Pretreatment of *Sophora flavescens* Residues to Produce Fermentable Sugars for Lactic Acid Production: Optimization and Mechanism Analysis

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The effects of three different pretreatments (H_2SO_4-heat, NaOH-heat, and hydrothermal pretreatment) on the enzymatic hydrolysis efficiency of *Sophora flavescens* residues (SFRs) were studied. After NaOH-heat pretreatment, the cellulose content of SFRs increased from 46.6% to 59.4%, and hydrolysis efficiency was 2.3 times higher than that of direct enzymatic hydrolysis without pretreatment. Moreover, the optimum pretreatment conditions were NaOH concentration of 1.00%, solid-liquid ratio of 1:10, temperature of 80 °C, and pretreatment time of 60 min. Under these conditions, 85.0% hydrolysis efficiency was obtained. When SFRs with NaOH-heat pretreatment were used as feedstocks in fermentation, the maximum lactic acid concentration was 33.7 g/L, which was twice that produced by SFRs without pretreatment. Mechanism analysis suggested that the lignin of SFRs was removed by NaOH solution, and the deacetylation reaction of hemicellulose occurred, which made the original smooth and dense structure become rough and loose, thus improving the accessibility of cellulose to enzymes.

Keywords: Pretreatment; Sophora flavescens residues; Lactic acid fermentation; Cellulose; Hemicellulose; Lignin

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INTRODUCTION

In recent years, the interest and demand for lactic acid has increased considerably. Lactic acid produced by fermentation is widely used in food, pharmaceutical, cosmetics, and leather industries; considerable attention to synthetic plastic pollution is driving the development of technologies for the production of biodegradable plastic poly-lactic acid (PLA) that is synthesized from lactic acid (Li *et al.* 2019; López-Gómez *et al.* 2019). At present, starch-based substances (corn, cassava, potatoes, *etc.*) and sugar-based substances (sugarcane, sugar beet, *etc.*) are the main raw materials in the process of lactic acid fermentation. Using these substances is expensive and can contribute to food shortage (Alves De Oliveira *et al.* 2018). If lactic acid could be fermented with renewable raw materials (such as lignocellulose wastes) as feedstock, the cost of lactic acid production could be greatly reduced (Gezae Daful and Görgens 2017; Kasmi *et al.* 2017; Djukic-

Vukovic *et al.* 2019; Li *et al.* 2019).

The rapid development of the traditional Chinese medicine industry and the enhancement of people's health awareness has increased the application of traditional Chinese herbal medicine and Chinese patent medicine worldwide. However, a large amount of waste herb residues are generated in the production of traditional Chinese medicine. The root of the perennial leguminous shrub *Sophora flavescens* Ait. contains various alkaloids, including matrine and oxymatrine (He *et al.* 2015). Matrine and oxymatrine have been widely used in clinical medicine due to their anti-allergic, anti-inflammatory, and hepatoprotective effects. *Sophora flavescens* residues (SFRs) are obtained after the extraction of matrine and oxymatrine from *S. flavescens* (Wang *et al.* 2017). The conversion of SFRs to value-added products, such as ethanol and methane, can alleviate the problem of energy shortage, but the price of the product is also low. For example, the current market price of anhydrous ethanol is 5000 to 6000 RMB/t (Ren *et al.* 2018; Ma *et al.* 2019). However, the market price of lactic acid is 9000 to 14000 RMB/t; thus, it is of great economic significance to explore SFRs to produce lactic acid by biological fermentation.

The SFRs contain high amounts of cellulose and hemicellulose, but a relatively low amount of lignin. The cellulose of SFRs can be converted into fermentable sugars by adding cellulase, but hemicellulose and lignin in the structural arrangement form a solid binding layer, which closely encapsulates cellulose and hinders the contact between cellulase and cellulose (Kumar *et al.* 2009; Chen *et al.* 2017). Therefore, aggressive pretreatment is important to remove lignin and separate cellulose and hemicellulose in order to increase the contact between cellulase and cellulose.

Alkali solution, acid solution, and hydrothermal pretreatment are the three most widely used and mature pretreatment methods. Alkali swells cellulose and destroys lignin, thus increasing the contact area between cellulose and cellulase; acid can dissolve hemicellulose and destroy the partial binding force of lignocellulose, while hydrothermal pretreatment can partially degrade hemicellulose and lignin without adding any chemical reagents (Yang and Wyman 2008; Ioelovich 2012; Munir *et al.* 2018). However, few studies have reported the selection and optimization of pretreatment process for SFRs.

In this study, hydrothermal pretreatment, acid pretreatment, and alkali pretreatment were applied in the field of lactic acid production from SFRs by fermentation. The best pretreatment method was selected by comparing the structural changes of SFRs, hydrolysis efficiency, and mass flow of the whole process before and after different pretreatment methods. The optimization and mechanism of the best pretreatment method was explored. Finally, the lactic acid concentration from SFRs with pretreatment and non-pretreatment was compared.

EXPERIMENTAL

Materials

Preparation of Sophora flavescens residues

The *Sophora flavescens* residues (SFRs) were provided by the Shanxi Zhendong Pharmaceutical Co., Ltd., in Shanxi Province, China. They were obtained after the extraction of alkaloids from *S. flavescens via* successive acetic acid soaking and decoction. The moisture content of SFRs were 68.4 ± 3.70%; the collected samples of SFRs were mostly round sheets with a diameter of 1 to 7 cm and a thickness of 3 to 6 mm. The SFRs

were air dried to a stable weight and then smashed to a uniform size of less than 1 mm in an electric grinder for subsequent use.

