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Cobalt-60 gamma-ray irradiation pretreatment and sludge protein for enhancing enzymatic saccharification of hybrid poplar sawdust

Yulin Xiang [a,*], Yuxiu Xiang [b], Lipeng Wang [a]

[a] College of Chemistry and Chemical Engineering, Yulin University, Yulin 719000, Shaanxi Province, China
[b] Department of Management Engineering, Qiqihar Institute of Engineering, Qiqihar 161005, Heilongjiang Province, China

HIGHLIGHTS

- Effects of gamma irradiation pretreatment on *HPS* were studied.
- The sludge proteins could improve enzymatic hydrolysis efficiency of *HPS*.
- Optimal factors were obtained for reducing sugar yield.
- The combined method is a promising method for *HPS* enzymolysis.

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ABSTRACT

In order to improve the enzymatic saccharification of hybrid poplar sawdust, gamma irradiation pretreatment and enzymatic hydrolysis in the presence of sludge protein were investigated. The cellulose crystallinity index were significantly decreased after irradiation pretreatment, and adding sludge protein improved enzyme activity and increased the reducing sugar yield. The conditions of irradiation pretreatment and enzymatic hydrolysis in the presence of sludge protein were systematically examined. The maximum reducing sugar yield was 519 mg/g under an irradiation dose of 300 kGy, a sludge protein dosage of 2 mg/mL, an enzymatic hydrolysis temperature of 45 °C, an enzymatic hydrolysis time of 84 h, and a 90 FPU/g enzyme loading. This work indicated that the combined method of gamma irradiation pretreatment and enzymatic hydrolysis in the presence of sludge protein was a promising potential for the saccharification of hybrid poplar sawdust.

1. Introduction

With highlighting problems of energy crisis and environmental pollution caused by use of traditional fossil fuels, alternative sustainable biofuels (e.g. biogas, biodiesel, cellulosic ethanol, etc.) have acted as an important research field all over the world (Kaouther et al., 2016). Lignocellulosic biomass is a plentiful natural resources of renewable energy on earth. The biofuel can be obtained from all kinds of lignocellulosic biomass, such as grass, tree, as well as many wastes from agriculture, forestry, municipal solids, and industry (Zhang et al., 2016a). Recently, it has been reported that lignocellulosic biomass have been successfully converted into biogases, bioethanol, and biodiesel (da Silva et al., 2016; Patel et al., 2015). Enzymatic hydrolysis is a key step from lignocellulosic biomass to biofuel (Mateusz et al., 2016). However, effective enzymatic hydrolysis of unpretreated biomass is not easy because of the compact structure of lignin, cellulose, and hemicellulose in lignocellulosic. Moreover, in terms of the preliminary conversion rate, the preliminary stage of the biorefinery, which is the conversion phase from cellulose to glucose, is a bottleneck (Lynd et al., 2008). To overfulfil this bottleneck, more process time and higher cellulose dosage will be used to improve the conversion rate. Complicated processing technology and high cost restrict wide-ranging production of biofuels.

A lot of pretreatment methods, such as hot compressed water pretreatment method (Akihiro et al., 2012), alkali pretreatment (Cai et al., 2016), dilute acid pretreatment (Jeong et al., 2016), organic solvent pretreatment (Zhou et al., 2016), ionic liquids (ILs) pretreatment (Chen et al., 2016), steam explosion pretreatment (Liu and Chen, 2016), and irradiation pretreatment method (Yin and Wang, 2016), have been exploited for strengthening enzymatic hydrolysis efficiency. Among these methods, irradiation pretreatment is considered as a promising pretreatment method because of its high efficiency for enhancing the enzymatic hydrol-

* Corresponding author.
E-mail address: yulinx@126.com (Y. Xiang).

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ysis of biomass (Liu et al., 2015, 2016; Jin et al., 2009). Irradiation pretreatment method can effectively decompose the stubborn lignocellulosic biomass into amorphous conformation and be favorable to the conversion of low molecular carbohydrates from cellulose (Liu et al., 2015; Cheng et al., 2013). Moreover, irradiation pretreatment has several characters as below: short process time, moderate temperature, and scarcely undesirable inhibitory substrates formed during chemical pretreatments (Liu et al., 2016; Chung et al., 2012). But the researches on the effect of γ-ray irradiation pretreatment on enzymatic saccharification of lignocellulosic biomass for biofuel production were very little and not systematic.

Simultaneously, enzymatic hydrolysis is enhanced when additives, such as surfactants or proteins, are present (Xu et al., 2016; Wang et al., 2015; Brethauer et al., 2011). However, most researches involved the effect of various surfactants, the reports about the effect of proteins are very little. Enhancing enzymatic saccharification efficiency by ^{60}Co γ-ray irradiation pretreatment and sludge protein as an additives has not been reported.

Therefore, one objective of this study was to investigate the effect of ^{60}Co γ-ray irradiation pretreatment on the physicochemical characteristics (including crystallinity and chemical structure) of hybrid poplar sawdust (*HPS*). A second objective was to study the feasibility and effectiveness of sludge protein as an additive for the improved enzymatic hydrolysis efficiency of *HPS* for biofuel production, and to determine the optimized operating conditions of enzymatic saccharification.

