Comparative Study on Pretreatment Processes for Different Utilization Purposes of Switchgrass

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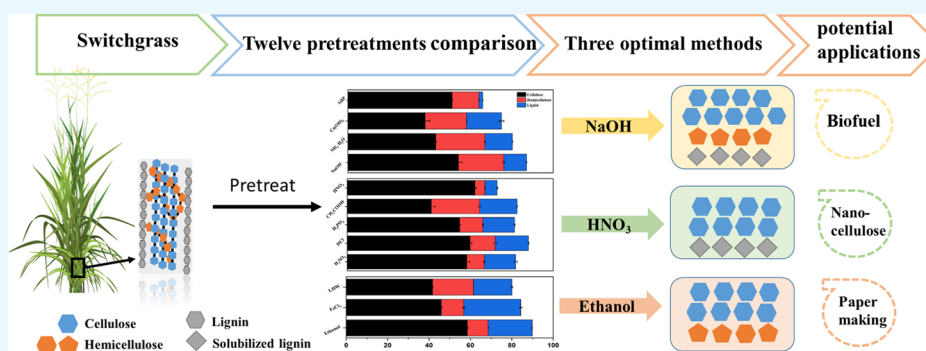
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ABSTRACT: Switchgrass (*Panicum virgatum*, L., Poaceae) with the advantages of high cellulose yield, and high growth even under low input and poor soil quality, has been identified as a promising candidate for production of low-cost biofuels, papermaking, and nanocellulose. In this study, 12 chemical pretreatments on a laboratory scale were compared for different utilization purposes of switchgrass. It was found that the pretreated switchgrass with sodium hydroxide showed considerable potential for providing mixed sugars for fermentation with 11.10% of residual lignin, 53.85% of residual cellulose, and 22.06% of residual hemicellulose. The pretreatment with 2.00% (v/v) nitric acid was the best method to remove 78.37% of hemicellulose and 39.82% of lignin under a low temperature (125 °C, 30 min), which can be used in the production of nanocellulose. Besides, a completely randomized design analysis of switchgrass pretreatments provided the alternative ethanol organosolv delignification of switchgrass for the papermaking industry with a high residual cellulose of 58.56%. Finally, scanning electron microscopy (SEM), X-ray diffraction (XRD), and Fourier-transform infrared spectroscopy (FT-IR) were carried out to confirm the changes in functional groups, crystallinity, and thermal behavior of the three materials, respectively, from the optimal pretreatments.

INTRODUCTION

Lignocellulosic biomass provides more than just mixed sugars for fermenting to biofuels and other biomaterials, which also plays an essential role in the pulp and paper industry as well as nanocellulose production.[1] These conversions are multistep processes involving pretreatment, which is critical to deconstruct the main recalcitrance of lignocellulosic plant cell walls due to the hemicellulose and lignin.[2] Moreover, the pretreatment has proved to lower the overall cost of lignocellulose conversion and to improve the process towards making the concept of biorefinery a reality.[3,4] In the last two decades, several pretreatments including physical, chemical, biological, and combinatorial methods have been developed to improve the conversion process of renewable biomass feedstocks.[5,6] The efficiency of the pretreatment step depends on several factors such as feedstock properties, reactor design, and reaction conditions. Usually, pretreatments conducted at relatively low temperatures and in a short time can be used to recover the cellulose, hemicellulose, and lignin of the biomass

for further conversion and valorization.[7] Different lignocellulosic biomass feedstocks require different pretreatment methods for overcoming the natural recalcitrance. An ideal pretreatment process should release more cellulose and make lignocellulose susceptible to the subsequent steps.[8]

Switchgrass (*Panicum virgatum*, L., Poaceae) is a promising biomass feedstock with a high yield and low input requirements. In addition, with the development of biotechnology, modified switchgrass is a dedicated energy crop to produce renewable chemicals and fuels.[9] Switchgrass has the potential to serve as a critical resource to produce a range of materials, chemicals, and energy products due to its typical composition

Table 1. Main Compositions of Switchgrass from Varying Pretreatments[a]