Microorganism and medium

The *Lactobacillus casei* CGMCC 1.6 used in the study was obtained from the China General Microbiological Culture Collection Center, Beijing, China. The stock culture was maintained at -80 °C in vials containing 15% (*v/v*) glycerol. Man-Rogosa-Sharpe (MRS) medium was used for cell growth and inoculum preparation, as previously reported (Zheng *et al.* 2017).

Methods

Pretreatment methods

The sulfuric acid pretreatment was performed at 120 °C with 1.5% (*v/v*) H_2SO_4 solution for 2 h at a solid-liquid ratio (*w/v*) of 1:10. The sodium hydroxide pretreatment was performed at 120 °C with 1.5% (*w/v*) NaOH solution for 2 h at a solid-liquid ratio (*w/v*) of 1:10. The hydrothermal pretreatment was performed at 120 °C with deionized water for 2 h at a solid-liquid ratio (*w/v*) of 1:10. The pretreatment experiments were all carried out in 250-mL BlueCap bottles with 100-mL working volume and 10.0 g SFRs powder. Moreover, nonionic surfactants, *e.g.* polyoxyethylene (80) sorbitan monooleate (POESM, Tween 80) were reported to improve the degradation of lignin, which enhances the hydrophilicity of the lignocellulosic biomass surface, thereby transferring the hydrophobic compound of lignin and hemicellulose into the aqueous phase (Kurakake *et al.* 1994; Kim *et al.* 2007). The addition of POESM was considered to promote removal efficiency of lignin and enhance the hydrolysis efficiency of residual cellulose sequentially (Qing *et al.* 2010). Further, to investigate the effect of the surfactant POESM on the pretreatment of SFRs, the pretreatment experiments were carried out after adding 3% (*v/v*) POESM under the above three pretreatment conditions. After pretreatment, the pretreated slurry was then centrifuged at 4000 rpm for 30 min. The solid part was washed with deionized water until it reached neutral pH, then air-dried and crushed for subsequent determination, analysis, and enzymatic hydrolysis. All experiments were done in triplicate, and the data are presented as mean value ± standard deviation. The related reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

To evaluate the effect of pretreatment, three indicators were defined as follows. The solid recovery of SFR after pretreatment was calculated with Eq. 1,

$$Solid\ recovery\ (\%) = WP/WI \times 100 \tag{1}$$

where *WI* (g) and *WP* (g) are the weight of the SFRs before and after pretreatment, respectively.

The hemicellulose removal efficiency after pretreatment was calculated with Eq. 2,

$$HRE = WHP/WHI \times 100 \tag{2}$$

where *HRE* is hemicellulose removal efficiency (%), and *WHI* (g) and *WHP* (g) are the hemicellulose weight of the SFRs before and after pretreatment, respectively.

The lignin removal efficiency after pretreatment was calculated with Eq. 3,

$$LRE = WLP/WLI \times 100 \tag{3}$$

where *LRE* is lignin removal efficiency (%), and *WLI* (g) and *WLP* (g) are the lignin weight of the SFRs before and after pretreatment, respectively.

Enzymatic hydrolysis methods

The enzymatic hydrolysis was performed at 50 °C under 150 rpm, with a cellulase (purchased from Jurong Food Ingredients Co. Ltd., Henan, China) loading of 25 FPU/g-dry matter for 72 h. The solid-liquid ratio was 1:10 using 50 mM sodium citrate buffer (pH 4.8). The hydrolysis experiments were performed in 50-mL flasks with 20-mL working volume and 2.0 g pretreated SFRs powder. After enzymatic hydrolysis, the slurry was centrifuged at 4000 rpm for 15 min, and the supernatant was analyzed for reducing sugar concentration. The hydrolysis efficiency was calculated with Eq. 4,

$$Hydrolysis\ efficiency\ (\%) = \frac{C_{RS} \times V_{Hydrolasate} \times 0.9}{W_{SFR} \times \omega_{cellulose}} \times 100 \qquad (4)$$

where C_{RS} indicated the reducing sugar concentration in the supernatant (g/L), $V_{Hydrolysate}$ is the volume of the SFRs hydrolysate (L), and W_{SFR} and $\omega_{cellulose}$ indicated the weight (g) and cellulose content (%) of the SFRs used for enzymatic hydrolysis, respectively.

Lactic acid production in open fermentation mode

Batch fermentations were performed in 250 mL flasks containing 10.0 g untreated or pretreated SFRs. The NaOH-heat pretreatment of SFRs was performed in 1% (*w/v*) NaOH solution with a solid-liquid ratio of 1:10 (*w/v*) at 80 °C for 60 min. Subsequently, enzymatic hydrolysis processes were performed for 72 h according to the last section. The SFRs hydrolysates were centrifuged at 4000 rpm for 15 min. Lactic acid productions were carried out in 100 mL of SFRs hydrolysates medium containing 10 g/L yeast extract and 2 g/L K_2HPO_4. The pH was adjusted to 7.0 with 2 M NaOH. The open batch fermentations were conducted anaerobically at 37 °C in an air-bath shaker at 150 rpm with 10% (*v/v*) inoculum. Samples were withdrawn periodically from the fermentation broths for glucose, cellobiose, and lactic acid measurements. Each experiment was performed in triplicate, and the result is presented as mean ± standard deviation. The related reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Analytical methods

