Thermo-carbide slag pretreatment of turfgrass pruning: Physical-chemical structure changes, reducing sugar production, and enzymatic hydrolysis kinetics

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**ABSTRACT**

Alkaline pretreatment shows a good effect on delignification of lignocellulosic biomass, and carbide slag, an alkaline industrial waste, was first applied to pretreat turfgrass pruning. Three thermo-alkaline pretreatment methods were compared to evaluate their ability to destroy the biomass structure and to enhance the enzymatic hydrolysis efficiency of turfgrass pruning, namely thermo-NaOH pretreatment, thermo-lime pretreatment, and thermo-carbide slag pretreatment. Results showed that the maximum net reducing sugar yield of 269.55 mg/g raw biomass was achieved by thermo-carbide slag pretreatment. Compared with raw biomass, the lignin and hemicellulose content of turfgrass pruning after thermo-carbide slag pretreatment reduced by 35.57% and 62.40%, respectively. SEM and FTIR analyses showed that the surface structure and chemical groups of turfgrass pruning were obviously destroyed by thermo-carbide slag pretreatment. There was a high positive correlation between the enzymatic hydrolysis efficiency and cellulose content of substrate. However, a high negative correlation was observed between the enzymatic hydrolysis efficiency and lignin content of substrate. The enzymatic hydrolysis kinetics with different pretreatments were well described by the fractal-like kinetics model, and the rate constant of enzymatic hydrolysis of thermo-alkali pretreated turfgrass pruning was about three times of that of the unpretreated turfgrass pruning. Overall, carbide slag is an ideal pretreatment material for enzymatic hydrolysis of turfgrass pruning in terms of hydrolysis efficiency and waste reuse.

1. Introduction

Along with the rapid development of urbanization, urban greening is more and more practiced. The expanding urban greening, especially lawn area, results in the production of large amounts of turfgrass pruning [1]. Turfgrass pruning as municipal solid waste may cause an adverse impact on the environment, so converting the turfgrass pruning to biofuel will be an environment-friendly technology [2]. With several advantages, such as fast growth, large production and renewability, the turfgrass pruning is a new feedstock for biofuel production. However, due to the complex inherent structure of lignocellulosic biomass, enzymatic hydrolysis of lignocellulosic biomass without pretreatment is difficult to achieve a high biofuel yield [3–5].

Therefore, various pretreatments have been introduced to promote the reducing sugar production from lignocellulosic biomass, including microwave irradiation [6], high pressure homogenization [7], thermo-alkaline pretreatment [8], thermo-acid pretreatment [9], biological pretreatment [10], and combined pretreatments [11,12]. Among these pretreatment methods, thermo-alkaline pretreatment is the most promising because of its high efficiency [13]. Thermo-alkali pretreatment of pine wood resulted in the highest methane yield with 8% NaOH at 100 °C for 10 min [14]. The maximum biogas yield from rice straw reached 574.5 ml/g VS with a 10% Ca(OH)2 pretreatment, 36.7% higher than that of the control [15]. Chen et al. reported that cumulative biogas yield from Spartina alterniflora increased by 68.17–153.29% after thermo-NaOH pretreatment [16]. Jin et al. compared thermo-NaOH and thermo-lime pretreatment of catalpa sawdust and found that thermo-lime pretreatment yielded more reducing sugar [17]. Although thermo-alkaline pretreatment is effective for lignocellulosic biomass, there are still some disadvantages, such as high alkali dosage and corresponding high cost of alkaline material [2].

Carbide slag, an industrial waste, is an alkaline mixture containing
more than 60% CaO [18]. To explore its utilization, many researchers have applied carbide slag as transesterification catalyst, neutralizing agent for acidic wastewater treatment, adsorbent for capturing CO₂, and raw material for cement production [19,20]. In this study, carbide slag was employed to pretreat the turfgrass pruning. The results will expand the utilization approach of carbide slag, and save the cost of lignocellulosic biomass pretreatment for biofuel production.

The objective of this study was to evaluate the efficiency of thermo-carbide slag pretreatment of turfgrass pruning for biofuel production, and the results were compared with those using thermo-water, thermo-NaOH, and thermo-lime pretreatment. The chemical composition and surface structure of turfgrass pruning after pretreatment were analyzed. Furthermore, the relationship between enzymatic hydrolysis efficiency and the structure change of turfgrass pruning was investigated, and the enzymatic hydrolysis kinetics was also studied.