pretreatments	cellulose (glucose)	xylan	arabinan	AIL	ASL
untreated switchgrass	39.42 ± 0.51	17.27 ± 0.04	2.98 ± 0.03	19.82 ± 0.62	1.40 ± 0.01
H_2SO_4 0.50% (v/v) 125 °C 30 min	52.30 ± 1.21	6.67 ± 0.14	0.80 ± 0.09	24.84 ± 0.66	0.96 ± 0.01
HCl 0.50% (v/v) 125 °C 30 min	53.72 ± 0.77	9.93 ± 0.54	1.00 ± 0.12	23.96 ± 0.18	1.46 ± 0.24
H_3PO_4 1.00% (v/v) 130 °C 20 min	49.20 ± 0.35	9.93 ± 0.04	0.82 ± 0.04	22.81 ± 0.61	1.22 ± 0.01
CH_3COOH 0.50% (v/v) 115 °C 20 min	36.75 ± 1.52	17.51 ± 0.49	3.63 ± 0.08	18.61 ± 0.24	1.66 ± 0.01
HNO_3 2.00% (v/v) 125 °C 30 min	55.84 ± 0.04	4.38 ± 0.01	0.00 ± 0.00	11.56 ± 0.25	1.21 ± 0.01
NaOH 1.00% (w/v) 75 °C 60 min	53.85 ± 1.84	18.96 ± 0.04	3.10 ± 0.03	10.03 ± 0.09	1.07 ± 0.11
NH_4OH 8.00% (v/v) 75 °C 60 min	42.85 ± 0.32	20.57 ± 0.01	3.26 ± 0.02	16.56 ± 0.32	1.45 ± 0.19
$Ca(OH)_2$ 0.25% (v/v) 30 °C 120 min	37.67 ± 2.18	18.21 ± 0.32	2.49 ± 0.43	19.14 ± 1.12	1.79 ± 0.03
AHP 8.00% (v/v) 130 °C 30 min	50.80 ± 0.27	11.36 ± 0.11	1.61 ± 0.02	12.87 ± 0.12	1.41 ± 0.02
C_2H_5OH 80.00% (v/v) 150 °C 40 min	58.56 ± 0.33	9.27 ± 0.13	0.73 ± 0.01	13.75 ± 0.29	1.41 ± 0.13
$FeCl_3$ 0.05 M 150 °C 30 min	46.00 ± 0.33	10.02 ± 0.43	0.74 ± 0.03	26.27 ± 0.25	0.97 ± 0.22
hot water 115 °C 30 min	41.77 ± 0.08	16.11 ± 0.04	3.08 ± 0.01	17.65 ± 0.84	0.97 ± 0.01

[a]Note: All compositions are presented as g/100 g of pretreated switchgrass besides the untreated raw switchgrass. AIL: acid-insoluble lignin. ASL: acid-soluble lignin.

of cellulose (32−45% wt), hemicellulose (21−31% wt), and lignin content (12−28% wt).[10−16] Nowadays, this energy crop is being used for several industrial processes with biotechnology applications such as renewable energy,[17] isoprenol production,[18] cellulose, and protein extraction.[19,20] However, there is still a lack of a comprehensive comparative study of different switchgrass pretreatment processes for further utilization of different purposes.

Going beyond of sugars from switchgrass, research studies also focus on papermaking and nanocellulose, but these technologies still require effective pretreatment to achieve desired switchgrass conversion. The ideal pretreatment to obtain fermentable sugars for production of biofuels and biochemicals is to eliminate lignin and preserve the maximum cellulose and hemicellulose, which can enhance their yields of fermentable sugars. In the production of pulp for papermaking, the choice of specific treatments is based on their delignification capacity,[21] suggesting the need to maximize lignin removal with the pretreatment. Switchgrass has attracted significant attention as a renewable source for nanocrystalline cellulose extraction, which is a two-step process including pretreatment and acid hydrolysis. The pretreatment process is to ensure the removal of hemicelluloses and releasing the cellulose that can be used for further effective hydrolysis into the nanocellulose material.[22,23]

Some studies have focused on various pretreatment technologies for the conversion of switchgrass into soluble sugars.[24] However, few research studies give a comprehensive insight into the comparison of composition details of chemical pretreatments on switchgrass. The present study tries to offer optimal and useful information on switchgrass chemical pretreatments on a laboratory scale, which will enable a better understanding of currently available pretreatments to improve switchgrass utilization. We characterized the physical and chemical changes of switchgrass when it was subjected to 12 pretreatments, including five dilute acid, four alkali, organosolv, ferric chloride, and hydrothermal pretreatments. Some pretreatments are applied for the first time. The practical details of the pretreatments presented in this study were used to choose the best strategies for enhancing the conversion process of switchgrass provide the guideline for better utilization of switchgrass.

■ RESULTS AND DISCUSSION

Composition of Switchgrass. The switchgrass raw material used in this study has a composition of 39.42 ± 0.52% w/w cellulose, 20.25 ± 0.01% w/w hemicellulose, and 21.22 ± 0.62% w/w lignin. However, the concentrations of cellulose, hemicellulose, and lignin obtained in this study had a marginal variation from the values reported by others,[25] which could be mainly attributed to species, cultivar differences, harvest maturity of the sample, and so on. The high cellulose and low lignin of switchgrass in this study indicate that this switchgrass is not only suitable as a feedstock for biofuel production but also an excellent resource for nanocellulose production and the pulp and paper industry.

Evaluation of the Influence of Various Parameters on the Dilute Acid Pretreatment Efficiency of Switchgrass. A completely randomized design with three replications was employed to compare the parameters of various mild dilute acid pretreatments of switchgrass, which was fundamentally ensured to retain cellulose and remove lignin. Further studies were conducted at the condition that the previous step no longer had a significant effect on residual cellulose and residual lignin. Table 1 shows the results of the five dilute acid pretreatments, and detailed experimental results are presented in the Supporting Information file.