The cellulose, hemicellulose, and Klason lignin content of the untreated and pretreated SFRs were determined according to the National Renewable Energy Laboratory Analytical Procedure (Sluiter *et al.* 2008). Accordingly, the SFRs samples were degraded by using 72% sulfuric acid at 30 °C for 1 h, then diluted to 4% sulfuric acid, and further degraded at 121 °C for 1 h. The content of cellulose and hemicellulose was quantified by the concentration of corresponding monomeric sugars, using an anhydro correction of 0.88 for pentose and a correction of 0.90 for hexose, respectively. The hemicellulose in SFRs was mainly composed of xylan, which was calculated based on xylose concentration. Moreover, the lignin content was measured by the method for placing SFRs into the muffle furnace at 575 °C for 4 h. Then they were transferred into a desiccator and cooled for 1 h. The reducing sugar concentration was measured using the dinitrosalicylic acid method with glucose as the standard (Miller 1959). The concentrations of sugars (xylose, glucose, and cellulose) and lactic acid were analyzed using high performance liquid chromatography (LC-20A, Shimadzu, Kyoto, Japan) with a Shodex SH1011 column (Shodex, Tokyo, Japan) and a refractive index detector (RID-10A, Shimadzu). A 0.25-Mm H_2SO_4 was used as the mobile phase at flow rate of 1.0 mL/min and 50 °C. In addition, the surface structures of SFRs before and after pretreatment were observed by using scanning electron microscopy (SEM) (Quanta 250, Thermo Fisher Scientific, Waltham, MA, USA). The changes of

chemical composition of SFRs before and after pretreatment were analyzed by infrared spectroscopy (FTIR) (Nicolet 6700, Thermo Fisher Scientific). The variations of crystalline components in SFRs were analyzed by X-ray diffractometer (XRD) (DMAX-rB, Rigaku, Tokyo, Japan), and the crystallinity index (CrI) of cellulose was calculated by Eq. 5,

$$CrI \ (\%) = \frac{I_{002} - I_{am}}{I_{am}} \times 100 \qquad (5)$$

where I_{002} denotes the diffraction intensity on 002 the surface, and I_{am} denotes diffraction intensity in the amorphous region when $2\theta = 18°$. The SFRs samples for SEM, FTIR, and XRD analysis were air-dried at 105 °C and mass to constant, then ground into powder. Particularly, for SEM analysis, the SFRs powder was sieved though a Φ 1 mm screen; for FTIR and XRD analysis, the SFRs powder was sieved though a Φ 0.074 mm screen.

RESULTS AND DISCUSSION

Composition Changes of SFRs before and after Pretreatment

To investigate the suitable pretreatment process, the effects of hydrothermal, NaOH-heat, and H_2SO_4-heat pretreatment methods on the composition changes of SFRs were compared. The results were shown in Table 1. The untreated SFRs contained 46.6% cellulose, 19.5% hemicellulose, and 14.9% Klason lignin. After different pretreatment methods, the solid recovery of SFRs ranged from 68.2% to 85.9%.

After NaOH-heat pretreatment, the content of lignin in SFR decreased the most (from 14.9% to 7.1%), and the LRE reached 67.5%. This result indicated that the alkali solution could dissolve most of lignin in SFRs. After H_2SO_4-heat pretreatment, the hemicellulose content decreased from 19.5% to 7.2%, and the HRE reached 73.0%. It also showed that the acid solution could dissolve most hemicellulose in SFR. The main mechanism of H_2SO_4-heat pretreatment to improve enzymatic hydrolysis efficiency was to dissolve hemicellulose, destroy its wrapping and winding of cellulose, thus increasing the contact area between cellulose and cellulase; the phenomenon has been reported by many researchers (Taherzadeh and Karimi 2007; Zheng *et al.* 2013; Sewsynker-Sukai *et al.* 2018). The HRE and LRE by hydrothermal pretreatment were 10.6% and 10.1% respectively; compared with H_2SO_4-heat and NaOH-heat pretreatment, the effect was not satisfactory.

Table 1. Changes of Cellulose, Hemicellulose and Lignin Contents of SFRs

Pretreatment	Compositions of SFRs (%, dry weight)			Solid recovery (%)	HRE[a] (%)	LRE[b] (%)
	Cellulose	Hemicellulose	Klason lignin			
Untreated SFRs	46.6 ± 0.6	19.5 ± 0.3	14.9 ± 0.7	/	/	/
Hydrothermal	48.5 ± 0.3	20.3 ± 0.6	15.6 ± 0.4	85.9 ± 0.5	10.6	10.1
Hydrothermal+PEOSM	48.9 ± 0.9	19.7 ± 0.2	15.1 ± 0.6	83.9 ± 0.3	15.2	15.0
NaOH-heat	59.4 ± 0.5	22.9 ± 0.7	7.1 ± 0.5	68.2 ± 0.9	19.9	67.5
NaOH-heat+ PEOSM	58.8 ± 2.2	21.5 ± 0.8	7.0 ± 0.4	69.7 ± 1.4	23.2	67.3
H_2SO_4-heat	54.7 ± 0.4	7.2 ± 0.6	17.4 ± 0.3	73.2 ± 0.8	73.0	14.5
H_2SO_4-heat+ PEOSM	55.2 ± 0.7	7.5 ± 0.2	17.9 ± 0.1	71.8 ± 1.1	72.4	13.7

[a]: HRE, hemicellulose removal efficiency;

[b]: LRE, lignin removal efficiency.

In addition, when 3% POESM was added into three different pretreatment process, the HRE and LRE were not significantly affected, only hydrothermal pretreatment increased by less than 5%. Due to the dissolution of hemicellulose and lignin in SFR by the six pretreatment methods, the relative content of cellulose in SFR increased with the decrease of solid mass. Among them, the content of cellulose after NaOH-heat pretreatment increased most significantly, from 46.6% to 59.4%.

