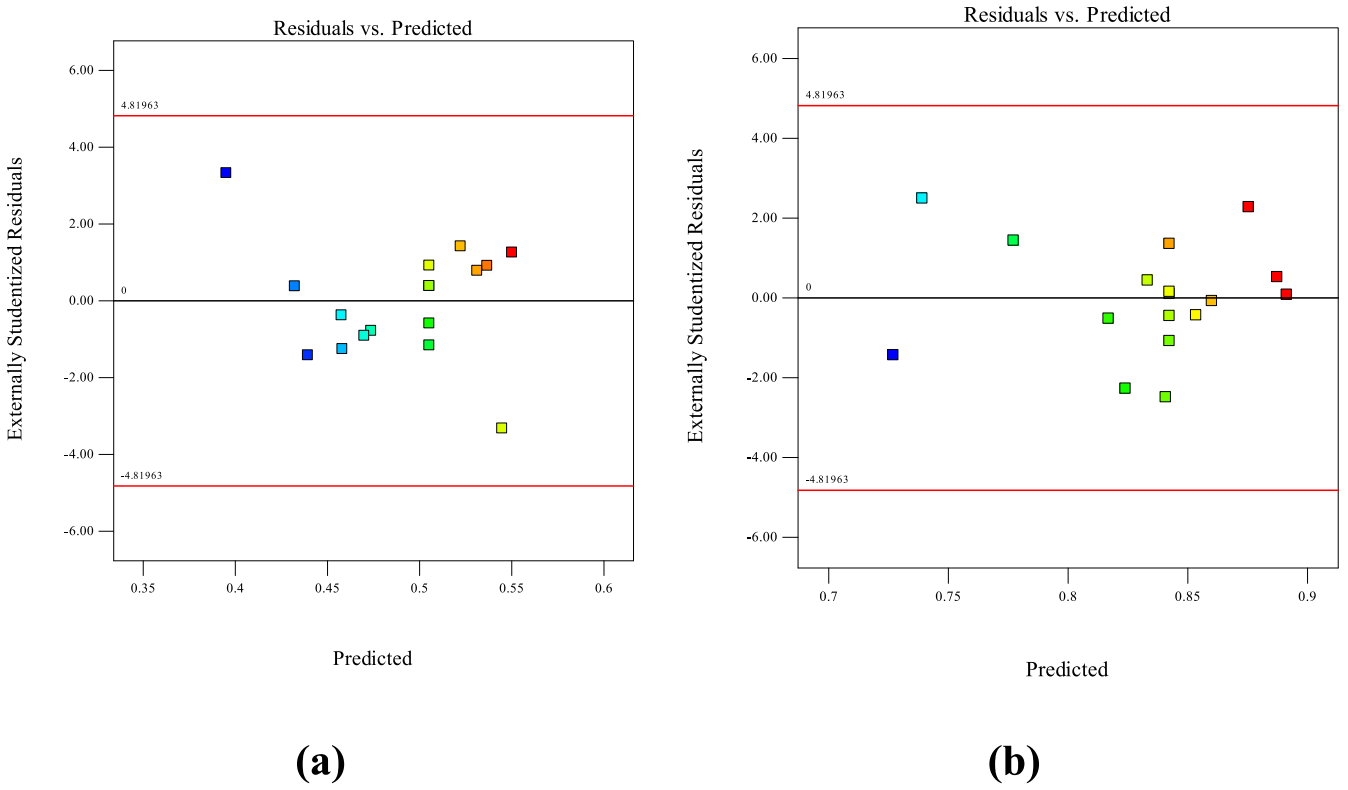

Figure 3. Comparison of residual and predicted response values. (a, b) Solid yield and lignin removal, respectively.

and solid-to-liquid ratio (C) on lignin removal. When NaOH concentration was certain, lignin removal decreased with the increase of the solid-to-liquid ratio. When the solid-to-liquid ratio was certain, lignin removal increased with the increase of NaOH concentration. Figure 5c shows the effects of time (B) and solid-to-liquid ratio (C) on lignin removal. When time was certain, lignin removal increased with the increase of the solid-to-liquid ratio. When the solid-to-liquid ratio was certain, lignin removal increased with the increase of time. The above analysis (Figure 5a−5c) indicated that the interactions among the NaOH concentration, time, and solid-to-liquid ratio had a certain effect on lignin removal.

The optimum values of the influencing factors were obtained by the analysis of Design-Expert software to achieve the highest solid yield and lignin removal. The optimum conditions were as follows: a NaOH concentration of 1%, a time of 60 min, and a solid-to-liquid ratio of 1:15. Under the optimum conditions, the theoretical maxima of the solid yield and lignin removal reached 56.5 and 84.1%, respectively. Then, the optimum solid yield and lignin removal under the optimal conditions were verified. The actual maxima of solid yield and lignin removal were 54.60 and 82.30%, respectively, which were consistent with the theoretical values. Therefore, the models were reliable and could be used to optimize the pretreatment conditions of SCB.

3.3. Optimization of Enzymolysis Conditions of Cellulase Using RSM. The yields of reducing sugar in the enzymatic hydrolysate are shown in Table S4 (Supporting Information). The regression equation reflected the relationship among the reducing sugar yield (R) and enzyme dose (A), time (B), and solid-to-liquid ratio (C).

$$R = 401.70 - 45.39A + 3.48B + 40.55C - 9.54AB - 15.46AC - 10.72BC - 59.46A^2 - 83.88B^2 - 44.27C^2$$

Table S7 (Supporting Information) shows the ANOVA of the model for reducing sugar yield. The F value (10.26, $P = 0.0028$), the lack of fit ($P = 0.0863$), and R^2 (0.9295) indicated the reliability of the model for reducing the sugar yield. As shown in Figure 6a, there were very small differences in the actual and predicted reducing sugar yields. As shown in Figure 6b, most of the points were in irregular distribution, which reflected the relationship between the predicted response values and the residuals. The results of Figure 6 showed the reliability of the model for reducing the sugar yield.