Dilute sulfuric acid (H_2SO_4) pretreatment has been studied extensively, which is efficient in the removal of hemicelluloses, rendering the cellulose more available.[8] A concentration of 0.50% v/v H_2SO_4 could yield 52.30% of residual cellulose and remove 62.62% of hemicelluloses (7.57 ± 0.23% w/w) with the low temperature of 125 °C for 20 min (Figure 1). There was a slight improvement in the residual cellulose of treated samples with the increase of the concentration of H_2SO_4 to 2.00% v/v (Table S1 and Figure S1). However, there was no significant difference in the lignin content, suggesting that the optimal switchgrass pretreatment was using the 0.50% v/v H_2SO_4 under the temperature of 125 °C for 20 min. Dilute H_2SO_4 pretreatment of switchgrass is generally performed at temperatures from 140 to 200 °C with an H_2SO_4 concentration of 0.50−2.00% and in a relatively short residence time (<60 min).[26] In our process, the cellulose residue and lignin degradation could occur due to the low temperature and acid concentration.

Inhibitory products, including furfural, hydroxymethylfurfural (HMF), and other organic acids, are commonly generated
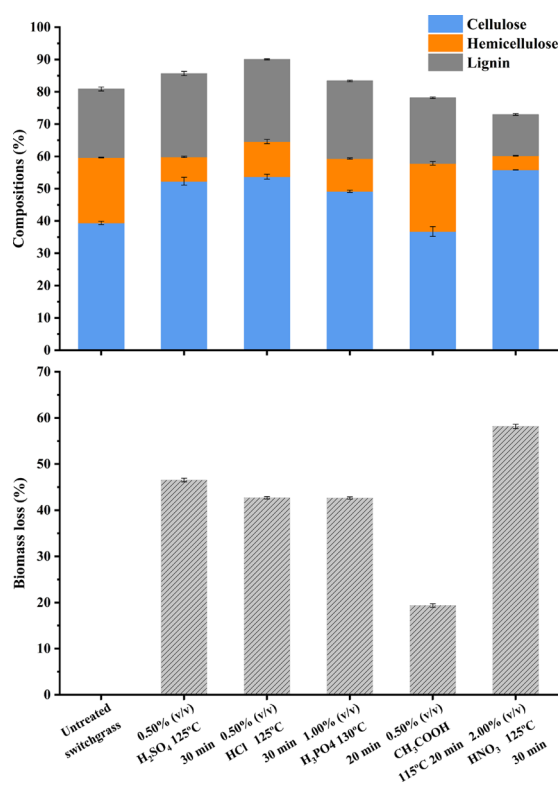
Figure 1. Chemical compositions and biomass loss of acid pretreated switchgrass.

during the hydrochloric acid (HCl) pretreatment process at higher pretreatment temperatures, which are needed for HCl pretreatment of some lignocellulose biomass. However, HCl is frequently used in hydrolyzing lignocellulose because it is volatile and easy to recover.[27] Little data is available on the HCl hydrolysis of switchgrass. In this article, we carried out a study to first evaluate the effect of different parameters on HCl pretreatment efficacy of switchgrass. Figure S2 shows the biomass loss, cellulose, hemicellulose, and lignin contents of switchgrass biomass samples after different dilute HCl pretreatments. Cellulose (53.72%) in pretreated switchgrass was retained with the 0.50% v/v HCl at 125 °C for 20 min (Figure 1). It is observed that as the temperature or acid concentration increases, the hemicellulose decreases continuously, as shown in Figure S2 and Table S2, which would be promising in nanocrystalline cellulose extraction.

Phosphoric acid (H_3PO_4) pretreatment of the lignocellulose biomass for biofuel production can increase the ethanol yields compared to other pretreatment strategies.[28] Besides, residual H_3PO_4 can be used as a fertilizer for microbial growth. Thus, it has been used for the pretreatment of switchgrass and other biomasses.[29] This study showed more details of H_3PO_4 pretreatment of switchgrass with four low temperatures (115, 120, 125, and 130 °C). The result signified that the maximum cellulose preservation and lignin degradation for switchgrass pretreatment was obtained with 1.00% v/v H_3PO_4 at 130 °C for 40 min, with a high residual cellulose of 49.20 and 35.16% of lignin removal (Figure 1). In this stage, the three parameters of H_3PO_4 pretreatment had almost no effect on lignin degradation. However, H_3PO_4 concentration had a more significant impact on the hemicellulose solubilization than the other parameters (Figure S3).

Acetic acid (CH_3COOH) attracts attention due to the autocatalysis occurring during the pretreatment of lignocelluloses.[30] It is reported to facilitate the release of natural organic acids from the biomass and the organic acids act as a natural catalyst for the rupture of the lignin−carbohydrate complex. A number of studies have reported that organic acid pretreatment of the biomass needs high temperature and pressure.[31] In this study, CH_3COOH pretreatment under low temperatures would reduce energy consumption, and we used the same conditions as those for the other acids to investigate the impact of various acids on the pretreatment of switchgrass. The result showed that CH_3COOH has little effect on the residual cellulose and lignin in treated samples (Figure 1). Similar results were reported for dilute CH_3COOH pretreatment of switchgrass under the temperature of 150 °C for efficient biobutanol production by Wang et al. However, they reported that the cellulose content would increase when the temperature reached 190 °C. CH_3COOH used in the pretreatment of the lignocellulosic biomass plays an essential role in enhancing microbial production of high-value biofuels.[17]