Comparison of Reducing Sugar Concentration by Enzymatic Hydrolysis after Different Pretreatments

The purpose of pretreatment is to improve enzymatic hydrolysis efficiency of SFRs. After pretreatment by different methods, enzymatic hydrolysis of SFRs was conducted according to the method described in method section. The concentration of reducing sugar in the enzymatic hydrolysate was determined, and the hydrolysis efficiency was also calculated. The results are shown in Table 2.

After enzymatic hydrolysis, the reducing sugar concentration of SFRs without pretreatment was 8.70 g/L, and the hydrolysis efficiency was only 33.5%. After different pretreatments, the concentration of reducing sugar increased, especially after NaOH-heat pretreatment, the highest concentration of reducing sugar was 25.5 g/L, and the hydrolysis efficiency reached 77.4%, which was 2.3 times higher than that of direct enzymatic hydrolysis without pretreatment. This might be due to the removal of most of the lignin from biomass by NaOH-heat pretreatment, which improved the accessibility of cellulose to enzymes and avoided the inactivation of cellulase by lignin adsorption. After hydrothermal pretreatment and H_2SO_4-heat pretreatment, 11.2 g/L and 19.8 g/L reducing sugars were obtained, and the hydrolysis efficiency was 41.4% and 65.2%, respectively, which were lower than that of NaOH-heat pretreatment. This might be because the extraction of alkaloids of *S. flavescens* had undergone acetic acid soaking and decoction, which was equivalent to acid pretreatment and hydrothermal pretreatment; thus the effects of these two pretreatments were very limited. In addition, no matter whether POESM was added in the pretreatment process, the concentration of reducing sugar and hydrolysis efficiency obtained after enzymatic hydrolysis did not change remarkably, which indicated that POESM in the pretreatment had little effect on hydrolysis.

Table 2. Reducing Sugar Concentration and Hydrolysis Efficiency of SFRs

Pretreatment	Reducing Sugar Concentration (g/L)	Hydrolysis Efficiency (%)
Untreated SFRs	8.70 ± 1.4	33.5
Hydrothermal	11.2 ± 0.4	41.4
Hydrothermal+POESM	12.4 ± 1.4	45.5
NaOH-heat	25.5 ± 2.7	77.4
NaOH-heat+ POESM	24.9 ± 2.0	76.2
H_2SO_4-heat	19.8 ± 0.7	65.2
H_2SO_4-heat+ POESM	19.6 ± 0.8	64.0

Mass Flow in the Pretreatment and Enzymatic Hydrolysis of SFRs

After different pretreatment, the cellulose content, solid recovery, and reducing sugar concentration were different. However, it was not advisable to use only one of the indexes to evaluate the advantages and disadvantages of the pretreatment method. Therefore, in this study, a certain quality of SFRs was pretreated and hydrolyzed; the reducing sugar yield was used to select the best pretreatment method.

The mass flow of 100 g SFRs after different pretreatment methods and enzymatic hydrolysis is shown in Fig. 1. Only 17.4 g of reducing sugar could be produced after enzymatic hydrolysis of 100 g SFRs without pretreatment, while reducing sugar yield obtained by hydrothermal pretreatment was similar to that of SFRs without pretreatment. Although the solid recovery after NaOH-heat pretreatment was slightly lower than that of hydrothermal and H_2SO_4-heat pretreatment, the reducing sugar yield after enzymatic hydrolysis was the highest, reaching 34.7 to 34.8 g/(100 g dry residue). Therefore, NaOH-heat pretreatment was chosen as the best pretreatment method for SFRs. In addition, POESM did not need to be added in the pretreatment of SFRs in order to save cost.

Conditions Optimization of NaOH-heat Pretreatment

According to the above results, the highest yield of reducing sugar was obtained by NaOH-heat pretreatment. Thus, the NaOH concentration (0.2 to 2.0 (*w/v*)), solid-liquid ratio (1:5 to 1:20 (*w/v*)), treatment temperature (30 °C to 120 °C), and time (30 to 240 min) were optimized to save energy, NaOH, and water consumption. After the pretreatment, the enzymatic hydrolysate of SFRs were obtained, and the reducing sugar concentration and hydrolysis efficiency were measured to evaluate the pretreatment effect.
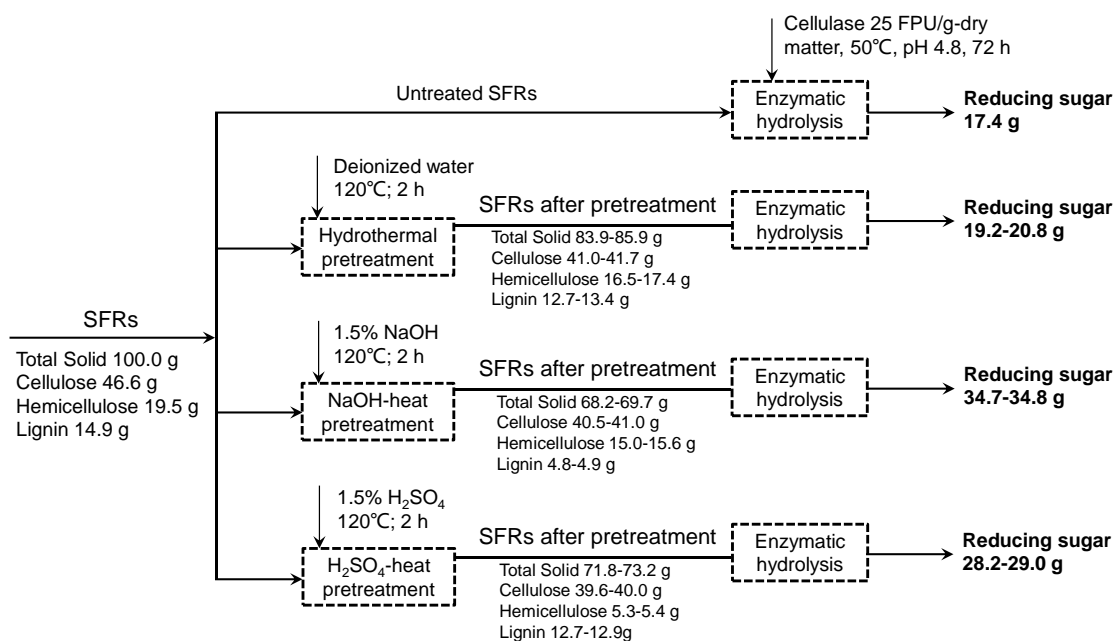