2. Materials and methods

2.1. Materials

In this study, turfgrass pruning (Poa pratensis) was collected from a local lawn in Beijing, China. After air-drying, the turfgrass pruning was milled by a laboratory mill (DF-25S, Dade, China), and screened to obtain a fraction of 20-60 mesh, then stored in plastic bags at room temperature. All chemicals were of analytical grade and purchased from Beijing Chemical Industry Group Co., China. The commercial enzyme was provided by Hunan Chemical Ltd. (China) and its filter paper activity was 200 FPU/g. Carbide slag was taken from a polyvinyl chloride production factory (China) and its components are listed in Table 1.

2.2. Pretreatment

For the pretreatments, 10.0 g dried turfgrass pruning was immersed in 200 ml distilled water, NaOH, lime and carbide slag solution of 1.75% (w/v) at 120 °C for 1 h in a sealed stainless-steel hydrothermal reaction kettle (Beijing Yanzheng Biotechnology co. LTD, China), respectively. The chemical dosage and heating temperature were chosen according to the literature and previous study [17,21,22], and the mixture pH was within a range from 12.43 to 13.19 with different alkaline pretreatments, which is the common pH used in alkaline pretreatments. After pretreatments, the mixture was cooled to room temperature, and the solid was separated from the liquor by filtration, and washed with tap water until a neutral pH. The solid fraction was dried at 65 °C and used as the feedstock for subsequent enzymatic hydrolysis. Raw turfgrass pruning was used as control without any pretreatment.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out for 72 h in 150 ml flasks with a solid loading of 2.5% (w/v) at a pH of 4.8, a temperature of 50 °C and a rotation speed of 150 r/min [2]. The samples were withdrawn at a certain time interval. The reaction mixture was filtered, and the liquid was used to analyze the reducing sugar yield. All enzymatic hydrolysis experiments were conducted in triplicates. Results were presented as mean value and standard deviations.

2.4. Analysis procedure

The chemical composition of turfgrass pruning samples were analyzed by a fiber analyzer (A2000i, Ankom, USA). The reducing sugar was measured using the 3,5-dinitrosalicylic acid method (DNS). The surface morphology feature of turfgrass pruning samples was scanned by scanning electron microscopy (S-3400 N II, Hitachi, Japan), and all samples were sputter-coated with gold before scanning. Fourier transformation infrared spectra (FTIR) of the samples were detected by a Fourier transform infrared spectrometer (Vertex 7.0, Bruker, USA) in the range from 400 to 4000 cm⁻¹.

The water absorption capacity was calculated by Eq. (1):

\[
\text{Water absorption capacity} = \frac{\text{Weight}_{0} - \text{Weight}_{1}}{\text{Weight}_{0}} \times 100\%
\]

\[
(1)
\]

where \(\text{Weight}_{0}\) is the weight of substrate before drying, and \(\text{Weight}_{1}\) is the weight of substrate after drying at 105 ± 3 °C for a minimum of 4 h.

The crystallinity index analysis of samples was carried out using an X-ray power instrument (Bruker, Germany), with Ni-filtered Cu Kα radiation (\(\lambda = 1.54 \text{ Å}\)) at 40 kV and 40 mA. Scattered radiation was detected in the 2θ range from 5° to 35° at a scan rate of 0.2°/min. The crystallinity index (CrI) was calculated based on Eq. (2) [23]:

\[
\text{CrI} = \frac{I_{002} - I_{am}}{I_{002}} \times 100\%
\]

\[
(2)
\]

where \(I_{002}\) represents the intensity of 002 lattice diffraction (2θ = 22.6°) and \(I_{am}\) represents the intensity of amorphous section (2θ = 18°).

The net reducing sugar yield based on 1 g raw material was calculated by Eq. (3):

\[
\text{Net reducing sugar yield} = \text{Reducing sugar yield (mg/g)} \times \text{Solid recovery (％)} \times 1 \text{ g (raw)}
\]

\[
(3)
\]

where Reducing sugar yield (mg/g) is the reducing sugar yield based on 1 g pretreated biomass, Solid recovery is the percentage of remaining solid after pretreatment per unit of raw biomass, and 1 g (raw) is the weight of raw biomass.

