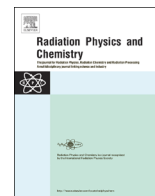




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Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Enhancement of enzymatic hydrolysis of wheat straw by gamma irradiation–alkaline pretreatment

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HIGHLIGHTS

- Pretreatment of wheat straw by gamma radiation and NaOH was investigated.
- Irradiation pretreatment can significantly decrease NaOH consumption.
- Reducing sugar yield reached 72.67% at 100 kGy and 2% NaOH treatment for 1 h.

ARTICLE INFO

Article history:

Received 29 January 2016

Received in revised form

14 February 2016

Accepted 16 February 2016

Available online 18 February 2016

Keywords:

Gamma irradiation

Wheat straw

Pretreatment

Enzymatic hydrolysis

ABSTRACT

Pretreatment of wheat straw with gamma irradiation and NaOH was performed to enhance the enzymatic hydrolysis of wheat straw for production of reducing sugar. The results showed that the irradiation of wheat straw at 50 kGy decreased the yield of reducing sugar, however, the reducing sugar yield increased with increasing dose from 50 kGy to 400 kGy. The irradiation of wheat straw at 100 kGy can significantly decrease NaOH consumption and treatment time. The reducing sugar yield could reach 72.67% after irradiation at 100 kGy and 2% NaOH treatment for 1 h. The combined pretreatment of wheat straw by gamma radiation and NaOH immersion can increase the solubilization of hemicellulose and lignin as well as the accessible surface area for enzyme molecules.

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1. Introduction

The research and development of renewable energy is one of the effective ways to address the energy shortages and environmental degradation. There is a great interest in exploring renewable biomass energy sources. Wheat straw is an abundant agricultural residue, and a potential substrate for biofuel production. However, its application for production of fuels and chemicals has been limited due to its highly crystalline structure, inaccessible morphology, and limited solubility. Therefore the pretreatment process is required to enhance the accessibility of the biomass to enzymes and/or chemical reagents (Driscoll et al., 2014).

The pretreatment of lignocellulosic materials has been studied for a long time (Yang et al., 2008). The pretreatment process can remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity (Sun and Cheng, 2002; Driscoll et al., 2014). Various technologies have been studied and applied for the pretreatment of lignocellulosic materials, including mechanical

milling (Tassinari et al., 1982), steam explosion (Ohgren et al., 2007), chemicals (Kim and Holtzappple, 2005), and irradiation (Chosdu et al., 1993; Takacs et al., 2000; Toth et al., 2003; Khan et al., 2006).

Ionizing radiation technology, such as electron beams and gamma rays can produce ionization in a material and then initiate chemical reactions and the cleavage of chemical bonds, which has been received increasing attention and application in recent years (Wang and Wang, 2007; Driscoll et al., 2014; Yin et al., 2014). The main principle of pretreatment of wheat straw by radiation technology is that, in the macromolecules of cellulose materials, free radicals are produced through rapid localization of the absorbed energy within the molecules, and resulting in the secondary degradation through chemical reactions such as chain scission, cross-linking, and so on (Khan et al., 2006; Chung et al., 2012; Karthika et al., 2012). When lignocellulosic materials are exposed to γ -ray radiation, the units of cellulose, hemicellulose and lignin have the similar probability of being affected by radiation. Lignin is a polyphenolic material, which can significantly affect the radical reactions through inter-molecular charge-transfer interactions (Barsberg et al., 2005; Lee et al., 2014).

The effect of adding water, ethanol and chlorine during the

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radiation of rice straw, chaff and sawdust was investigated (Kumakura and Kaetsu, 1979). The results showed that the presence of water and alcohol had no significant function to decrease the required absorbed dose for effective acceleration of enzymatic hydrolysis, but the presence of chlorine could greatly enhance enzymatic hydrolysis and increase the yield of sugar. Kumakura and Kaetsu (1984) studied the radiation of chaff in the presence of various acids, such as sulfuric, hydrochloric, and acetic acid, the results showed that glucose yield increased with increasing concentration of acids, in which hydrochloric acid was the most effective for the acceleration of the enzymatic hydrolysis. Compared with acid treatment, alkaline treatment can decrease the sugar degradation (Kaar and Holtzapfle, 2000; Foldvary et al., 2003). Furthermore, the combined pretreatment of radiation with sodium hydroxide was also been studied. Lu and Kumakura (1993) investigated the radiation pretreatment of rice straw in the presence of low concentration of NaOH. High degradation of lignocellulose at a relatively low dose was attributed to the weakness of the chemical links between lignin, hemicellulose, and cellulose units as well as the change of compositions through alkali swelling. Chosdu et al. (1993) found that the glucose yield increased to 43% after treated with electron beam irradiation at 500 kGy and 2% NaOH treatment, it was 20% for untreated sample of corn stalk.