To visually reflect the interactions among influence factors, the effects of three variables on the reducing sugar yields of the enzymatic hydrolysate were shown in the 3D response surface plot (Figure 7). Figure 7 shows the effects of enzyme dose (A), time (B), and solid-to-liquid ratio (C) on reducing sugar yields. As shown in Figure 7a, when enzyme dose (A) was certain, the reducing sugar yield first increased and then decreased with the increase of time (B). When time (B) was certain, the reducing sugar yield increased with the increase of enzyme dose (A). As shown in Figure 7b, when the solid-to-liquid ratio (C) was certain, the reducing sugar yield increased with the increase of enzyme dose (A). When enzyme dose (A) was certain, the reducing sugars yield increased with the increase in the solid-to-liquid ratio (C). As shown in Figure 7c, when the solid-to-liquid ratio (C) was certain, the reducing sugar yield first increased and then decreased with the increase of time (B). When time (B) was certain, the reducing sugar yield increased with the increase of the solid-to-liquid ratio (C). The above analysis (Figure 7a−7c) showed that the interactions among enzyme dose, time, and

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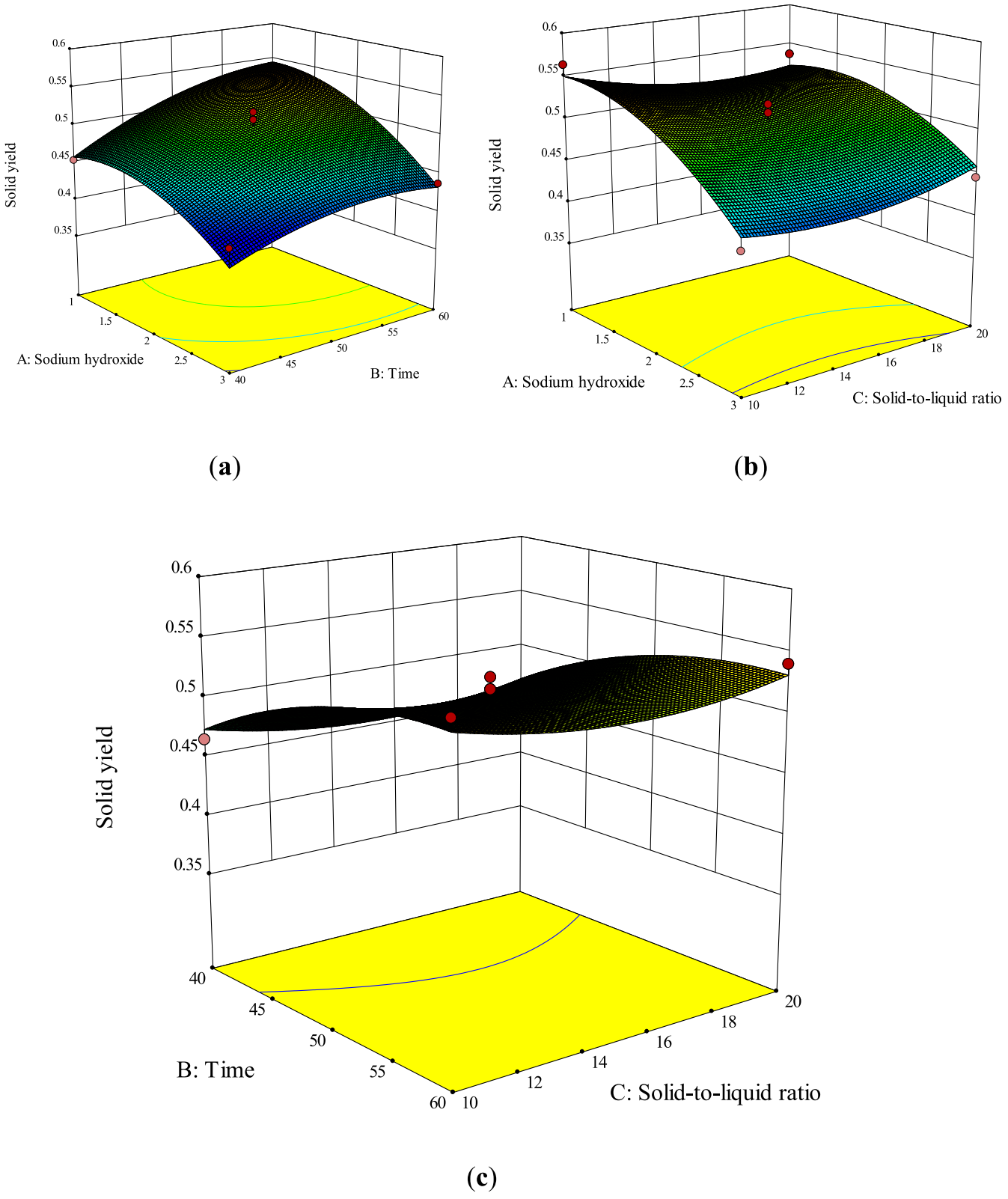

Figure 4. Effects of (a) NaOH concentration and time, (b) NaOH concentration and solid-to-liquid ratio, and (c) time and solid-to-liquid ratio on solid yield.

solid-to-liquid ratio had a certain effect on reducing the sugar yield of the enzymatic hydrolysate.

The optimum values of the influencing factors were obtained by the analysis of Design-Expert software to achieve the highest yield of reducing sugar in the enzymatic hydrolysate. The optimum enzymatic conditions were as follows: an enzyme dose of 18 FPU/g, a time of 64 h, and a solid-to-liquid ratio of 1:30. Under the optimum conditions, the theoretical maximum of reducing sugar yield was 466.59 mg/g original SCB. Then, the highest reducing sugar yield under the optimal conditions was