The fractal-like kinetics model can be used to describe the enzymatic hydrolysis of biomass, and the reducing sugar concentration can be deduced as Eq. (4):

\[
P = \frac{S_{0}}{0.9} \left[1 - \exp \left(-\frac{0.9 \times k \cdot t}{1 - h}\right)\right]
\]

\[
(4)
\]

where \(P\) is the concentration of reducing sugar, \(S_{0}\) is the initial concentration of reducing sugar, \(h\) is the fractal dimension describing the substrate fractal, 0.9 is the conversion coefficient from cellulose to reducing sugar, \(k\) is the rate constant presenting the reaction rate between substrates and enzymes, and \(t\) is the enzymatic hydrolysis time.

3. Results and discussion

3.1. Solid recovery and composition of turfgrass pruning after pretreatments

Thermo-alkaline pretreatment solubilizes part of biomass, mainly lignin and hemicellulose. Thus the solid recovery of lignocellulosic biomass decreased after pretreatment. In this study, solid recovery
represents the percentage of remaining solid weight after pretreatment. As shown in Fig. 1, the solid recovery after different thermo-alkaline pretreatments was from 33.3% to 74%, indicating significantly different effects of alkaline chemicals during the pretreatment. The maximum solid recovery of 74% was achieved after thermo-carbide slag pretreatment, while only 33.3% solid recovery was obtained after thermo-NaOH pretreatment. The low solid recovery was undesirable for the subsequent reducing sugar production.

To increase the cellulose content and decrease the lignin and hemicellulose content is the main theme of turfgrass pruning pretreatment, since the reducing sugar was mainly produced from cellulose. Jin et al. also demonstrated that thermo-lime and thermo-carbide slag pretreatment increased the CrI value by 62.38%, 49.01% and 46.04%, respectively. The increase of CrI value by lime and thermo-carbide slag pretreatment could enhance the enzymatic hydrolysis of sugarcane bagasse increased by 13.93%, and the subsequent enzymatic hydrolysis increased more than two fold. The crystallinity index (CrI) represents the ratio of crystalline cellulose to the amorphous region (often lignin and hemicellulose) [28]. Crystalline cellulose in lignocellulosic biomass is highly recalcitrant because of the intermolecular hydrogen bonding within lignocellulosic biomass, thus preventing the biomass degradation [10]. Previous studies reported that high CrI value could improve the enzymatic hydrolysis. Chen et al. reported that after NaOH pretreatment, the CrI value of sugarcane bagasse increased by 13.93%, and the subsequent enzymatic hydrolysis increased more than two fold [29].

Table 2 reports the CrI of turfgrass pruning before and after pretreatments. The thermo-water pretreatment did not enhance the CrI value owing to its mild condition. However, thermo-NaOH, thermo-lime and thermo-carbide slag pretreatment increased the CrI value by 62.38%, 49.01% and 46.04%, respectively. The increase of CrI value by thermo-alkaline pretreatment could be attributed to the removal of amorphous region (lignin and hemicellulose) of lignocellulosic biomass [30,31], which was in accordance with the results from Fig. 2 that thermo-alkaline pretreatment solubilized most of lignin and part of hemicellulose.

3.4. Accessible surface area of turfgrass pruning after pretreatments

The accessible surface area of biomass is a determining factor for enzymatic hydrolysis, and increasing the accessible surface area significantly benefits the enzymatic hydrolysis [32]. The accessible surface area of lignocellulosic biomass can be evaluated by water absorptive capacity [7]. The higher the water absorptive capacity is, the higher the accessible surface area is. Thus, increasing water absorption capability was a determining method to promote the enzymatic hydrolysis [7]. The changes of water absorption capacity of turfgrass pruning before and after pretreatments were summarized in Table 2. The water absorption capability of turfgrass pruning pretreated by thermo-NaOH, thermo-lime and thermo-carbide slag was higher than 90% (Table 2), and respectively increased by 17.58%, 12.27% and 11.14%, compared with that of the raw biomass. Jin et al. reported that the water absorption capability of biomass after high-pressure homogenization pretreatment was much higher than that of raw biomass [33]. The thermo-alkaline pretreatments could enhance the enzymatic hydrolysis of turfgrass pruning through increasing the water absorption capability.
3.5 FTIR spectra of turfgrass pruning before and after carbide slag pretreatment

