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Enhanced enzymatic hydrolysis of poplar bark by combined use of gamma ray and dilute acid for bioethanol production

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ABSTRACT

Pretreatment of poplar bark with a combination of sulfuric acid (3%, w/w, H_2SO_4) and gamma irradiation (0–1000 kGy) was performed in an attempt to enhance enzymatic hydrolysis for bioethanol production. The yields of reducing sugar were slightly increased with an increasing irradiation dose, ranging from 35.4% to 51.5%, with a 56.1% reducing sugar yield observed after dilute acid pretreatment. These results clearly showed that soluble sugars were released faster and to a greater extent in dilute acid-pretreated poplar bark than in gamma irradiation-pretreated bark. When combined pretreatment was carried out, a drastic increase in reducing sugar yield (83.1%) was found compared with individual pretreatment, indicating the possibility of increasing the convertibility of poplar bark following combined pretreatment. These findings are likely associated with cellulose crystallinity, lignin modification, and removal of hemicelluloses.

1. Introduction

Lignocellulosic biomass has the potential to serve as a low cost and renewable feedstock for bioconversion into fermentable sugars, which can be further utilized for biofuel production. However, untreated lignocelluloses are difficult to hydrolyze because of the crystalline structure of the cellulose and the presence of hemicelluloses and lignin. Therefore, a prerequisite step before the bioconversion process is required for the breakdown or modification of lignin and hemicelluloses, and the disruption of the crystalline structure of cellulose, thereby enhancing enzyme accessibility to cellulose during the hydrolysis step (Niu et al., 2009; Alvira et al., 2010). Among different pretreatment methods such as biological, physical, chemical, and physico-chemical pretreatments, chemical pretreatment using dilute acid as a catalyst, which has been extensively evaluated for treating a variety of lignocellulosic feedstocks, has been reported to be one of the leading pretreatment technologies (Qi et al., 2010). Researchers have reported that the yields of reducing sugars were 52.3%, 61.4%, and 76.5% when rye straw, wheat straw, and olive trees were subjected to sulfuric acid pretreatment, respectively (Sun and Cheng, 2005; Cara et al., 2008; Qi et al., 2010). During dilute acid pretreatment, solubilization of hemicelluloses and fracture of lignin occurs, however, most of the lignin remain in the pretreated solid residue. The remaining lignin fraction possesses a higher affinity for cellulolytic enzyme components than carbohydrates, thus resulting in reduced hydrolysis efficiency. Therefore, further removal or modification of lignin after dilute acid pretreatment may be an effective approach to promoting enzymatic digestibility of lignocellulosic materials (Qi et al., 2010).

Ionizing radiation can easily penetrate the lignocellulosic structure and undoubtedly produce free radicals useful in the modification of the lignin structure, as well as the breakdown of cellulose crystal regions. After the termination of irradiation, radicals produced in amorphous regions will quickly become extinct, whereas those trapped in the crystalline and semi-crystalline regions of the cellulose structure decay slowly over time and cause further degradation of the lignocellulosic biomass. The presence of the polyphenolic material lignin will affect overall radiochemical events. Phenoxy radicals appear to be important radical intermediates in lignin formation and are ultimately transformed into lignin *O*-quinonoid structures (Chunping et al., 2008). Therefore, ionizing radiation such as a gamma ray may be an excellent alternative to enzymatic hydrolysis for permitting access of the derivative enzymes to the cellulose. In previous studies, irradiation-induced degradation of

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various lignocellulosic materials for increasing sugar yield has been reported, including in wheat straw (Chunping et al., 2008), rice straw (Xin and Kumakura, 1993), bagasse (Han et al., 1981), corn stalks, peanut husks (Chosdu et al., 1993), and oil palm empty fruit bunches (Matsuhashi et al., 1995). However, several researchers have reported that irradiation doses of more than 1000 kGy were required to induce a noticeable effect on sugar production from rice straw and sugarcane bagasse by enzymatic hydrolysis (Kumakura and Kaetsu, 1979; Han et al., 1983).

The effect of the combined use of dilute acid and gamma ray on enzymatic hydrolysis of lignocellulosic biomass has seldom been assessed. One report showed that the IVRD (*in vitro* rumen digestibility) of sugarcane bagasse increased after combination treatment with sulfuric acid and gamma ray (Han et al., 1983).

Therefore, the combine effects of acid and gamma ray treatment on enzymatic hydrolysis in various lignocellulosic materials should be further examined, as performed in this study using poplar bark. In addition, this investigation provides deeper insight into the efficiency of enzymatic hydrolysis and the changes in the physico-chemical characteristics of the poplar bark biomass.

2. Materials and methods

2.1. Pretreatment

Poplar trees (*Populus alba* L.) were harvested at Chonnam National University (Jeonnam Province, Korea) in early October 2010, and the poplar bark was air-dried at ambient temperature, ground in a Wiley mill, and passed through a 420 μm sieve. The chemical composition of poplar bark is shown in Table 1. Poplar bark samples (100 g) were autocleaved with 3% (w/w) sulfuric acid at 121 °C for 60 min and exposed to gamma ray at 25–1000 kGy, which was generated by a gamma irradiator (^{60}Co, ca. 111TBq, MDS Nordion, Ottawa, Canada). Thereafter, water insoluble solids were washed for removal of the remaining acid with deionized water until the washing water reached around pH 7; the solids were dried in vacuo. The sample was stored at −4 °C until analyzed.