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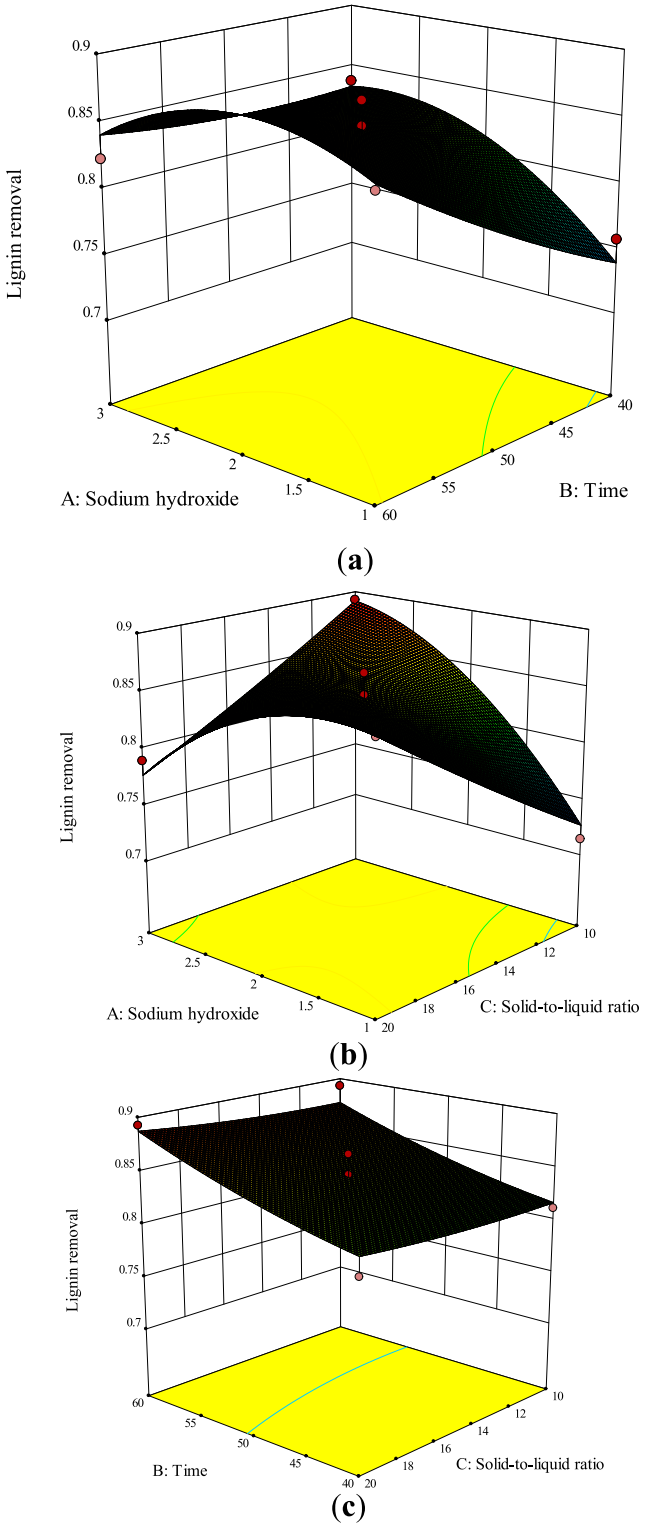

Figure 5. Effects of (a) NaOH concentration and time, (b) NaOH concentration and solid-to-liquid ratio, and (c) time and solid-to-liquid ratio on lignin removal.

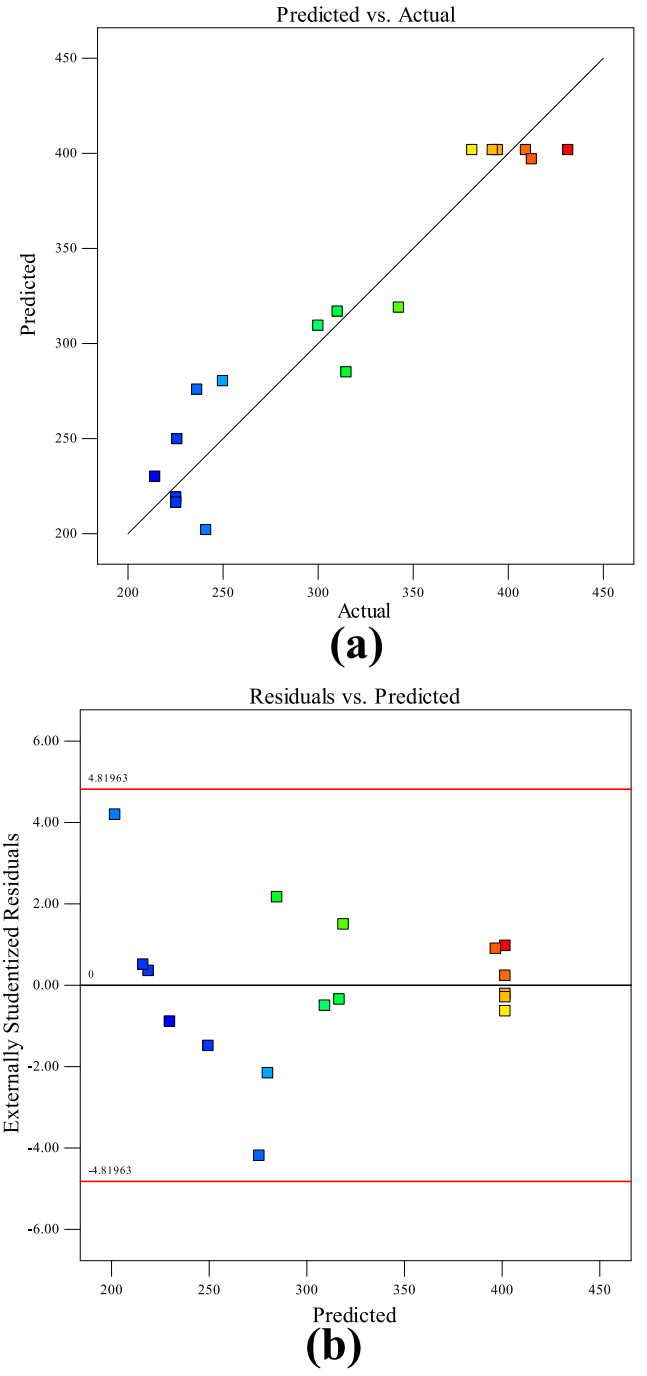

Figure 6. Graphs of reducing sugar yields. (a) Predicted and actual reducing sugar yields. (b) Residual and predicted response values for reducing sugar yields.

verified. The actual maximum of reducing sugar yield was 443.52 mg/g original SCB, which was consistent with the theoretical value. Therefore, the model was reliable and could be used to optimize enzymolysis conditions of NaOH-pretreated SCB.