FTIR spectra have been widely applied to investigate the chemical functional groups of lignocellulosic biomass [34]. As shown in Fig. 4, after thermo-carbide slag pretreatment, the absorbance peak at 3000–3600 cm\(^{-1}\) assigning to the hydrogen bonds weakened, since the hydrogen bonds in biomass could be disrupted by the carbide slag. Several peaks associated with lignin and hemicellulose, such as the characteristic peaks of 1725, 1600 and 1250 cm\(^{-1}\), were clearly observed (Fig. 4). Compared with raw biomass, their intensities sharply reduced after thermo-carbide slag pretreatment, which strongly supported the results in Fig. 2 and Fig. 3 that hemicellulose and lignin were destroyed and removed after thermo-alkaline pretreatments. Besides, a significant reduction occurred at 898 cm\(^{-1}\) (\(\beta\)-1,4-glucosic bond vibration), indicating that the bond between cellulose and hemicellulose was broken [22]. These results confirmed that thermo-carbide slag pretreatment had a very positive effect on the removal of lignin and hemicellulose, and could enhance the interactions between enzymes and celluloses. Similar results were obtained in sugarcane bagasse pretreatment by microwave assisted glycerol [35].

### Table 2
CrI value and water absorption capacity of raw biomass and pretreated turfgrass pruning.

<table>
<thead>
<tr>
<th>Pretreatments</th>
<th>Raw biomass</th>
<th>Thermo-water</th>
<th>Thermo-NaOH</th>
<th>Thermo-lime</th>
<th>Thermo-carbide slag</th>
</tr>
</thead>
<tbody>
<tr>
<td>CrI value (%)</td>
<td>20.2</td>
<td>16.1</td>
<td>32.8</td>
<td>30.1</td>
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<td>Water</td>
<td>81.07</td>
<td>80.98</td>
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<td>90.10</td>
</tr>
<tr>
<td>absorption</td>
<td>capacity (%)</td>
<td></td>
<td></td>
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</tbody>
</table>

#### 3.6 Enzymatic hydrolysis of turfgrass pruning with and without pretreatments

The aim of biomass pretreatment is to improve the reducing sugar production. Compared with the raw turfgrass pruning, thermo-lime and thermo-carbide slag pretreatments effectively improved the net reducing sugar yield (as shown in Fig. 5). The maximum net reducing sugar yield was achieved with the thermo-carbide slag pretreatment.
yield of 269.55 mg/g turfgrass pruning was obtained with the thermo-carbide slag pretreatment, which increased by 58.35% compared with that from the raw biomass. Many studies reported that alkali had a good effect for lignocellulosic biomass pretreatment. Lin et al. reported that water hyacinth produced 296.0 mg/g reducing sugar after NaOH pretreatment [36], and Wang et al. reported that corn straw produced 241.17 mg/g reducing sugar after lime pretreatment [37]. In this study, thermo-NaOH pretreatment showed higher proportion of cellulose and lower proportion of lignin and hemicellulose in the solid after pretreatment (Fig. 2), severer structural damage (Fig. 3) and higher water absorption capacity (Table 2). However, the net reducing sugar yield after thermo-NaOH pretreatment was significantly lower, even lower than that of the raw biomass (Fig. 5). The main reason was that the solid recovery of thermo-NaOH pretreatment was significantly lower than that of other pretreatments (as shown in Fig. 1). The cellulose of 0.168, 0.128, 0.203 and 0.249 g/g turfgrass pruning was obtained after thermo-water, thermo-NaOH, thermo-lime, and thermo-carbide slag pretreatment, respectively. The better effect of thermo-carbide slag than thermo-lime and thermo-NaOH might be also attributed to its composition. The main component of carbide slag was CaO, which counted for 70.06% of the solid, other components included Al₂O₃, Fe₂O₃, MgO and so on (as shown in Table 1). The synergetic effects of these metallic oxides might contribute to breaking down of lignocellulosic structure, and the detailed mechanisms should be studied in the future.

Since the aim of biomass pretreatment is to improve enzymatic hydrolysis, enhance net reducing sugar production and reduce cost. Therefore, the thermo-carbide slag pretreatment is chosen because of the highest solid recovery and the highest net reducing sugar production, rather than the thermo-lime and thermo-NaOH pretreatment. Furthermore, the cost of carbide slag for turfgrass pruning pretreatment in biofuel production is low, meanwhile, the handling cost of carbide slag will be saved. Using carbide slag in lignocellulosic biomass pretreatment is promising.

