Thermo-carbide slag pretreatment of energy plants for enhancing enzymatic hydrolysis

Xue Tao[a], Guangming Zhang[b], Panyue Zhang[a,*], Siqi Wang[a], Mohammad Nabi[a], Hongjie Wang[a]

[a] College of Environmental Science and Engineering, Beijing Forestry University, Beijing 100083, China
[b] School of Environment & Natural Resources, Renmin University of China, Beijing 100872, China

ABSTRACT

Energy plants have several advantages, such as, low lignin, fast growth, large production and perenniality, and present great potential for biofuel production. To enhance its biodegradability, in this study, carbide slag as a novel pretreatment agent was employed to pretreat four typical energy plants, namely *Medicago sativa, Panicum virgatum, Triarrhena lutarioriparia,* and *Arundo donax.* These energy plants were all pretreated in 1.75% (w/v) carbide slag solution at 120 °C for 1 h, and then were hydrolyzed to produce reducing sugar within 72 h by an enzymes loading of 30 FPU/g substrate. The results showed that thermo-carbide slag remarkably disrupted the surface microstructure from a high compact and tight structure to a poly-ravine and distorted structure of four energy plants. Thermo-carbide slag pretreatment significantly facilitated the removal of hemicellulose and lignin, and increased the crystallinity indexes. After thermo-carbide slag pretreatment, four energy plants yielded more than 220 mg/g of reducing sugar. The maximum reducing sugar yield of 297.87 mg/g was produced from pretreated *Panicum virgatum*, which is 7.9 times higher compared with that of raw *Panicum virgatum*. The significant improvement of enzymatic hydrolysis may be attributed to the low lignin content, higher retention of cellulose, and low crystallinity after the thermo-carbide slag pretreatment. Thermo-carbide slag pretreatment is a promising pretreatment method, especially for *Panicum virgatum*. Carbide slag, as the abundant and cheap alkaline waste, can reduce the processing cost of energy plant bioconversion for further biofuel production. The study provides a novel research direction for further biofuel production through waste treating waste.

1. Introduction

In recent years, utilization of renewable biomass as biofuel production is becoming more attractive, and energy plants are the most potential feedstock for conversion to biofuels and chemicals. Energy plants have been characterized as low lignin content, fast growth, large production and perenniality, showing great potential for biofuel production (Mohapatra et al., 2017). Moreover, they need lower energy inputs than annual crops and avoid competition for land use with food crops (Wi et al., 2015). Energy plants also benefits the environment through soil and water conservation, carbon sequestration, and providing habitat for wildlife (Kou et al., 2017). The biomass yield, composition and biodegradability of lignocellulosic biomass are important factors that affect biofuel production and cost (Kou et al., 2017; Seunggon et al., 2015). As the most abundant renewable energy feedstock, lignocellulosic biomass is mainly composed of cellulose, hemicellulose, and lignin. The chemical composition and tight structure of lignocellulosic biomass contribute to native biomass recalcitrance to

enzyme access (Siripong et al., 2016).

In general, the conversion efficiency of lignocellulosic biomass to biofuels is very low without any pretreatment due to its tight structure. For biofuel production, a three-step process is necessary to convert lignocellulosic biomass to biofuels: pretreatment, enzymatic hydrolysis and fermentation (Ye et al., 2016). Enzymatic hydrolysis is the rate-limiting process, and thus various pretreatment methods are employed to enhance its efficiency. Chemical pretreatments (Yoo et al., 2017), physical pretreatments (Singh et al., 2017), hydrothermal pretreatments (Sun et al., 2015), biological pretreatments (Baba et al., 2017), and combinations of these methods (Al-Mallahi et al., 2016; Moodley and Kana, 2017) have been applied to disrupt the lignocellulosic biomass structure and increase enzyme access to cellulose. Among various pretreatment methods, alkaline pretreatment has been considered as a promising pretreatment method due to its high effectiveness at breaking ester bonds between lignin and polysaccharides (Jin et al., 2016; Martínez et al., 2015). It is reported that alkaline pretreatment is the most widely used processing method for the effective removal of

lignin from lignocellulosic biomass (Sun et al., 2015). Jin et al. (2015a,b) reported that enzymatic hydrolysis of grass clipping increased by 148.8% after the thermo-NaOH pretreatment. Kamireddy et al. (2013) reported that alkaline pretreatment effectively removed lignin from lignocellulosic biomass. After the 20% thermo-Ca(OH)$_2$ pretreatment, glucose production from *Eucalyptus* improved to 90% compared with 64% for raw biomass (Ishiguro and Endo, 2015). Under optimal pretreatment conditions (50 °C, 24 h, 0.10 g Ca(OH)$_2$/g raw biomass), reducing sugar production reached 433.40 mg/g (Xu et al., 2010). However, in previous research, these alkaline chemicals were expensive, and the pretreatment process was time-consuming and not environmentally friendly.

Carbide slag, an alkaline industrial waste, is a mixture containing more than 60% of CaO. Carbide slag of about 1.5 ton is generated in production of 1 ton polyvinyl chloride (Cheng et al., 2009), and more than 40 million tons of carbide slag is produced worldwide every year. To explore its utilization, many researchers have applied carbide slag as trans-esterification catalyst, neutralizing agent of acidic wastewater, adsorbent for capturing CO$_2$, and raw material for cement (Li et al., 2012a,b; Liu et al., 2015). In this work, thermo-carbide slag is employed to pretreat energy plants. The study is expected to expand the utilization of carbide slag, and save the pretreatment cost of energy plants for further biofuel production.