3.4. Bioethanol Production by Fermenting the Enzymolysis Product by *Bacillus* sp. EtOH. The ethanol-producing strain EtOH obtained from our previous study was inoculated into a fermentation medium containing cellulase-treated hydrolysate of SCB (final reducing sugar content of 20 g/L). The change in the ethanol concentration in the fermentation medium was observed. As shown in Figure S1 (Supporting Information), ethanol concentration quickly increased from 0 to 24 h and reached the maximum (2.769 g/L) at 36 h. Ethanol concentration showed a decreased trend from 36 to 48 h, had a slight increase from 48 to 60 h, and then continuously decreased from 60 to 84 h.

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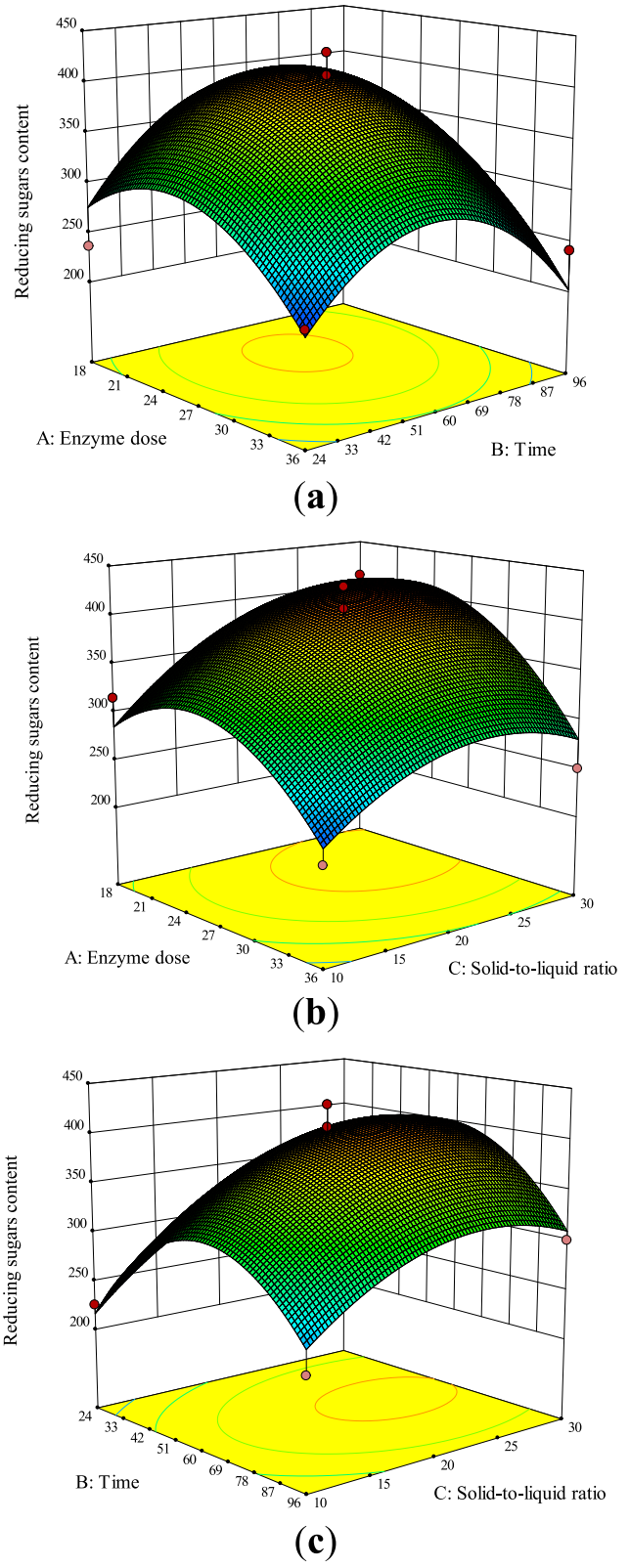

Figure 7. Effects of (a) enzyme dose and time, (b) enzyme dose and solid-to-liquid ratio, and (c) time and solid-to-liquid ratio on reducing sugar yield.

4. DISCUSSION

Nowadays, the primary issue is the shortage of conventional energy sources (such as petroleum, natural gas, and coal) and the harsh environmental impact. Therefore, the alternative new energy from lignocellulosic biomass has attracted great attention. The agricultural wastes such as SCB emerged as the interesting substrates for the microbial fermentation due to the accessibility and composition (cellulose, hemicellulose, and lignin).[36] Sugarcane (1.6 billion tonnes per year) is one of the most widely distributed biomasses in the world, and the reasonable use of its waste SCB (such as bioethanol production) may play an important role in relieving energy-related problems.[37] In recent years, China's sugarcane production accounts for about 6% of global sugarcane production. SCB accounts for about 35% of the weight of the sugarcane. Therefore, the utilization of SCB is an important issue in China.

The most difficult problem of the utilization of SCB is the conversion process (i.e., the transformation of SCB into value-added products).[38] The transformation can be completed through pretreatment, hydrolysis, and microbial fermentation. The biological pretreatment methods are time-consuming compared with chemical pretreatment methods.[39] In this study, the effects of three chemical pretreatment methods (NaOH, H_2SO_4, and sodium percarbonate/glycerol) on solid yield and lignin removal were investigated. The solid yield and lignin removal were 51.15 and 75.52% under the treatment of 2% NaOH, a time of 40 min, and a solid-to-liquid ratio of 1:10, respectively (Figure 1). The delignification efficiency of 75.52% was higher than that of 57% under the treatment of 1.2% H_2SO_4 and 90% glycerol.[40] A combined process of dilute acid and ionic liquid treatments achieved 80.2% lignin removal, which was slightly higher than our unoptimized result (75.52%).[41]

