Radiation Physics and Chemistry 94 (2014) 231–235

Contents lists available at ScienceDirect

Radiation Physics and Chemistry

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

Improved enzymatic hydrolysis of wheat straw by combined use of gamma ray and dilute acid for bioethanol production

Sung Hyun Hong [a], Jae Taek Lee [b], Sungbeom Lee [a], Seung Gon Wi [c], Eun Ju Cho [a], Sudhir Singh [a], Seung Sik Lee [a], Byung Yeoup Chung [a,*]

[a] Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute, Jeongeup 580-185, Republic of Korea
[b] Department of Bioenvironmental Chemistry, Chonbuk National University, Jeonju 561-756, Republic of Korea
[c] Department of Bioenergy Science and Technology, Chonnam National University, Gwangju 500-757, Republic of Korea

HIGHLIGHTS

- The glucose yield was significantly affected by combined pretreatment.
- Increasing enzymatic hydrolysis is due to decrease in their structures.
- Combined pretreatment is the most effective method for the enzymatic hydrolysis.

ARTICLE INFO

Article history:
Received 5 December 2012
Accepted 2 May 2013
Available online 5 June 2013

Keywords:
Wheat straw
Gamma irradiation
Bioethanol
Lignocelluloses
Dilute acid

ABSTRACT

Pretreating wheat straw with a combination of dilute acid and gamma irradiation was performed in an attempt to enhance the enzymatic hydrolysis for bioethanol production. The glucose yield was significantly affected by combined pretreatment (3% sulfuric acid-gamma irradiation), compared with untreated wheat straw and individual pretreatment. The increasing enzymatic hydrolysis after combined pretreatment is resulting from decrease in crystallinity of cellulose, loss of hemicelluloses, and removal or modification of lignin. Therefore, combined pretreatment is one of the most effective methods for enhancing the enzymatic hydrolysis of wheat straw biomass.

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 (Niu et al., 2009; Alvira et al., 2010; Chung et al., 2012b). Among different pretreatment methods such as biological, physical, chemical, and physico-chemical pretreatments, many researchers using sulfuric acid have been extensively studied (Sun and Cheng, 2005; Cara et al., 2008; Qi et al., 2010) and there have been numerous reports on the relationship between radiation dose and cellulose fiber degradation (Bouchard et al., 2006; Stupinska et al., 2007; Yang et al., 2008). However, it seems these technologies are not yet mature enough to be profitable for commercial application. In addition, there are only two reports on the effects of combined use of gamma ray and dilute acid. One report showed that the *in vitro* rumen digestibility of sugarcane bagasse increased after combined treatment (Han et al., 1983) and another report showed the combined effects of enzymatic hydrolysis using poplar bark (Chung et al., 2012a). Therefore, more information of combined effects on acid and gamma ray treatment on enzymatic hydrolysis in different types of lignocellulosic biomasses should be necessary.

2. Materials and methods

2.1. Pretreatment

The wheat (*Triticum aestivum* L.) straw harvested at Advanced Radiation Technology Institute (ARTI), Korea Atomic Energy Research Institute (KAERI) (Jeonbuk Province, Korea) in April 2011. The wheat straw was air-dried at ambient temperature, ground in a Wiley mill, and passed through a 420 μm sieve. The wheat straw was determined to have 77.4 ± 5.2% neutral sugar, 18.8 ± 0.4% lignin, and 3.6 ± 0.2% ash. The ground wheat straw

* Corresponding author. Tel.: +82 63 570 3331; fax: +82 63 570 3339.
E-mail address: bychung@kaeri.re.kr (B. Yeoup Chung).