The aim of this study is to evaluate the effects of thermo-carbide slag pretreatment on enhancing enzymatic hydrolysis of four typical energy plants. In addition, the chemical composition and physical-chemical structure changes of four energy plants were also investigated.

2. Materials and methods

2.1. Materials

Medicago sativa (MS), *Panicum virgatum* (PV), *Triarrhena lutariori-paria* (TL), and *Arundo donax* (AD), four typical energy plants, were harvested at the Grass and Biofuels Center in Beijing, China. These energy plants were dried in an oven at 105 °C for one hour and then 65 °C to a constant mass, and then milled using a laboratory hammer mill (DF-25S, Dade, China) and screened using a 20–80 mesh sieve shaker. In this study, the commercial cellulose enzyme generated by *Trichoderma reesei* was supplied by Hunan Chemical Ltd. (Hunan, China), and its filter paper activity was 200 FPU/g. Carbide slag was taken from Hengyang Ruijie Trade Co., Ltd. (Hunan, China), and its components analyzed by X-ray Fluorescence Spectrometer (AXIOS, Netherlands) are listed in Table 1.

2.2. Thermo-carbide slag pretreatment

For the thermo-carbide slag pretreatment, 10.0 g dried energy plant sample was immersed in 200 ml 1.75% (w/v) carbide slag solution. The suspension was heated at 120 °C for 1 h in a sealed stainless-steel hydrothermal reaction kettle (Beijing Yanzheng Biotechnology co. LTD, Beijing, China) (Dien et al., 2006; Tao et al., 2018). After the pretreatment, the mixture of substrates was cooled in the cold rinse bank to room temperature, and the solid was separated from the liquor via filtration, and then washed with tap water until a neutral pH. The solid fraction was dried at 65 °C and then used as a feedstock for the subsequent enzymatic hydrolysis. Raw energy plants were used as the control without any pretreatment.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of samples was conducted in a shaking incubator at 50 °C and 100 rpm for 72 h in 150-ml flasks with constant temperature incubator. The reaction system contained 1 g sample and 40 ml citrate buffer (0.1 mol/L, pH = 4.8), and the enzyme loading of substrate was 30 FPU/g. Samples were taken at certain time intervals for the determination of reducing sugar yield. All enzymatic hydrolysis experiments were carried out in triplicates.

2.4. Analysis methods

The compositions of energy plants were analyzed using a fiber analyzer (A200i, Ankom, USA). Microstructure of the surface was examined using scanning electron microscopy (S-3400N II, Hitachi, Japan). All samples were sputter-coated with gold before scanning, and the acceleration voltage was 10 kV. Fourier transformation infrared spectra (FTIR) of the samples were detected using Fourier transform infrared spectrometer (Vertex 7.0, Bruker, Germany) in the range from 4000 to 400 cm^{-1} to analyze the structural changes of chemical groups after the thermo-carbide slag pretreatment. Reducing sugar concentration was measured using the 3,5-dinitrosalicyclic acid method (Miller, 1959).

The fractal-like kinetics model was used to describe the reactions of lignocellulosic biomass that were prepared as fractals. The reactions fitted the fractal-like kinetics model as Eq. (1) (Auxenfans et al., 2014):

$$P = \frac{S_0}{0.9}\left[1 - \exp\left(-\frac{0.9 \times k}{1-h}t^{1-h}\right)\right] \tag{1}$$

where P is the concentration of reducing sugar (g/L), S$_0$ is the initial concentration of reducing sugar (g/L), h is the fractal dimension that describes the substrate fractal, 0.9 is the conversion coefficient from biomass to reducing sugar, k is the rate constant (h^{-1}), presenting the reaction rate between the substrates and enzyme, and t is the enzymatic hydrolysis time (h). The model has been proved effective with good convergence for product formation.

The crystallinity index of samples was determined via X-ray diffractometer (XRD) (D8 Advance, Bruker, Germany). Samples were scanned at 6 s/step from $2\theta = 5°$–30° with a step of 0.2°. Crystallinity index (CrI) was calculated based on Eq. (2) provided by Segal et al. (1959):

$$CrI = 100 \times \frac{I_{002} - I_{amorphous}}{I_{002}} \tag{2}$$

where I$_{002}$ is the diffraction intensity of crystalline structure ($2\theta \approx 22.6°$), and I$_{amorphous}$ is the diffraction intensity of amorphous fraction ($2\theta \approx 16.3°$).

3. Results and discussion

3.1. Chemical composition of four energy plants

Lignocellulosic biomass is mainly composed of cellulose, hemicellulose and lignin. Low lignin content in lignocellulosic biomass is beneficial for the enzymatic digestibility of lignocellulosic biomass (Yang et al., 2014). Table 2 shows the chemical composition content of four energy plants and other common agro-forestry solid wastes. It showed that different lignocellulosic biomass had little difference in

Table 1
Components of carbide slag.

Components	CaO	SiO$_2$	Fe$_2$O$_3$	Al$_2$O$_3$	MgO	SO$_3$	P$_2$O$_5$	Na$_2$O	LOI
(%)	70.06 ± 4.36	3.03 ± 0.39	0.40 ± 0.06	0.99 ± 0.09	0.11 ± 0.01	0.38 ± 0.04	0.01 ± 0.00	0.08 ± 0.00	24.94 ± 4.60

Notes: LOI is loss on ignition. All data are presented as means ± standard deviations.