### 3.7. Fractal-like kinetics modeling

The kinetics of enzymatic hydrolysis of pretreated turfgrass pruning was fitted with the fractal-like kinetics model, and the results were reported in Table 3. Compared to other models, the advantages of fractal-like kinetics model include an effective analytical solution, a good convergence for product formation, and simple equation (only two easily determined parameters, i.e. fractal dimension and rate constant) [38]. The correlated coefficients (R²) were all above 0.95 (Table 3), indicating that the fractal-like kinetics model was suitable for studying the enzymatic hydrolysis of turfgrass pruning. The greater the rate constant (k) was, the stronger the binding capacity between biomass and enzymes was [39]. The rate constants of turfgrass pruning pretreated by three thermo-alkaline methods were all higher than that of raw biomass, which indicated that the alkali enhanced the enzymatic hydrolysis of biomass. All fractal dimensions were in the range of 0–1, and the lower the fractal dimension, the more easily the biomass was hydrolyzed. The fractal dimension was distributed in a range from 0.604 to 0.683 after thermo-alkaline pretreatments and there was no significant difference.

### 3.8. Relationship between turfgrass pruning component and enzymatic hydrolysis

To further reveal the effect of chemical component change after pretreatments on the enzymatic hydrolysis, the relationship between enzymatic hydrolysis efficiency and chemical component of turfgrass pruning was investigated. As shown in Fig. 6, the enzymatic hydrolysis was more obviously influenced by the content of cellulose, lignin and hemicellulose with a R² > 0.94. The reducing sugar was mainly produced from cellulose, so the enzymatic hydrolysis efficiency of biomass showed a positive correlation with the cellulose content. The presence of lignin and hemicellulose reduced the accessibility of lignocellulosic biomass to enzymes and limited further biomass degradation [40]. Mosier et al. also summarized that the biodegradation of lignocellulosic biomass was strongly influenced by the presence of lignin, which resisted the enzymatic hydrolysis process [41]. Therefore, decreasing the lignin content was beneficial to improving subsequent enzymatic hydrolysis of lignocellulosic biomass.

The CrI value was positively related to the enzymatic hydrolysis efficiency, and the R² reached to 0.988, which could be attributed to the disruption of amorphous region (lignin and hemicellulose) after thermo-alkaline pretreatments (as shown in Fig. 2 and Table 2). Similarly, Zhu et al. reported that the enzymatic hydrolysis efficiency benefited from the high biomass crystallinity [42]. There was also a positive correlation (R² = 0.959) between water absorption capacity and enzymatic hydrolysis. The more the specific surface area was, the higher the enzymatic hydrolysis efficiency was.

### Table 3

<table>
<thead>
<tr>
<th>Pretreatments</th>
<th>Enzymatic hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate constant (k)</td>
</tr>
<tr>
<td>Raw material</td>
<td>0.030</td>
</tr>
<tr>
<td>Thermo-water</td>
<td>0.026</td>
</tr>
<tr>
<td>Thermo-NaOH</td>
<td>0.095</td>
</tr>
<tr>
<td>Thermo-lime</td>
<td>0.097</td>
</tr>
<tr>
<td>Thermo-carbide slag</td>
<td>0.087</td>
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</tbody>
</table>

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4. Conclusions
Thermo-alkaline pretreatments effectively enhanced the Crl value, water absorption capacity, and disrupted the surface structure of turfgrass pruning, thus greatly enhancing the reducing sugar production from turfgas pruning. Thermo-carbide slag pretreatment was optimal in terms of solid recovery (74%) and net reducing sugar yield (269.55 mg/g turfgas pruning). There was a high positive correlation between the enzymatic hydrolysis efficiency and cellulose content of biomass, while a high negative correlation between the enzymatic hydrolysis efficiency and lignin content. The fractal-like kinetics model was suitable for fitting the enzymatic hydrolysis kinetics of turfgas pruning, and the rate constant of enzymatic hydrolysis of turfgas pruning pretreated with thermo-alkaline methods was about three times of that of the control. In summary, the carbide slag, an industrial waste, is a promising and economic alkaline material to pretreat lignocellulosic biomass for subsequent biofuel production.

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