This work evaluated the effect of subjecting switchgrass to nitric acid (HNO_3) pretreatment with different time periods, temperatures, and concentrations. After optimal HNO_3 pretreatment, a relative increase in the cellulose content (55.84 ± 0.04% w/w) and a significant decrease in hemicellulose (4.38 ± 0.01% w/w) and lignin contents (12.77 ± 0.27% w/w) were observed (Figure 1). Up to 40.00% of lignin was removed when the acid concentration was increased to 2.00% (v/v) (Figure S5), which showed that the strength of HNO_3 was a determinant factor for the HNO_3 pretreatment of switchgrass. In addition, it was reported that the nitrate formed during the HNO_3 pretreatment was a promising nitrogen source in the fermentation process.[32]

It is well known that acid pretreatment of lignocelluloses is a highly active process to obtain a suitable structure. Furthermore, dilute acid is a promising method to extract hemicellulose,[7] which is also proved by this study. The most significant cellulose residue and delignification were observed on the HNO_3 pretreatment of switchgrass, which can be used for nanocellulose or fermented sugar production. Besides, H_3PO_4 and HNO_3 pretreatments of switchgrass show the significant potential for the production of low-cost biofuels and high-value biochemicals because the residual acid can be used as a fertilizer for microbial growth.

Comparison of the Alkaline Pretreatments of Switchgrass. In this study, the impact of the residence time (min), temperature (°C), and concentration of the alkali (% v/v or % w/v) was examined for cellulose, hemicellulose, and lignin levels of the switchgrass biomass with the completely randomized design. The results of the four alkaline pretreatments are presented in Table 1 and in the Supporting Information file. They have been widely used in the pretreatment of biomass for production of paper and mixed sugars due to the solubilization of lignin in these methods.

In this work, the process designed not only maximizes cellulose release and lignin removal but also augments and identifies the optimal parameters. The experiment with 1.00% w/v sodium hydroxide (NaOH) at 75 °C for 60 min results in 53.85% of residual cellulose and 11.10% of residual delignification in pretreated switchgrass (Figure 2). The high temperature was beneficial for lignin removal in the switchgrass pretreatment (Figure S6 and Table S6). Those results obtained
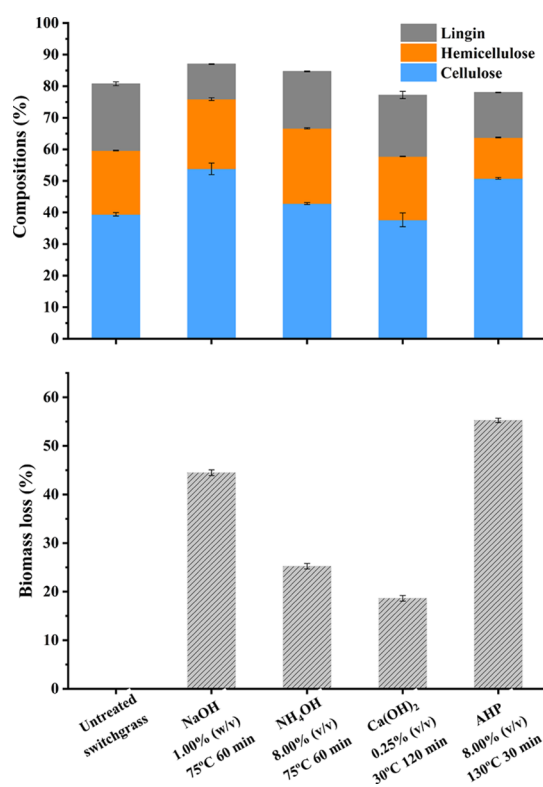
Figure 2. Chemical compositions and biomass loss of alkali pretreated switchgrass.

in the NaOH pretreatment study of switchgrass are consistent with Gao's findings.[33]

Chemical pretreatment with ammonia (NH_4OH) can remove lignin with a minimal effect on hemicellulose degradation.[34] Figure S7 and Table S7 show the details of the NH_4OH pretreatment of switchgrass at varying treatment factors. The optimal condition for the NH_4OH pretreatment is at a temperature of 75 °C for 60 min at a concentration of 8.00% (v/v) (Figure 2). NH_4OH pretreatment does not cause hemicellulose degradation compared to other alkaline agents. A similar result was displayed by Cayetano et al.[35] Additionally, previous studies have found that residual NH_4OH concentrations would be toxic to bacteria,[35,36] which would be an obstacle for bioconversion of lignocellulosic biomass.

Although lime [$Ca(OH)_2$] is not strong enough for pretreatment by itself, it is much cheaper and it is possible to recover calcium that can be used to improve the economic promise of alkaline pretreatment at an ambient temperature.[37] Thus, $Ca(OH)_2$ pretreatment of lignocellulosic biomass is drawing increasing attention. $Ca(OH)_2$ pretreatment parameters of switchgrass were evaluated in this work, and the detailed results are presented in Figure S8. The poor performance of lime probably resulted from the poor solubility of lime in water and the lower pretreatment time used in this work.[38] Future studies should be conducted with $Ca(OH)_2$ and other reagent mixtures to improve the cost-effectiveness of switchgrass for biochemical processes and the pulp and paper manufacturing process.