To further improve the solid yield and lignin removal, RSM was utilized to optimize the NaOH pretreatment conditions (NaOH concentration, solid-to-liquid ratio, and time). RSM is a sophisticated method to optimize the process. In our previous studies, RSM was successfully used to optimize the processes including the removal of heavy metal chromium, carotenoid extraction, and culture medium composition.[42−47] The solid yield and lignin removal were greatly affected by the NaOH concentration and time in this study (Figures 4 and 5). The optimal NaOH pretreatment conditions were a NaOH concentration of 1%, a time of 60 min, and a solid-to-liquid ratio of 1:15. Under the optimal conditions, the solid yield and lignin removal were 54.60 and 82.30%, respectively. The contact between the microbe/enzyme and the active ingredients of SCB is hindered by lignin and the crystallinity of cellulose.[48] The objective of the pretreatment is to eliminate lignin/hemicelluloses, increase the area of contact between cellulose and the enzyme, and decrease the crystallinity of cellulose. NaOH significantly changed the structure of SCB and made it more compatible with cellulase. Previous studies suggested that alkali pretreatment was more suitable for SCB, which was consistent with our study.[49,50,15]

After NaOH pretreatment, RSM was used to optimize the enzymatic hydrolysis conditions (cellulase dose, time, and solid-to-liquid ratio) that affected the yield of reducing sugar. Under the optimal enzymatic hydrolysis conditions (an enzyme dose of 18 FPU/g, a time of 64 h, and a solid-to-liquid ratio of 1:30), the actual yield of reducing sugar in the enzyme-treated hydrolysate was 443.52 mg/g original SCB with a cellulose conversion rate of 85.33%. By optimizing the percentages of cellulase/xylanase/β-glucanase/pectinase, 88.5% of cellulose in NaOH-treated SCB was hydrolyzed.[51] 10% SCB pretreated with 1% H_2SO_4 was hydrolyzed by 20 FPU/g cellulase at 50 °C for 96 h, and a cellulose conversion rate of about 65% could be obtained.[52] The

https://doi.org/10.1021/acsomega.4c02485
ACS Omega 2024, 9, 29566−29575

alkali-pretreated SCB was treated with 10 FPU/g cellulase and a solid-to-liquid ratio of 33:100 for 120 h, and the final conversion rate of cellulose was 60%.[53] The main bottleneck for the industrial hydrolysis of cellulose is the cost of cellulase production. In our study, through the optimization of the enzymatic hydrolysis conditions by RSM, a high conversion rate (85.33%) was obtained, which reduced the production cost.

Yeast, especially *S. cerevisiae*, is the industrial workhorse to produce ethanol.[54] In addition, some bacteria can produce a small amount of ethanol. Ethanol-producing bacteria include *Bacillus*, *Zymomonas mobiliz*, *Leuconostoc*, and Thermoanaerobacterales.[55,56] Our previous study showed that the dominant fungi included *Aspergillus, Zygosaccharomyces, Issatchenkia, Monascus, Millerozyma, Thermoascus, Thermomyces, Hyphopichia, Rhizomucor, Lichtheimia*, and *Cladosporium* in three samples from Danquan Baijiu production (HTD samples "dqjq_ck" and "dqjqcp", and the fermented grain sample dqjp3).[57] Our previous study also found that with the higher HTD-making temperature, most yeasts and molds were destroyed, and the microbial community of HTD primarily propagated thermophilic bacteria such as *Bacillus*.[58] In addition, *Bacillus* was the dominant bacterium in sample "dqjqcp".[58] Therefore, we suggested that *Bacillus* was primarily responsible for producing ethanol rather than yeast in the Danquan Baijiu production. Therefore, we isolated the bacterial strain *Bacillus* sp. EtOH from the HTD of Danquan Baijiu in this study. This strain can produce ethanol and Baijiu aroma substances such as tetramethylpyrazine (an important active substance). When the strain EtOH was cultured for 36 h in a fermentation medium containing cellulase-treated SCB hydrolysate, yeast extract, and peptone, ethanol concentration reached 2.769 g/L (0.353%, v/v). The yields of sugar-to-ethanol and SCB-to-ethanol were 13.85 and 11.81% in this study, respectively. The ethanol concentrations and yields are summarized in Table S8 (Supporting Information). As shown in Table S8, the ethanol yield of strain EtOH was lower than that of a variety of yeasts.[59−63] However, strain EtOH can produce tetramethylpyrazine (an important active substance). Our study provided a suitable way for bioethanol production from SCB, which achieved the efficient resource utilization of the agricultural waste SCB.

5. CONCLUSIONS

Among the three pretreatment methods, NaOH pretreatment was the most suitable method based on the solid yield and delignification efficiency. After optimization by RSM, the solid yield and lignin removal were 54.60 and 82.30% under the treatment of 1% NaOH, a time of 60 min, and a solid-to-liquid ratio of 1:15, respectively. Under the optimal enzymatic hydrolysis conditions (an enzyme dose of 18 FPU/g, a time of 64 h, and a solid-to-liquid ratio of 1:30), the actual yield of reducing sugar in the enzyme-treated hydrolysate was 443.52 mg/g original SCB with a cellulose conversion rate of 85.33%. A bacterium, namely, *Bacillus* sp. EtOH, which produced ethanol and Baijiu aroma substances, was isolated from the HTD of Danquan Baijiu in our previous study. When the strain EtOH was fermented for 36 h in a fermentation medium (cellulase-treated SCB, yeast extract, and peptone), ethanol concentration reached 2.769 g/L (0.353%, v/v). The yields of sugar-to-ethanol and SCB-to-ethanol were 13.85 and 11.81% in this study, respectively. In brief, after NaOH pretreatment, 1 g of original SCB produced 0.5460 g of NaOH-pretreated SCB, and then after the enzymatic hydrolysis, the highest reducing sugar yield (443.52 mg/g original SCB) was obtained. Our strategy provided a suitable way for bioethanol production from SCB.

■ ASSOCIATED CONTENT

Data Availability Statement

The data are available throughout the manuscript and supporting files.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c02485.

Factors and levels of BBD for NaOH pretreatment conditions (Table S1); groups of BBD, solid yield, and lignin removal for NaOH pretreatment conditions (Table S2); results of reducing sugar yield in cellulase-treated hydrolysate (Table S3); results of reducing sugar yield in cellulase-treated hydrolysate (Table S4); analysis of variance for the model (solid yield) (Table S5); analysis of variance for the model (lignin removal) (Table S6); analysis of variance for the model (reducing sugar yield) (Table S7); comparison of recent data with the present study on ethanol production (Table S8); and change of ethanol concentration in the fermentation medium (Figure S1) (PDF)

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https://doi.org/10.1021/acsomega.4c02485
ACS Omega 2024, 9, 29566−29575

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work was funded by Guangxi Key Research and Development Program (Nos. AB21220057 and 2021AB27009), Innovation Project of Guangxi Graduate Education (YCSW2024164).