Fig. 1. Mass flow of SFRs after different pretreatment methods and same enzymatic hydrolysis

As shown in Fig. 2, with the concentration of NaOH increasing from 0.2% to 1.0%, the concentration of reducing sugar also increased, and hydrolysis efficiency increased from 67.3% to 83.6%. However, when the NaOH concentration continued to increase, the concentration of reducing sugar and hydrolysis efficiency increased slightly. When the solid-liquid ratio increased from 1:5 to 1:10, hydrolysis efficiency reached the highest value, which was 83.6%. If the solid-liquid ratio continued to increase, hydrolysis efficiency remained basically stable. When the pretreatment temperature was 80 °C, the reducing sugar concentration in the enzymatic hydrolysate reached the maximum, and hydrolysis efficiency decreased when the temperature continued to increase. This might be because the NaOH solution could not only remove lignin and hemicellulose from biomass,

but also it could destroy cellulose at high temperatures. This was not conducive to the contact between enzymes and cellulose, resulting in the decrease in enzymatic hydrolysis efficiency (Nlewem and Thrash 2010). In addition, the concentration of reducing sugar increased with the increase of pretreatment time. When the pretreatment time was 60 minutes, the hydrolysis efficiency reached 85.0%. When the pretreatment time continued to increase, the enhancement of the reducing sugar concentration was not notably.
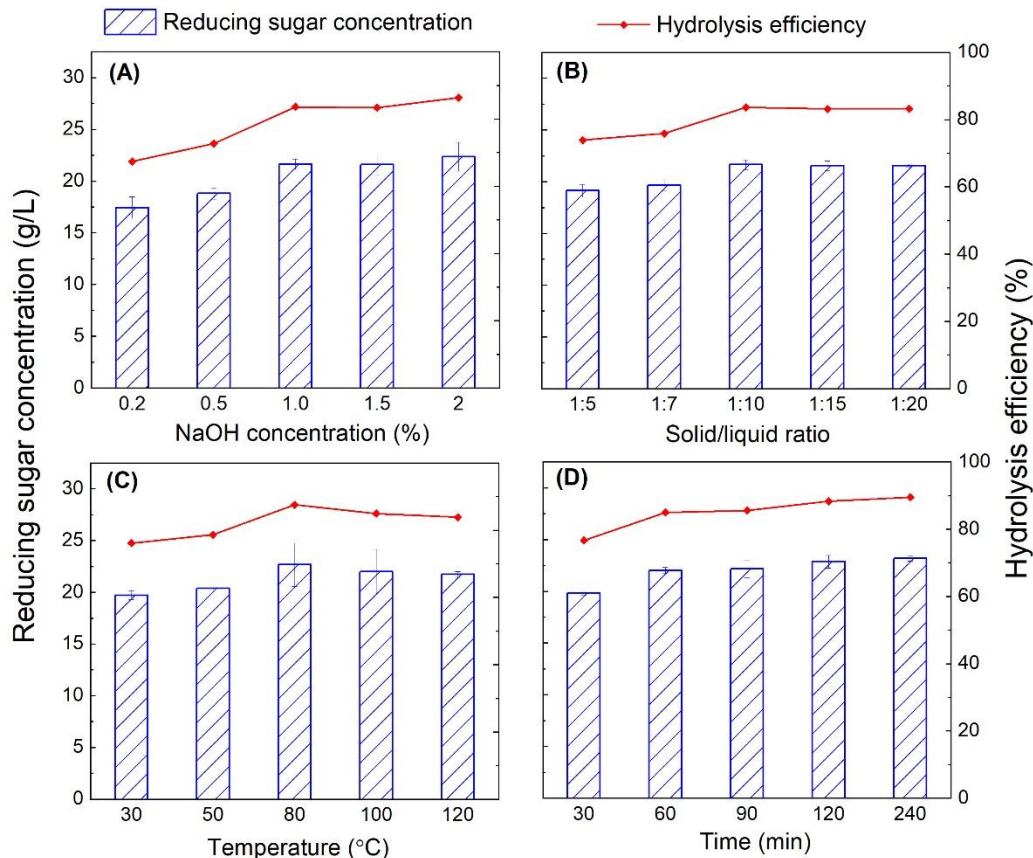

Fig. 2. Effects of each experimental conditions on NaOH-heat pretreatment of SFRs. (A) NaOH concentration; (B) Solid-liquid ratio; (C) Temperature; (D) Time.

Therefore, from the point of view of saving energy consumption and improving the pretreatment efficiency of SFRs, the optimum pretreatment conditions for SFR were 1% NaOH concentration, 1:10 solid-liquid ratio, 80 °C temperature, and 60 min pretreatment time. Under the optimum conditions, 22.0 g/L reducing sugar was obtained from enzymatic hydrolysate of SFR, and the hydrolysis efficiency was 85.0%.