The alkaline hydrogen peroxide (AHP) was regarded as an effective pretreatment for grass, corn stover, and other materials.[39] We first explored several key parameters to test the potential of AHP for further improvement of switchgrass pretreatment. A cellulose yield of 50.80% is obtained using

pretreatment of 8.00% (v/v) AHP at 130 °C for 30 min (Figure 2). It could be seen that the successful pretreatment processes with a high cellulose yield were achieved in the pretreatment with a high AHP concentration. Other authors have reported the same behavior for different biomass pretreatments.[40]

Although the retention of cellulose was lower in the alkaline pretreatments, most alkaline pretreatments can prevent hemicellulose loss and remove lignin at low temperatures. For the alkali pretreatments of switchgrass, NaOH was one of the best choices for optimizing lignin removal while minimizing carbohydrate loss, which could be successfully used in papermaking and biofuel production.

Study on Various Parameters of the Three Other Switchgrass Pretreatments. In the present study, the three other chemical pretreatments were also compared for the switchgrass utilization. Organosolv pretreatment has been proposed to modify the substrate chemically and physically. Figure S10 and Table S10 outline our strategies to improve the pretreatment of switchgrass using ethanol (C_2H_5OH) with a catalyst of H_2SO_4. The C_2H_5OH pretreatment removed 28.55% of lignins and 51.62% of hemicelluloses with 80% (v/v) C_2H_5OH at 150 °C for 40 min (Figure 3). Cateto et al.
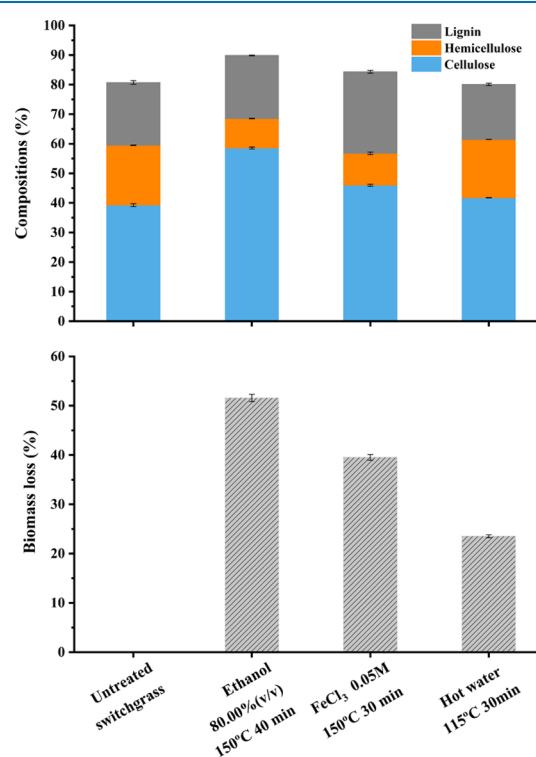
Figure 3. Chemical compositions of the three other pretreated switchgrass.

employed 75% of C_2H_5OH to treat Kanlow switchgrass and the pretreatment was performed at 180 °C for 1 h resulting in a ~60.5% of lignin and ~74.0% of hemicellulose loss.[41] In this study, there was a slight increase in cellulose release and lignin removal with the increasing of the temperature and pretreatment duration. In addition, C_2H_5OH could be recovered by precipitation of pretreatment effluents,[42] which makes the process much more sustainable and promising.

Although ferric chloride ($FeCl_3$) pretreatment hardly removes the lignin, it can efficiently remove the hemicellulose
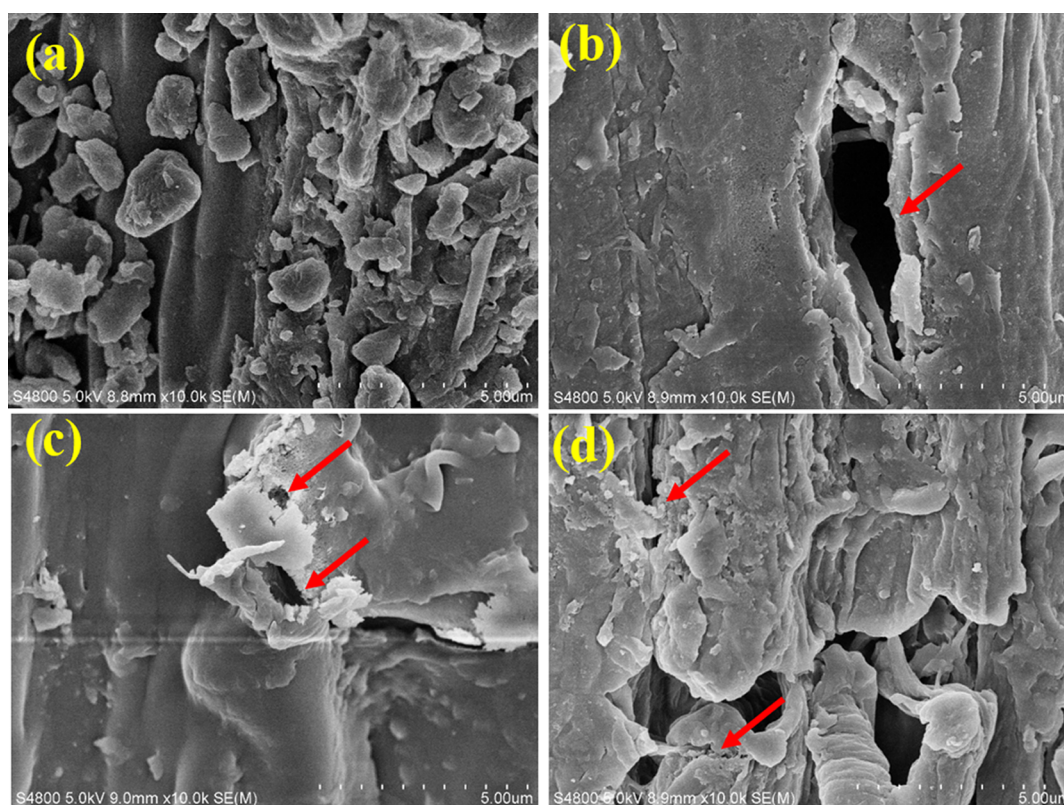
Figure 4. SEM images of untreated and pretreated switchgrass: (a) untreated switchgrass; (b) HNO_3 pretreated switchgrass; (c) NaOH pretreated switchgrass; (d) C_2H_5OH pretreated switchgrass at 10 000× magnification.