The objective of this study was to investigate the effect of integrating γ radiation and NaOH pretreatment on enzymatic hydrolysis of wheat straw, and to probe the possible mechanism of this combining pretreatment process.

2. Materials and methods

2.1. Wheat straw

Wheat straws were collected in the field located in Changping District of Beijing, China. After collection they were cut into 1 cm length, washed with deionized water, dried at 60 °C and stored in sealed plastic bags at room temperature. The moisture and ash content of the straw were determined by the gravimetric methods (Foyle et al., 2007). The carbohydrate and the lignin content were determined using 2 M hydrochloric acid hydrolysis at 100 °C followed by concentrated sulfuric acid at room temperature to hydrolyze the hemicellulose and cellulose. The remaining acid-insoluble lignin was measured by weighing method. The content of the components was expressed as dry matter (Yang et al., 2008).

2.2. Mechanical crushing

Raw wheat straws were crushed with a high-speed (24,000 rpm) pulverizer for 8 min, and the powder smaller than 140-mesh was collected for use.

2.3. Radiation pretreatment

Wheat straw powders below 140-mesh were sealed in polyethylene bags and irradiated with dose rate of 245 Gy/min by a Co-60 γ source at room temperature. After radiation, the further treatment process (e.g. NaOH swelling or enzymatic hydrolysis) was carried out soon to avoid the aftereffect of radiation.

2.4. NaOH pretreatment

NaOH pretreatment of wheat straw was carried out in 200-mL glass beaker. Each glass beaker was loaded with 10 g wheat straw, and 100 mL NaOH solution (with different concentrations, in terms of percentage, w/v) was added and mixed, then the mixture was left for a certain time. After the swelling pretreatment, NaOH

solution and wheat straw powder were totally transferred to a 250-mL centrifuge bottle and centrifuged for 10 min at 4000 rpm. The supernatant was decanted, 200 mL of deionized water was added, then pH was adjusted to about 7.0 using hydrochloric acid (1:5, v/v). After repeated the centrifugation step, the supernatant was discarded. The solids were transferred to 100 mL weighed glass beaker, dried at 60 °C. Before and after NaOH swelling the masses of the samples were carefully measured to determine the weight loss. The dried powder below 140-mesh was used for enzymatic hydrolysis or other analyses.

2.5. Enzymatic hydrolysis

For enzymatic hydrolysis reaction, sample (1.0 g) was added into a flask containing 50 mL of 0.1% cellulase solution and reacted at 50 °C (150 rpm) for 48 h. The cellulase (1646 FPU/g) used in this study was Onozuka R-10 (Yakult Mfg. Co., Ltd.), which was dissolved in 0.1 M acetate buffer solution at pH 4.8. After enzymatic hydrolysis, the sample was centrifuged immediately at 12,000 rpm for 10 min, then the supernatant was collected for analysis. The reducing sugar was analyzed with 3,5-dinitrosalicylic acid reagent. Reducing sugar yield (%) was expressed as the ratio of reducing sugar to sample mass (dry matter). Each sample was analyzed in triplicate ($n=3$), and the mean values and standard deviations (SD) were calculated.

3. Results and discussion

3.1. Wheat straw composition

Cellulose was the major component of untreated wheat straw, which was 47.0%, while hemicellulose content was 27.0%. These values are similar to the values reported by Sun and Cheng (2002), which are 30% of cellulose and 50% of hemicellulose. The wheat straw contained 12.4% acid-insoluble lignin and 4.5% ash.

3.2. Effect of absorbed dose on enzymatic hydrolysis

Wheat straw powder below 140-mesh was irradiated at 50, 100, 200, 300 and 400 kGy, the effect of dose on reducing sugar yield is shown in Table 1.

Table 1 shows that the reducing sugar yield increased linearly with increasing dose from 50 kGy to 400 kGy, it was 27.73% at 400 kGy, which is approximately 130% of the untreated sample, indicating that radiation can enhance the enzymatic hydrolysis of wheat straw. When wheat straw was irradiated at 50 kGy, the reducing sugar yield was lower than for the unirradiated one, possibly because the radiation-induced cross-linking reaction was predominant over chain scission in the macromolecules of the lignocellulose material. In previous study, it was found that the

Table 1
Effect of radiation dose on reducing sugar yield.

Dose (kGy)	Reducing sugar yield (%)
0	21.51 (0.12)
50	20.73 (0.08)
100	21.88 (0.19)
200	23.88 (0.24)
300	26.45 (0.17)
400	27.73 (0.37)

All samples were performed in triplicate (standard deviations are presented in parenthesis).

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