NaOH-heat Pretreatment Mechanism Analysis of SFRs
Infrared spectrum analysis of SFRs before and after NaOH-heat pretreatment

Because the functional groups and chemical bonds of cellulose have specific frequencies in infrared spectroscopy, the changes of structure and composition of cellulose before and after pretreatment can be qualitatively and semi-quantitatively analyzed by infrared spectroscopy. Table 3 lists the functional groups corresponding to different wavenumbers and their modes of vibration (Xu *et al.* 2015). The infrared spectrum of SFRs before and after NaOH-heat pretreatment was shown in Fig. 3.
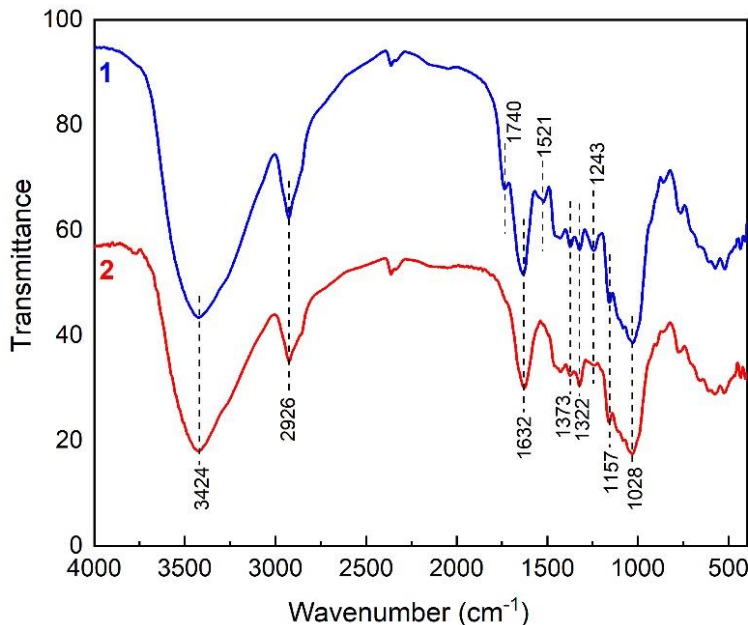
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Fig. 3. Infrared spectrum of SFRs before and after NaOH-heat pretreatment. (1-before pretreatment; 2-after pretreatment). The pretreatment of SFRs was performed in 1% (*w/v*) NaOH solution with a solid-liquid ratio of 1:10 (*w/v*) at 80 °C for 60 min.

For lignocellulosic substances, the absorbance at wavenumber 3424 cm^{-1} can be attributed to the stretching vibration of hydroxyl group forming hydrogen bond, and wavenumber 2926 cm^{-1} is for the C-H stretching vibration of methyl and methylene. These two functional groups exist not only in cellulose, but also in hemicellulose and lignin. The wavenumber 1157 cm^{-1} corresponds to the asymmetric stretching vibration of C-O-C in cellulose and hemicellulose molecules. The wavenumber 1028 cm^{-1} corresponds to C-O stretching in cellulose and hemicellulose molecules. After NaOH-heat pretreatment, the absorption peaks of cellulose and hemicellulose did not change significantly, indicating that the main chain structure of cellulose and hemicellulose did not change significantly after pretreatment.

Wavenumber 1740 cm^{-1} is the characteristic peak of hemicellulose and the stretching vibration absorption peak of C=O on acetyl and carboxyl groups of hemicellulose. As shown in Fig. 3, the peak disappeared after NaOH-heat pretreatment. This was due to the deacetylation of hemicellulose molecule by NaOH-heat pretreatment, and the acetyl group broke from the hemicellulose molecule chain to produce acetic acid (Castro *et al.* 2017). After NaOH-heat pretreatment, the concentration of acetic acid in the pretreated wastewater reached 4.40 g/L (data not shown), which was consistent with the results of infrared spectroscopy analysis.

Wavenumber 1632 cm^{-1} is the stretching vibration of C-O connected with aromatic ring, 1521 cm^{-1} is the stretching vibration of benzene ring skeleton, 1373 cm^{-1} is the stretching vibration of phenolic hydroxyl group, 1243 cm^{-1} is the C-O stretching vibration of syringaldehyde benzene ring, which all belong to the characteristic absorption peaks of lignin. After NaOH-heat pretreatment, the intensity of these peaks decreased or even disappeared, indicating that the lignin structure of SFR was destroyed and lignin was effectively removed after NaOH-heat pretreatment. This was consistent with the results of removing 67.5% lignin from SFR by NaOH-heat pretreatment (Table 1).

Table 3. Wavenumber and Spectrum Attribution of Infrared Spectra

Wavenumber (cm⁻¹)	Spectrum Attribution
3424	Stretching vibration of O-H associated with hydrogen bond (polysaccharide, lignin)
2926	C-H stretching vibration of methyl and methylene (polysaccharide, lignin)
1740	C=O stretching vibration (hemicellulose)
1632	Stretching vibration of C-O connected with aromatic rings (lignin)
1521	Skeleton telescopic vibration of benzene ring (lignin)
1373	Stretching vibration of phenolic hydroxyl groups (lignin)
1322	C-H vibration (cellulose), C-O stretching (lignin, xylan)
1243	C-O stretching vibration of syringaldehyde benzene ring
1157	Asymmetric telescopic vibration of C-O-C (cellulose, hemicellulose)
1028	Stretching peak of C-O (cellulose, hemicellulose)

Based on the above analysis, the main mechanism of NaOH-heat pretreatment was to effectively remove lignin and acetyl from hemicellulose. It was suggested that the delignification and deacetylation improved the accessibility of cellulose to enzymes and enzymatic hydrolysis (Zhu *et al.* 2008; Castro *et al.* 2017).