and break ether and ester linkages between lignin and carbohydrates. Moreover, it is recyclable.[43] It is useful in the pretreatment of rice straw, bagasse, wood fiber, and *Pennisetum alopecuroides.*[44] The effects of pretreatment durations, temperatures, and $FeCl_3$ concentrations on the $FeCl_3$ pretreatment of switchgrass were first studied, and the results are displayed in Figure 3. Samples pretreated with 0.05 M $FeCl_3$ solution under 150 °C for 30 min exhibited maximum cellulose retention and hemicellulose removal.

Hot water pretreatment does not need catalysts and it is environment friendly.[8] Moreover, due to its ability to separate nearly pure hemicellulose from the rest of the feedstock, hot water pretreatment has been widely used as part of overall processes in fractionating the components of the lignocellulosic biomass. In the present study, lower temperatures (105−135 °C) and short pretreatment durations (15−60 min) were used on the hot water pretreatment of switchgrass, and the results are displayed in Figure 3, Table S12, and Figure S12. The composition change of switchgrass was not apparent, which was consistent with the report of hot water pretreatment of brewers' spent grain.[45]

Structural Changes in the Three Optimal Pretreatments for Switchgrass. In this work, HNO_3, NaOH, and C_2H_5OH pretreatments were found to be the most effective switchgrass pretreatments. The effects on the surface morphology and crystalline structure of these three methods, at the investigated optimal conditions, were further studied. These results provided the most crucial evidence that the intact structures of raw switchgrass were disrupted and the cellulose, hemicellulose, and lignin were changed after pretreatments.

Scanning electron microscopy (SEM) images show the surface of untreated and treated switchgrass samples in Figure 4. The untreated sample had a compact, rough, and nonuniform surface, while the treated samples had some discernible changes that specifically manifested in the appearance of abrasion and a smooth surface, as well as some layering and scaling, which was possibly caused by lignin removal or hemicellulose degradation.[46] The morphological structures of the pretreated samples were thus destroyed, making them advantageous for further utilization.

The crystalline structure could give the evidence of inherent components in the lignocellulosic biomass, and crystallinity in the biomass is mostly attributed to the cellulose. The crystallinity of switchgrass samples was assessed by X-ray diffraction in this work[47] (Figure 5a). There were no new peaks that appeared in pretreated samples, indicating that the three pretreatments did not bring changes in the cellulose crystalline allomorph.[46] According to Reddy et al.,[48] diffraction peaks near 15−16, 22.5, and 35° were originated from cellulose I. The results show that the peaks near 15−16, 22.5° became sharper in pretreated switchgrass, which implied that the crystallinity was increased. This may be correlated with the removal of lignin or hemicellulose, which is consistent with the results of the chemical composition analysis of pretreated samples.