2.2. X-ray diffraction analysis

The crystallinity index (CrI) of each sample was measured using a High Resolution X-Ray Diffractometer (X'Pert PRO Multi Purpose X-Ray Diffractometer, PANalytical, Almelo, The Netherlands) with Cu Kα radiation at 50 kV and 300 mA. Samples were scanned over a scattering angle (2θ) from 5° to 40° at a rate of 2θ min^{-1}. The CrI was calculated using the intensities of diffraction of the crystalline structure (plane 002, 2θ=22.5°) and the amorphous fraction (2θ=18°) (Segal et al., 1959):

$$CrI = \left\{ \frac{I_{002} - I_{amorphous}}{I_{002}} \right\} \times 100$$

Table 1
Chemical compositon of poplar bark (contents are shown as % dry weight).

Chemical composition (%)	Poplar bark
Total neutral sugar	72.3 ± 8.4
Lignin	20.2 ± 1.7
Nitrogen	0.8 ± 0.0
Ash	0.6 ± 0.0
Alcohol–benzene extract	1.2 ± 0.1

2.3. Enzymatic hydrolysis

The cellulase (2900 U/g) used was Onozuka R-10 (Yakult, Japan) from *Trichoderma viride* and endo-β-(1–4) xylanase (X2753, Sigma, USA) with 2500 U/g was from *Thermomyces lanuginosus* produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism. The β-glucosidase (63.9 U/g) was Novozyme 188 (Sigma, USA) derived from *Aspergillus niger*. Freeze-dried poplar bark powders (50 mg) were soaked in 5 mL of 50 mM sodium citrate buffer (pH 5.0) with the antibiotic sodium azide (0.2%) to inhibit microbial contamination. Enzymatic hydrolysis was performed at 37 °C and 150 rpm with an enzyme loading of 3.2 mg cellulase per gram of biomass, 540 μg β-glucosidase per gram of biomass, and 540 μg endo-β-(1–4) xylanase per gram of biomass for 96 h. Aliquots of 1.0 mL were taken at the termination of enzymatic hydrolysis after 96 h, immediately chilled on iced water and were centrifuged at 16100 *g* for 15 min. Levels of reducing sugars (glucose and xylose) in the supernatant solution were determined by high performance liquid chromatography (Waters, USA) using a column (Phenomenex Rezex RPM-monosaccharide, 300 × 7.8 mm) at 85 °C; distilled water was used as an eluent at a flow rate of 0.6 mL min^{-1}. A refractive detector was used for carbohydrates (Wi et al., 2009).

2.4. Chemical analyses

Alcohol–benzene extracts were gravimetrically determined. The ground sample (5 g) was extracted 3 times with 150 mL of ethanol–benzene (1:2) using a Soxhlet apparatus for 12 h. The content of the extracts was determined as the difference in weight of the dry sample before and after the extraction. Ash content was quantified through combustion for 3 h at 700 °C in a muffle furnace to reach a constant mass and nitrogen content was determined using a CHN micro-analyzer (Perkin Elmer 240, USA) (Chung et al., 2003). Lignin content was determined colorimetrically using the acetyl bromide procedure (Kim et al., 2004) and the chemical structure of lignin was examined by alkaline nitrobenzene oxidation (NBO), according to a procedure modified by Iiyama and Lam (1990).

Neutral sugar composition was analyzed using a modified alditol-acetate procedure, as described by Blakeney et al. (1983). The cell wall residue sample (20 mg) was treated with 72% sulfuric acid (w/w, 125 μL) for 45 min at an ambient temperature, and the suspension was diluted with distilled water to give a 4% sulfuric acid concentration. After hydrolysis for 1 h at 121 °C, the solution was neutralized with 15 M ammonia solution. *myo*-Inositol (0.1 mL of 1 g per 50 mL solution) was added as an internal standard. An aliquot (0.2 mL) was reduced using sodium tetrahydroborate (2 g) dissolved in 100 mL anhydrous dimethylsulfoxide, and the excess sodium tetrahydroborate was decomposed with 18 M acetic acid (0.1 mL). Alditol was acetylated with methylimidazole (0.2 mL) as a catalyst, followed by acetic anhydride (2 mL). After 10 min at an ambient temperature, distilled water (5 mL) was added to decompose any excess acetic anhydride. Alditol acetate was extracted with dichloromethane (1.5 mL). Tough Cap-17 (TC-17) capillary column chromatography was performed on a Shimadzu GC-18A system (Tokyo, Japan) equipped with a flame-ionization detector. The injector and detector temperature was 250 °C and the column temperature was 210 °C.

3. Results and discussion

The release of reducing sugars such as xylose and glucose was slightly increased with an increasing irradiation dose, ranging