2. Materials and methods

2.1. Collection and preparation of materials

HPS (*Aigeiros* of *Populus*) was collected from a wood plant located in Yulin, China. The sawdust was naturally dried at atmospheric condition for 1 week, then crushed and sieved (40 mesh). The obtained sawdust was sealed in plastic bags and stored at room temperature. Sludge protein was fresh-made. Excess sludge from Yuyang zone wastewater treatment plant in Shaanxi (China) was kept in storage tank after diluted with water, and the sample was stirred for 10 min. Experiments were carried out under the action of ultrasonic in a reactor. The effective volume of the reactor was 1 L, working pressure 9.8 MPa. The working power and frequency of ultrasound-assisted extraction were fixed at 108 W and 20.024 kHz, respectively. Extraction time was 40 min, pH was 9, and temperature was 35 °C. After extraction, the flask was immediately cooled to room temperature by using chilled water. Soluble protein in the supernatant was obtained by centrifuging the disintegrated sludge at 3000 r/min for 10 min at ambient temperatures of 21–25 °C. Supernatant was dried at 35 °C for 1 week into protein powder. The powder was mainly consisted of protein, peptides, oligopeptide, amino acid, and a little other material such as polysaccharide (Xiang et al., 2011). Cellulase (derived from and Choi Biotechnology Co., Ltd., Ningxia, China, which was generated by Acremonium cellulolyticus). Activity is 1040 μ/g (pH 5.7).

2.2. Irradiation pretreatment

The ^{60}Co-source was supplied by Jin-Pengyuan Radiation Research Center (Tianjin, China). 50 g of the preparative *HPS* sample was placed in a 100 mL glass bottle, and then the bottle was exposed to the designated doses (0–500 kGy at 50 kGy increments) in a ^{60}Co irradiator (source intensity: 9.99×10^{15} Bq). In order to achieve uniform target doses, the sample was rotated 360° continuously under the irradiation process. Finally, the sample was cooled to room temperature (about 25 °C) in a water bath before analysis.

2.3. Enzymatic hydrolysis

The pretreated *HPS* of 0.5 g was respectively mixed with 10 mL of 0.075 mol/L (pH 4.8) citric acid-sodium citrate buffer in a 50 mL flask, and then cellulose and a certain amount of sludge protein was blended into the flask. Enzymatic hydrolysis was performed in a shaking incubator at 160 r/min and 50 °C for 48 h. After hydrolysis, the samples were withdrawn at certain time interval for the analysis of reducing sugar by DNS method (Hu et al., 2008). Enzyme activity was measured by IUPAC method (Zou and Guo, 2010), and filter paper size was $1 \times 1\,cm^2$ square piece.

2.4. Analysis methods

2.4.1. SDS-PAGE method

SDS-PAGE was performed based on the modified method of Laemmli (Fu and Sapirstein, 1996). Protein solution was centrifuged at 3000*g* for 10 min. Sludge protein solution (2.5 mg/mL) was mixed 1:1 with Lammeli buffer (25% glycerol, 0.01% bromophenol blue, 2% SDS, 62.5 mM Tris-HCl, pH 6.8) to a final concentration of 1 mg/mL. Electrophoresis buffer contained 0.2 M glycine, 0.1% SDS, and 20 mM Tris-HCl at pH 8.3. SDS-PAGE was run with 4 °C running buffer at room temperature. Protein solutions were loaded onto gels and run at 100 V for 40 min using a Mini-Protean III cell (Bio-Rad) and PowerPac Basic power supply. Gels were stained using a colloidal blue stain kit followed by destaining for 3–5 h. Protein molecular weights were analyzed using the software Gel-Pro analyzer.

2.4.2. Brunauer, Ennett and Teller (BET) analysis

The specific surface area of pretreated/untreated *HPS* was analyzed using the surface area and pore size analyzer (ASAP-2000, MIC, USA), and computed according to BET mothod (Liu et al., 2015).

2.4.3. FT-IR

FT-IR spectra of the *HPS* before and after pretreatment were recorded using a Fourier Transform Spectrometer (IR Prestige-21). It is used to investigate the component changes of pretreated and untreated *HPS*. The wavenumber range of the spectrometer is 4000–500 cm^{-1} using 100 scans at 4 cm^{-1} resolution. The mass ratio of KBr and *HPS* (10 mg) in was 100:1.

2.4.4. XRD

The crystallinity of *HPS* was determined on a X-ray diffractometer (D/MAX-2400) using Cu kα radiation source carried out at 30 mA and 40 kV over a 2θ range from 5° to 40° with a step size of 0.2° (2θ) and a Scan speed 2°/s. The crystalline index (CrI) was estimated by Eq. (1) (Segal et al., 1959):

$$\mathrm{CrI} = 100 \times \frac{I_{002} - I_{\mathrm{am}}}{I_{002}} \quad (1)$$

where I_{002} is maximum intensity of crystalline structure at 2θ = 22.6°; I_{am} is intensity of the amorphous portion at 2θ = 18.0°.

2.5. Statistical analysis

Enzymatic hydrolysis experiments were performed in triplicate. In order to minimize the systematic error, each experimental measurement was replicated 3 times. The differences were less than 5%, and the results were analyzed using the Origin8.0 and SAS 9.0 software.