The chemical structure of the lignocellulosic biomass is measured by FT-IR to identify the functional groups in the samples. Figure 5b shows the FT-IR spectra of different switchgrass samples. The result analysis was conducted with the related public literature data.[45,46,49] There was no significant difference between untreated switchgrass and pretreated switchgrass in the spectra, which implied that the three pretreatments did not affect the overall structure of switchgrass. The peaks at 1060 cm^{-1}, which was characteristic
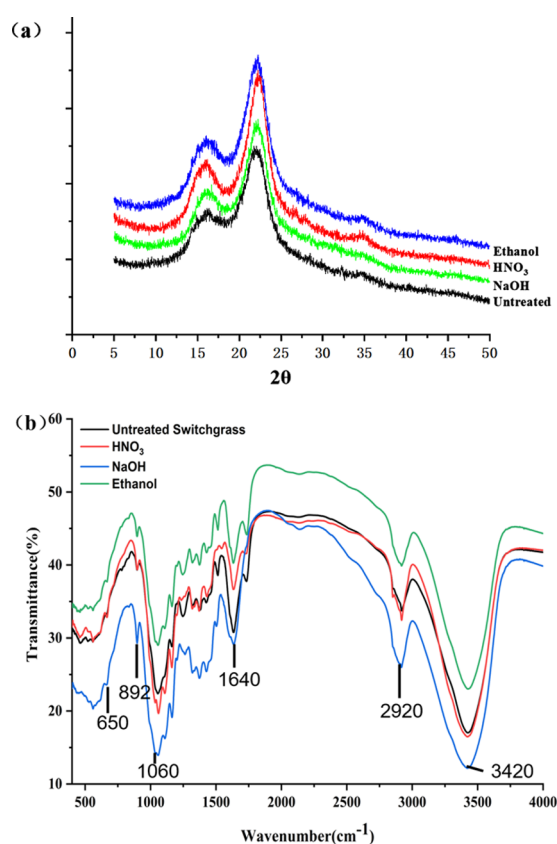
Figure 5. X-ray diffraction (XRD) pattern (a) and Fourier-transform infrared spectroscopy (FT-IR) spectra (b) of untreated and pretreated samples via HNO_3, NaOH, and C_2H_5OH.

of cellulose, were observed after pretreatments. Peaks at wavelengths of 3420 and 2920 cm^{-1} can be used to evaluate the alteration of the cellulose structure. A decrease of these bands resulted from the breakdown of intermolecular hydrogen bonding in cellulose and hemicellulose, which might cause the changes in crystallinity in pretreated switchgrass.

CONCLUSIONS

Twelve different chemical pretreatment techniques were comparatively investigated to enhance switchgrass utilization and reduce energy consumption. SEM, XRD, and FT-IR analyses were further conducted to confirm the structural changes in three optimal pretreated samples. Besides, HCl, AHP, and $FeCl_3$ were employed first to investigate the effect on pretreatment of the switchgrass biomass. In conclusion, NaOH-pretreated switchgrass had a high residual cellulose and low residual lignin under the low temperature while the HNO_3 and C_2H_5OH pretreatments also had the potential to be suitable pretreatment methods for different utilization purposes of switchgrass. Furthermore, HNO_3 and H_3PO_4 were highly recommended in pretreatment of switchgrass for biofuels.

EXPERIMENTAL SECTION

Sample Processing. Switchgrass was donated by the Key Laboratory of Biofuels, Shandong Provincial Key Laboratory of Energy Genetics, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences. Switchgrass was dried at 42 °C for 72 h and after that the dry samples were ground and sieved using a 425 μm opening

sieve. It was then stored at a temperature of 20−25 °C in a dry place for further experiments.

Pretreatment. *Acid Hydrolysis.* The acid hydrolysis of switchgrass samples was carried out with H_2SO_4, HCl, H_3PO_4, CH_3COOH, and HNO_3[8,45,52] to investigate the effect of various acids on switchgrass. First, the ground samples (2.50 g) were dispersed into 50 mL of 0.50% (v/v) acid solutions and then exposed to steam in a pressure vessel at 120 °C for distinct periods (20, 30, 40, and 50 min), respectively. After the pretreatment, the sample was collected by filtration, washed thoroughly with three volumes of distilled water (500 mL) and dried at 42 °C for the biomass loss analysis and chemical composition analysis. Further, the samples (2.50 g) were mixed with 50 mL of 0.50% (v/v) acid solutions and then exposed to steam in a pressure vessel at four different temperatures (115, 120, 125, and 130 °C) for the best pretreatment period from chemical composition analysis of previous experiments. After the pretreatment, the solids were thoroughly washed with deionized water to neutrality, collected by filtration and dried at 42 °C for the biomass loss analysis and chemical composition analysis. Finally, the biomass (2.50 g) was added into 50 mL of varying concentrations of the acid (0.25, 0.50, 1.00, and 2.00% v/v) and exposed to steam in a pressure vessel using the optimal pretreatment period and temperature from the chemical composition analysis of previous experiments. After the pretreatment, the resulting sample was collected by filtration and washed thoroughly with three volumes of distilled water (500 mL) and dried at 42 °C for the biomass loss analysis and chemical composition analysis. Each experiment was repeated in triplicate under the same conditions to ensure the reproducibility of the results, which were expressed as mean values ± standard deviation (SD).

Alkaline Hydrolysis. The effects of NaOH concentrations (0.50, 1.00, 1.50, and 2.00% w/v), temperatures (65, 75, 85, and 95 °C), and time periods (30, 60, 90, and 120 min) on the yield of cellulose, hemicellulose. and lignin were studied with the single factor experiment. The experimental processes were the same as acid hydrolysis, whose details are illustrated in Table S6. The effects of NH_4OH concentrations (6.00, 8.00, 10.00, and 12.00% w/v), temperatures (65, 75, 85, and 95 °C), time periods (30, 60, 90, and 120 min), and the impacts of $Ca(OH)_2$ concentrations (0.25, 0.50, 1.00, and 2.00% w/v), temperatures (25, 30, 35, and 40 °C), time periods (2, 4, 6, and 8 h), as well as, the effects of AHP concentrations (0.50, 1.00, 6.00, 7.00 and 8.00% v/v), temperatures (65, 75, 85, and 95 °C), and time periods (30, 60, 90, and 120 min) on the yield of cellulose, hemicellulose, and lignin were studied in the same way. The H_2O_2 solution is adjusted to pH 11.0 with 6 mol/L NaOH to prepare AHP. Each experiment was repeated in triplicate under the same conditions to ensure the reproducibility of the results, expressed as mean values ± standard deviation (SD).