X-ray diffraction analysis of SFRs before and after NaOH-heat pretreatment

Cellulose is a high polymer linked by D-glucose with β-1,4 glycoside bonds. Cellulose can be divided into a crystalline region and an amorphous region according to whether there is a tight arrangement of its molecular structure. The crystallinity index (CrI) of cellulose can reflect the degree of crystallization when cellulose aggregates, and it is also an important factor affecting the enzymatic hydrolysis of cellulose (Smuga-Kogut *et al.* 2016). X-ray diffraction is a common method to analyze the crystallinity of cellulose. According to the X-ray diffraction pattern, the value of CrI can be calculated by Eq. 5, which indicates the change of cellulose crystallinity of SFRs before and after pretreatment.
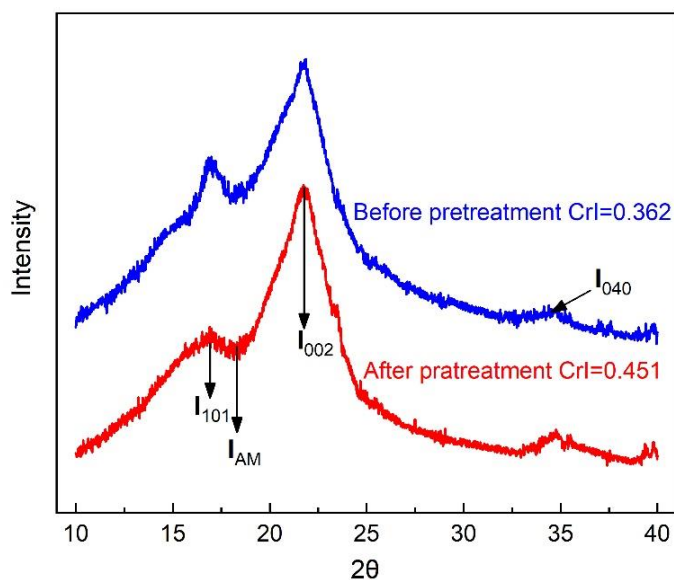

Fig. 4. X-ray diffraction patterns of SFRs before and after NaOH-heat pretreatment. The pretreatment of SFRs was performed in 1% (*w/v*) NaOH solution with a solid-liquid ratio of 1:10 (*w/v*) at 80 °C for 60 min.

The X-ray diffraction pattern of SFRs before and after NaOH-heat pretreatment is shown in Fig. 4. There were three peaks in the diffraction pattern of original SFRs (2θ=16.8º, 22.0º and 34.4º), which belong to (101), (002) and (040) lattice planes of cellulose (I), respectively. After NaOH-heat pretreatment, these peak positions remained unchanged, indicating that NaOH-heat pretreatment did not destroy the crystalline structure of cellulose, and the typical crystal form (cellulose I) was still shown.

The CrI of the original SFRs was 0.362, which increased to 0.451 after NaOH-heat pretreatment. On the one hand, the improvement of CrI was due to the removal of most lignin and some hemicellulose from SFRs by NaOH-heat pretreatment, and the removal of these amorphous components increased the CrI value. However, some amorphous cellulose might be recrystallized to form crystalline cellulose due to the swelling effect of alkali solution, which led to the increase of its crystallinity. The phenomenon that alkali solution pretreatment increased CrI of lignocellulosic materials had also been reported in other literatures (Deng *et al.* 2013; Kan *et al.* 2018).

Scanning electron microscopic analysis of SFRs before and after NaOH-heat pretreatment

Scanning electron microscopy (SEM) could be used to intuitively understand the changes of fiber structure of SFRs before and after pretreatment. Niu *et al.* (2009) found that the smooth and orderly surface of rice straw was damaged by NaOH solution with photocatalysis-assisted method, and the surface area of cellulose was increased, which made enzymatic hydrolysis easier.

SEM images of SFRs samples before and after NaOH-heat pretreatment are shown in Fig. 5. The untreated SFRs samples showed a smooth surface with fine structure and orderly arrangement in microscopy (Fig. 5(A)). After NaOH-heat pretreatment, the surface of SFRs was damaged obviously, rough and uneven, and the compact structure became loose. Meanwhile, many pores were exposed to the surface of the sample (Fig. 5(B)). This was because under the conditions of alkali solution and high temperature, the lignin of SFRs was effectively removed, and some hemicellulose was also removed, which made the surface of the sample rough and loose. In addition, the pores exposed to the surface could improve the permeability of the material, make the cellulase more easily contact with the cellulose, thereby improving the enzymatic hydrolysis efficiency (Vancov and McIntosh 2011; Murciano Martínez *et al.* 2015).
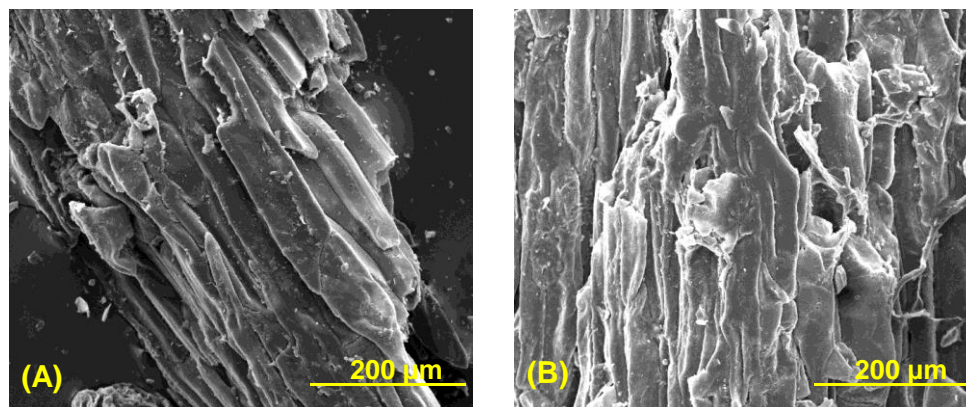