■ REFERENCES

(1) Nazos, A.; Grammelis, P.; Sakellis, E.; Sidiras, D. Acid-catalyzed wet torrefaction for enhancing the heating value of barley straw. *Energies* **2020**, *13*, 1693.

(2) Elshout, P. M. F.; van Zelm, R.; van der Velde, M.; Steinmann, Z.; Huijbregts, M. A. J. Global relative species loss due to first-generation biofuel production for the transport sector. *GCB Bioenergy* **2019**, *11*, 763−772, DOI: 10.1111/gcbb.12597.

(3) Sims, R. E.; Mabee, W.; Saddler, J. N.; Taylor, M. An overview of second generation biofuel technologies. *Bioresour. Technol.* **2010**, *101*, 1570−1580.

(4) Maia, J. L. D.; Cardoso, J. S.; Mastrantonio, D. J. D. S.; Bierhals, C. K.; Moreira, J. B.; Costa, J. A. V.; Morais, M. G. Microalgae starch: A promising raw material for the bioethanol production. *Int. J. Biol. Macromol.* **2020**, *165*, 2739−2749.

(5) Liao, H.; Ying, W.; Lian, Z.; Xu, Y.; Zhang, J. H. One-step sodium bisulfate hydrolysis for efficient production of xylooligosaccharides from poplar. *Bioresour. Technol.* **2022**, *355*, No. 127269.

(6) Pollegioni, L.; Tonin, F.; Rosini, E. Lignin-degrading enzymes. *FEBS J.* **2015**, *282*, 1190−1213.

(7) Schneider, W. D. H.; Goncalves, T. A.; Uchima, C. A.; Couger, M. B.; Prade, R.; Squina, F. M.; Dillon, A. J. P.; Camassola, M. *Penicillium echinulatum* secretome analysis reveals the fungi potential for degradation of lignocellulosic biomass. *Biotechnol. Biofuels* **2016**, *9*, 66.

(8) Hassan, S. S.; Williams, G. A.; Jaiswal, A. K. Emerging technologies for the pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **2018**, *262*, 310−318.

(9) Antoniêto, A. C. C.; Nogueira, K. M. V.; Mendes, V.; Maués, D. B.; Oshiquiri, L. H.; Zenaide-Neto, H.; de Paula, R. G.; Gaffey, J.; Tabatabaei, M.; Gupta, V. K.; Silva, R. N. Use of carbohydrate-directed enzymes for the potential exploitation of sugarcane bagasse to obtain value-added biotechnological products. *Int. J. Biol. Macromol.* **2022**, *221*, 456−471.

(10) Zhang, H. D.; Huang, S. H.; Wei, W. Q.; Zhang, J. J.; Xie, J. Investigation of alkaline hydrogen peroxide pretreatment and Tween 80 to enhance enzymatic hydrolysis of sugarcane bagasse. *Biotechnol. Biofuels* **2019**, *12*, 107.

(11) Ferreira, J. A.; Taherzadeh, M. J. Improving the economy of lignocellulose-based biore fineries with organosolv pretreatment. *Bioresour. Technol.* **2020**, *299*, No. 122695.

(12) Giri, R.; Sharma, R. K. Fungal pretreatment of lignocellulosic biomass for the production of plant hormone by *Pichia fermentans* under submerged conditions. *Bioresour. Bioprocess* **2020**, *7*, 30.

(13) Bensah, E. C.; Mensah, M. Chemical pretreatment methods for the production of cellulosic ethanol: Technologies and innovations. *Int. J. Chem. Eng.* **2013**, *2013*, No. 719607.

(14) Wang, Q.; Wang, W.; Tan, X.; Zahoor; Chen, X.; Guo, Y.; Yu, Q.; Yuan, Z.; Zhuang, X. Low-temperature sodium hydroxide pretreatment for ethanol production from sugarcane bagasse without washing process. *Bioresour. Technol.* **2019**, *291*, No. 121844.

(15) Wang, C.; Qi, W.; Liang, C.; Wang, Q.; Wang, W.; Wang, Z.; Yuan, Z. Impact of alkaline pretreatment condition on enzymatic hydrolysis of sugarcane bagasse and pretreatment cost. *Appl. Biochem. Biotechnol.* **2021**, *193*, 2087−2097.

(16) Hou, Q.; Ju, M.; Li, W.; Liu, L.; Chen, Y.; Yang, Q. Pretreatment of lignocellulosic bio mass with ionic liquids and ionic liquid-based solvent systems. *Molecules* **2017**, *22*, 490.

(17) Zhu, J. Q.; Qin, L.; Li, W. C.; Zhang, J.; Bao, J.; Huang, Y. D.; Li, B. Z.; Yuan, Y. J. Simultaneous saccharification and co-fermentation of dry diluted acid pretreated corn stover at high dry matter loading: Overcoming the inhibitors by non-tolerant yeast. *Bioresour. Technol.* **2015**, *198*, 39−46.

(18) Zhuang, X.; Wang, W.; Yu, Q.; Qi, W.; Wang, Q.; Tan, X.; Zhou, G.; Yuan, Z. Liquid hot water pretreatment of lignocellulosic biomass for bioethanol production accompanying with high valuable products. *Bioresour. Technol.* **2016**, *199*, 68−75.

(19) Anugwom, I.; Eta, V.; Virtanen, P.; Mäki-Arvela, P.; Hedenström, M.; Yibo, M.; Hummel, M.; Sixta, H.; Mikkola, J. P. Towards optimal selective fractionation for Nordic woody biomass using novel amine−organic superbase derived switchable ionic liquids (SILs). *Biomass Bioenergy* **2014**, *70*, 373−381.