C_2H_5OH Treatment. Organosolv pretreatment was performed as described by Ravindran et al.[45] with an optimization process. Briefly, the samples (2.50 g) were dispersed into 50 mL of 60.00% (v/v) C_2H_5OH solution, which was made with 1% H_2SO_4 (v/v) as a catalyst, in a 500 mL pressure bottle and then heated at 150 °C for distinct periods (20, 30, 40, and 50 min), respectively. After the pretreatment, the solids were thoroughly washed with deionized water to neutrality, collected by filtration and dried at 42 °C for the biomass loss analysis and chemical composition analysis. Further, the

samples (2.50 g) were mixed with 60.00% (v/v) C_2H_5OH solution (50 mL) and then heated at four different temperatures (140, 150, 160, and 170 °C) for the best pretreatment period from the chemical composition analysis of previous experiments. After the pretreatment, the solids were thoroughly washed with deionized water to neutrality, collected by filtration and dried at 42 °C for the biomass loss analysis and chemical composition analysis. Finally, the biomass (2.50 g) was added into 50 mL varying concentrations of C_2H_5OH (60.00, 70.00, 80.00, and 90.00% v/v) and then heated with the optimal pretreatment period and temperature from the chemical composition analysis of previous experiments. After the pretreatment, the solids were thoroughly washed with deionized water to neutrality, collected by filtration and dried at 42 °C for the biomass loss analysis and stored in a cool and dry place until further chemical composition analysis. Each experiment was repeated in triplicate under the same conditions to ensure the reproducibility of the results, expressed as mean values ± standard deviation (SD).

FeCl₃ Pretreatment. FeCl₃ pretreatment of switchgrass was implemented according to the procedure described by Chen et al.[43] Raw biomass (2.50 g) was treated with 50 mL of FeCl₃ solution and the effects of FeCl₃ concentrations (0.01, 0.05, 0.10, and 0.2 M), temperatures (140, 150, 160, and 170 °C), and time periods (20, 30, 40, and 50 min) were investigated. The experiment processes were the same as those in C_2H_5OH treatment, whose details can be found in Table S11. Each experiment was repeated in triplicate under the same conditions to ensure the reproducibility of the results, expressed as mean values ± standard deviation (SD).

Hot Water Treatment. Hot water pretreatment of switchgrass was performed as described by Ravindran et al.[45] with a slight modification. Switchgrass (2.50 g) was moistened with 50 mL of water. Moreover, the effects of pretreated temperatures (105, 115, 125, and 135 °C) and time periods (15, 30, 45, and 60 min) in a stainless-steel autoclave were studied. The experiment processes were the same as those in C_2H_5OH treatment and the details can be found in Table S12. Each experiment was repeated in triplicate under the same conditions to ensure the reproducibility of the results, expressed as mean values ± standard deviation (SD).

Pretreated Switchgrass Characterization. SEM, X-ray diffraction, and FT-IR were used to observe the changes in the surface, crystallinity, and chemical structure of the four optimal pretreatments of the switchgrass biomass, respectively, using the methods mentioned by Wang et al.[46] The surface structures of untreated and pretreated switchgrass samples were analyzed with a SEM (Hitachi S-4800, Hitachi, Ltd.). XRD analysis was conducted using a Bruker D8 Advance XRD system (Germany). FT-IR analysis was carried out using a Nicolet 6700 FT-IR spectrometer (Thermo Fisher). Untreated and pretreated samples mixed with spectroscopic grade potassium bromide (1:20) were obtained within the spectral range of 400−4000 cm^{-1}.

Chemical Composition Measurement. Chemical compositions of the native and pretreated switchgrass samples were performed according to the National Renewable Energy Laboratory (NREL) protocol with a slight modification.[50,51] Briefly, 300 ± 10 mg of the sample was mixed into 3.00 ± 0.01 mL of 72% (w/w) sulfuric acid and shaken at 30 °C for 60 min. Then, adding 84.00 ± 0.04 mL of deionized water, the mixture was incubated at 121 °C for 60 min. When the mixture

was cooled to room temperature, the residue was removed by filtration to determine the acid-insoluble lignin (AIL) content. High-performance liquid chromatography (HPLC) determined the supernatant with a refractive index detector (RID). The Bio-Rad Aminex HPX-87H column (7.8 mm × 300 mm, 9 μm) was used for monosaccharide determination at 60 °C. A final amount of 5 mmol/L H_2SO_4 was used as the mobile phase at a flow rate of 0.6 mL/min. Besides, the acid-soluble lignin (ASL) content in the liquid was detected using a UV−visible spectrophotometer.

■ ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01047.

> Tables and figures of the results of 12 pretreatment optimization processes (PDF)

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Notes

The authors declare no competing financial interest.

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