Fig. 5. SEM images of SFRs before and after NaOH-heat pretreatment. (A) SFRs before pretreatment; (B) SFRs after pretreatment. The pretreatment of SFRs was performed in 1% (*w/v*) NaOH solution with a solid-liquid ratio of 1:10 (*w/v*) at 80 °C for 60 min.

Lactic acid production by fermentation of SFRs before and after pretreatment

In order to explore the effect of pretreatment on the lactic acid production, the same amount of SFRs with non-pretreatment and pretreatment were used to hydrolyze and ferment. The changes of sugar concentration, lactic acid concentration, and pH are shown in Fig. 6. In the enzymatic hydrolysis stage, the final concentration of glucose and cellobiose produced by SFR with NaOH-heat pretreatment were 31.7 g/L and 14.2 g/L, respectively, which were more than twice the sugar concentration of SFRs without NaOH-heat pretreatment. In addition, by observing the changes of sugar concentration during enzymatic hydrolysis, it was found that the concentration of glucose and cellobiose increased most rapidly in the first 24 h, regardless of whether the SFRs was pretreated with NaOH or not, because the accumulation of glucose and cellobiose would produce feedback inhibition, which would make the inactivity of cellulase.

During the lactic acid fermentation stage, the glucose and cellobiose were consumed rapidly by *L. casei* in 24 h; the lactic acid concentration increased rapidly in the first 24 h and then slowly. The maximum lactic acid concentration produced by SFRs with NaOH-heat pretreatment could reach 33.7 g/L, which was twice the concentration of lactic acid produced by SFRs without pretreatment. This result showed that after dissolving most lignin by NaOH-heat pretreatment, the cellulase could be contacted with the cellulose of SFRs better, resulting in better enzymatic hydrolysis effect, higher sugar production and ultimately increased lactic acid concentration. Therefore, NaOH-heat pretreatment greatly improved the final fermentation results.
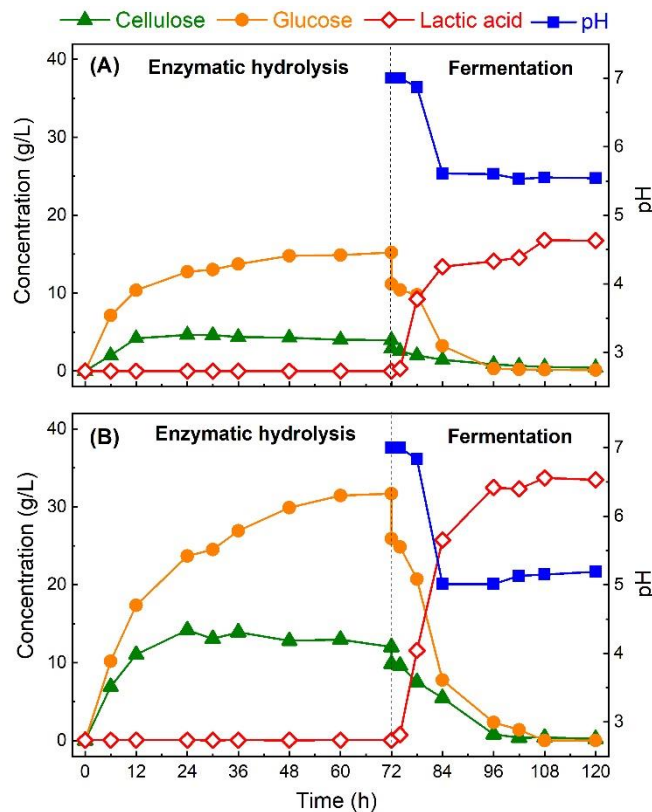

Fig. 6. The effect of NaOH-heat pretreatment on lactic acid fermentation. (A) SFRs without NaOH-heat pretreatment as substrate; (B) SFRs with NaOH-heat pretreatment as substrate. The pretreatment of SFRs was performed in 1% (w/v) NaOH solution with a solid-liquid ratio of 1:10 (w/v) at 80 °C for 60 min.

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CONCLUSIONS

1. The yield of reducing sugar per 100 g of *Sophora flavescens* residues (SFRs) after NaOH-heat pretreatment was 34.8 g, which was 81.3% and 20.0% higher than that of hydrothermal and H_2SO_4-heat pretreatment, respectively.

2. Under the optimum NaOH-heat pretreatment conditions (1% (w/v) NaOH solution; 1:10 (w/v) solid-liquid ratio; 80 °C temperature; 60 min treatment time), 85.0% hydrolysis efficiency was obtained.

3. Mechanism analysis suggested that the lignin of SFRs could be removed by NaOH solution, and the deacetylation reaction of hemicellulose occurred, which made the original smooth and dense structure become rough and loose.

4. Lactic acid concentration (33.7 g/L) produced from SFRs with NaOH-heat pretreatment was twice as high as that of SFRs without pretreatment.

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