(20) Jogi, R.; Mäki-Arvela, P.; Virtanen, P.; Kumar, N.; Hemming, J.; Russo, V.; Samikannu, A.; Lestander, T. A.; Mikkola, J. P. Understanding the formation of phenolic monomers during fractionation of birch wood under supercritical ethanol over iron based catalysts. *J. Energy Inst.* **2020**, *93*, 2055−2062.

(21) Nguyen, T. C.; Anne-Archard, D.; Fillaudeau, L. Rheology of lignocellulose suspensions and impact of hydrolysis: A review. *Filaments Bioprocesses* **2015**, *149*, 325−357, DOI: 10.1007/10_2015_323.

(22) Ogier, J. C.; Leygue, J. P.; Ballerini, D.; Pourquie, J.; Rigal, L. Production d'ethanol a partir de biomasse lignocellulosique. *Oil Gas Sci. Technol.* **1999**, *54*, 67−94, DOI: 10.2516/ogst:1999004.

(23) Joyjamras, K.; Chaotham, C.; Chanvorachote, P. Response surface optimization of enzymatic hydrolysis and ROS scavenging activity of silk sericin hydrolysates. *Pharm. Biol.* **2022**, *60*, 308−318.

(24) Hunsakul, K.; Laokuldilok, T.; Sakdatorn, V.; Klangpetch, W.; Brennan, C. S.; Utama- Ang, N. Optimization of enzymatic hydrolysis by alcalase and flavourzyme to enhance the antioxidant properties of jasmine rice bran protein hydrolysate. *Sci. Rep.* **2022**, *12*, No. 12582.

(25) Walker, G. M.; Walker, R. S. K. Enhancing yeast alcoholic fermentations. *Adv. Appl. Microbiol.* **2018**, *105*, 87−129.

(26) Sommer, P. Conversion of Hemicellulose and D-xylose into Ethanol by the Use of Thermophilic Anaerobic Bacteria, PhD Thesis; Technical University of Denmark1998.

(27) Ahring, B. K.; Jensen, K.; Nielsen, P.; Bjerre, A. B.; Schmidt, A. S. Pretreatment of wheat straw and conversion of xylose and xylan to ethanol by thermophilic anaerobic bacteria. *Bioresour. Technol.* **1996**, *58*, 107−113, DOI: 10.1016/S0960-8524(96)00090-9.

(28) Klinke, H. B.; Thomsen, A. B.; Ahring, B. K. Potential inhibitors from wet oxidation of wheat straw and their effect on growth and ethanol production by *Thermoanaerobacter mathranii*. *Appl. Microbiol. Biotechnol.* **2001**, *57*, 631−638.

(29) Han, P. J.; Luo, L. J.; Han, Y.; Song, L.; Zhen, P.; Han, D. Y.; Wei, Y. H.; Zhou, X.; Wen, Z.; Qiu, J. Z.; Bai, F. Y. Microbial community affects Daqu quality and the production of ethanol and flavor compounds in Baijiu fermentation. *Foods* **2023**, *12*, 2936.

(30) Wu, H.; Huang, L.; Li, J.; Yang, D.; Shang, C. Optimization of the conditions for the growth and ethanol production of strain *Bacillus* sp. EtOH. *J. Biobased Mater. Bioenergy* **2024**, *18*, 333−338, DOI: 10.1166/jbmb.2024.2375.

(31) Uppal, S. K.; Kaur, R.; Sharma, P. Optimization of chemical pretreatment and acid saccharification for conversion of sugarcane bagasse to ethanol. *Sugar Tech* **2011**, *13*, 214−219.

(32) Rabelo, S. C.; Andrade, R. R.; Filho, R. M.; Costa, A. C. Alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis and fermentation of sugarcane bagasse to ethanol. *Fuel* **2014**, *136*, 349−357.

(33) Raffrenato, E.; Van Amburgh, M. E. Technical note: improved methodology for analyses of acid detergent fiber and acid detergent lignin. *J. Dairy Sci.* **2011**, *94*, 3613−3617.

(34) Zhang, X. B. H.; Zhang, T.; Xie, W.; Zhu, M. J.Impacts of NaOH pretreatment on enzymolysis and fermentation performance of sugarcane bagasse *Biomass Chem. Eng.* 2016; Vol. *50*, pp 9−16.
(35) Lv, X. J.; Wu, Y. H.; Jiang, X. H.; Cheng, X. A.; Li, J. T.Optimization of enzymatic hydrolysis conditions of sugar cane bagasse pretreated by sodium hydroxide based on response surface methodology *China Brewing* 2022; Vol. *41*, pp 161−166.
(36) Ng, H. S.; Kee, P. E.; Yim, H. S.; Chen, P. T.; Wei, Y. H.; Chi-Wei Lan, J. Recent advances on the sustainable approaches for conversion and reutilization of food wastes to valuable bioproducts. *Bioresour. Technol.* **2020**, *302*, No. 122889.
(37) Jugwanth, Y.; Sewsynker-Sukai, Y.; Gueguim Kana, E. B. Valorization of sugarcane bagasse for bioethanol production through simultaneous saccharification and fermentation: optimization and kinetic studies. *Fuel* **2020**, *262*, No. 116552.
(38) Shabbirahmed, A. M.; Haldar, D.; Dey, P.; Patel, A. K.; Singhania, R. R.; Dong, C. D.; Purkait, M. K. Sugarcane bagasse into value-added products: a review. *Environ. Sci. Pollut. Res.* **2022**, *29*, 62785−62806.
(39) Sarker, T. R.; Pattnaik, F.; Nanda, S.; Dalai, A. K.; Meda, V.; Naik, S. Hydrothermal pretreatment technologies for lignocellulosic biomass: a review of steam explosion and subcritical water hydrolysis. *Chemosphere* **2021**, *284*, No. 131372.
(40) Zhao, X.; Li, S.; Wu, R.; Liu, D. Organosolv fractionating pretreatment of lignocellulosic biomass for efficient enzymatic saccharification: chemistry, kinetics, and substrate structures. *Biofuels, Bioprod. Biorefin.* **2017**, *11*, 567−590, DOI: 10.1002/bbb.1768.
(41) Diedericks, D.; van Rensburg, E.; Görgens, J. F. Fractionation of sugarcane bagasse using a combined process of dilute acid and ionic liquid treatments. *Appl. Biochem. Biotechnol.* **2012**, *167*, 1921−1937.
(42) Gong, D.; Ye, F.; Pang, C.; Lu, Z.; Shang, C. Isolation and characterization of *Pseudomonas* sp. Cr13 and its application in removal of heavy metal chromium. *Curr. Microbiol.* **2020**, *77*, 3661−3670, DOI: 10.1007/s00284-020-02162-5.
(43) Pang, B.; Lv, L.; Pang, C.; Ye, F.; Shang, C. Optimization of growth conditions of *Acinetobacter* sp. Cr1 for removal of heavy metal Cr using central composite design. *Curr. Microbiol.* **2021**, *78*, 316−322.
(44) Li, Y.; Huang, X.; Luo, L.; Shang, C. Optimization of extraction conditions of carotenoids from *Dunaliella parva* by response surface methodology. *Molecules* **2022**, *27*, 1444.
(45) Gan, S.; Liang, S.; Zou, Q.; Shang, C. Optimization of carotenoid extraction of a halophilic microalgae. *PLoS One* **2022**, *17*, No. e0270650.
(46) Li, Y.; Gan, S.; Luo, L.; Yang, W.; Mo, L.; Shang, C. Optimization of molasses and soy bean meal content to enhance tetramethylpyrazine yield by *Bacillus* sp. TTMP20. *Molecules* **2023**, *28*, 6515.
(47) Gan, S.; Luo, L.; Ding, X.; Li, Y.; Yang, D.; Shang, C. Optimization of growth medium of *Bacillus* sp. TTMP20 by response surface methodology. *J. Biobased Mater. Bioenergy* **2023**, *17*, 783−789, DOI: 10.1166/jbmb.2023.2328.
(48) Rocha, G. J.; Martín, C.; da Silva, V. F.; Gómez, E. O.; Gonçalves, A. R. Mass balance of pilot-scale pretreatment of sugarcane bagasse by steam explosion followed by alkaline delignification. *Bioresour. Technol.* **2012**, *111*, 447−452.
(49) Ejaz, U.; Muhammad, S.; Ali, F. I.; Hashmi, I. A.; Sohail, M. Methyltrioctylammonium chloride mediated removal of lignin from sugarcane bagasse for themostable cellulase production. *Int. J. Biol. Macromol.* **2019**, *140*, 1064−1072.
(50) Kim, J. S.; Lee, Y. Y.; Kim, T. H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresour. Technol.* **2016**, *199*, 42−48.
(51) Chen, P.; Tao, S.; Zheng, P. Efficient and repeated production of succinic acid by turning sugarcane bagasse into sugar and support. *Bioresour. Technol.* **2016**, *211*, 406−413.
(52) Borges, D. G.; Junior, A. B.; Farinas, C. S.; Giordano, R. D.; Tardioli, P. W. Enhanced saccharification of sugarcane bagasse using soluble cellulase supplemented with immobilized β-glucosidase. *Bioresour. Technol.* **2014**, *167*, 206−213, DOI: 10.1016/j.biortech.2014.06.021.
(53) Gao, Y.; Xu, J.; Yuan, Z.; Zhang, Y.; Liu, Y.; Liang, C. Optimization of fed-batch enzymatic hydrolysis from alkali-pretreated sugarcane bagasse for high-concentration sugar production. *Bioresour. Technol.* **2014**, *167*, 41−45.
(54) Gambacorta, F. V.; Dietrich, J. J.; Yan, Q.; Pfleger, B. F. Rewiring yeast metabolism to synthesize products beyond ethanol. *Curr. Opin. Chem. Biol.* **2020**, *59*, 182−192.
(55) Klinke, H. B.; Thomsen, A. B.; Ahring, B. K. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Appl. Microbiol. Biotechnol.* **2004**, *66*, 10−26, DOI: 10.1007/s00253-004-1642-2.
(56) Li, X. F.; Tian, S.; Pan, Y. P.; Yang, X. S.Development of ethanol production from pentose and hexose by bacterium *Microbiol. China* 1992, *30*, 101−105.
(57) Wu, H.; Li, J.; Zhang, J.; Wu, S.; Xiang, X.; Lu, Z.; Shang, C. Analysis of fungal diversity of three samples (dqjq_ck, dqjqcp and dqjp3) from Danquan distillery. *J. Biobased Mater. Bioenergy* **2023**, *17*, 196−202, DOI: 10.1166/jbmb.2023.2267.
(58) Shang, C.; Li, Y.; Zhang, J.; Gan, S. Analysis of bacterial diversity in different types of Daqu and fermented grains from Danquan distillery. *Front. Microbiol.* **2022**, *13*, No. 883122.
(59) Orrego, D.; Zapata-Zapata, A. D.; Kim, D. Ethanol production from coffee mucilage fermentation by *S. cerevisiae* immobilized in calcium-alginate beads. *Bioresour. Technol. Rep.* **2018**, *3*, 200−204.
(60) Karagöz, P.; Özkan, M. Ethanol production from wheat straw by *Saccharomyces cerevisiae* and *Scheffersomyces stipitis* co-culture in batch and continuous system. *Bioresour. Technol.* **2014**, *158*, 286−293.
(61) Menon, V.; Prakash, G.; Prabhune, A.; Rao, M. Biocatalytic approach for the utilization of hemicellulose for ethanol production from agricultural residue using thermostable xylanase and thermotolerant yeast. *Bioresour. Technol.* **2010**, *101*, 5366−5373.
(62) Singhvi, M. S.; Deshmukh, A. R.; Kim, B. S. Cellulase mimicking nanomaterial- assisted cellulose hydrolysis for enhanced bioethanol fermentation: an emerging sustainable approach. *Green Chem.* **2021**, *23*, 5064−5081.
(63) Kim, M.; Singhvi, M. S.; Kim, B. S. Eco-friendly and rapid one-step fermentable sugar production from raw lignocellulosic biomass using enzyme mimicking nanomaterials: A novel cost-effective approach to biofuel. *Chem. Eng. J.* **2023**, *465*, No. 142879.

https://doi.org/10.1021/acsomega.4c02485
ACS Omega 2024, 9, 29566−29575