0969-806X/$ - see front matter
http://dx.doi.org/10.1016/j.radphyschem.2013.05.056

(50 g) with 3% (w/w) of sulfuric acid was autocleaved at 121 °C for 60 min and exposed to gamma radiation before and after dilute acid pretreatment from 0–1000 kGy. Gamma irradiation was carried out at ambient temperature using a high-level cobalt-60 irradiator (point source AECL, IR-79, MDS Nordion International Co., Ltd., Ottawa, ON, Canada) at Advanced Radiation Technology Institute, Korea Atomic Energy Research Institute (Jeongeup, Korea). A cobalt-60-γ-source with an activity of approximately 215 kCi (7.96×10^{15} Bq) with a dose rate of 12 kGy/h was used for irradiation. Dosimetry was performed using 5 mm diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany) (Lee et al., 2012b). The dosimeters were calibrated against an International Standard Set by the International Atomic Energy Agency (Vienna, Austria). The residues were washed for removal of remaining acid with deionized water until washed water reached around pH 7 and dried in vacuo. The sample was stored at −4 °C until used (Chung et al., 2012a).

2.2. Enzymatic hydrolysis

Enzymatic hydrolysis was performed at 37 °C and 150 rpm with an enzyme loading of 3.2 mg cellulase g^{-1} biomass, 540 μg β-glucosidase g^{-1} biomass and 540 μg endo-β-(1–4) xylanase g^{-1} biomass for 96 h in 250 ml flasks using 50 mM sodium citrate buffer (pH 5.0) and wheat straw powders (50 mg) were soaked in 5 ml of sodium azide with antibiotics (0.2%) to inhibit microbial contamination. The commercial enzymes [R-10 cellulase (Onozuka, Japan), β-glucosidase (Sigma, USA) and endo-β-(1–4) xylanase (Sigma)] used in this study for hydrolysis. After the enzymatic hydrolysis reaction, the mixture was centrifuged immediately at 16100 g for 20 min. The glucose content in the hydrolysate was determined by high-performance liquid chromatography (Shimadzu, Japan) equipped with a RI detector using a column (Rezex RPM-monosaccharide, 300 × 7.8 mm) at 65 °C and distilled water was used as an eluent at a flow rate of 0.6 ml min^{-1} (Chung et al., 2012b).

2.3. Other analyses

The morphological changes of wheat straw after pretreatments were observed by a scanning electron microscope (JSM-6390 SEM; JEOL Ltd., Tokyo, Japan) (Chung et al., 2009). The trapped reactive oxygen species (ROS) were detected by an ESR spectrometer (JES-FA 200; JEOL Ltd., Tokyo, Japan) (Lee et al., 2012a). The crystallinity index (CrI) of each sample was measured by X-ray diffraction using a diffractometer (Almelo, Netherland) (Chung et al., 2012b). The structural features of lignin polymers after pretreatments were analyzed by a FTIR Bruker Vertex 70 Spectrometer (Germany) at a resolution of 4 cm^{-1} with 64 scan times (Chung et al., 2008).

The lignin content was measured by acetyl bromide procedure (Chung et al., 2012b) and the chemical structure of lignin was examined by alkaline nitrobenzene oxidation (NBO) (Chung et al., 2012b). Neutral sugar composition was performed using a modified alditol-acetate procedure (Chung et al., 2012b).

3. Results and discussion

SEM revealed morphological changes in wheat straw following various pretreatments. When compared to the surface structure of control sample, which has a continuous, even and smooth flat surface, the gamma irradiated wheat straw has a rugged, unsmooth, and little broken face. Wheat straw treated with dilute acid was dimly visible that cells would be tangled resulting from their collapsed shape. The pretreated wheat straw with combined use of dilute acid and gamma ray clearly showed severely rugged on surface and leaf split up many pieces (Fig. 1).

This would be due to bombardment with reactive oxygen species (ROS) produced by gamma irradiation. The relative unit (r.u.) is expressed as a signal ratio of ROS (Lee et al., 2012a). A dose-dependent increase in the peak intensity of EPR was clearly observed in irradiated wheat straw ranging from 1.64 r.u. to 8.54 r.u. In addition, sample of combined pretreatment showed 9.78 r.u. together with 2.08 r.u. in acid treated sample (Fig. 2). Although a

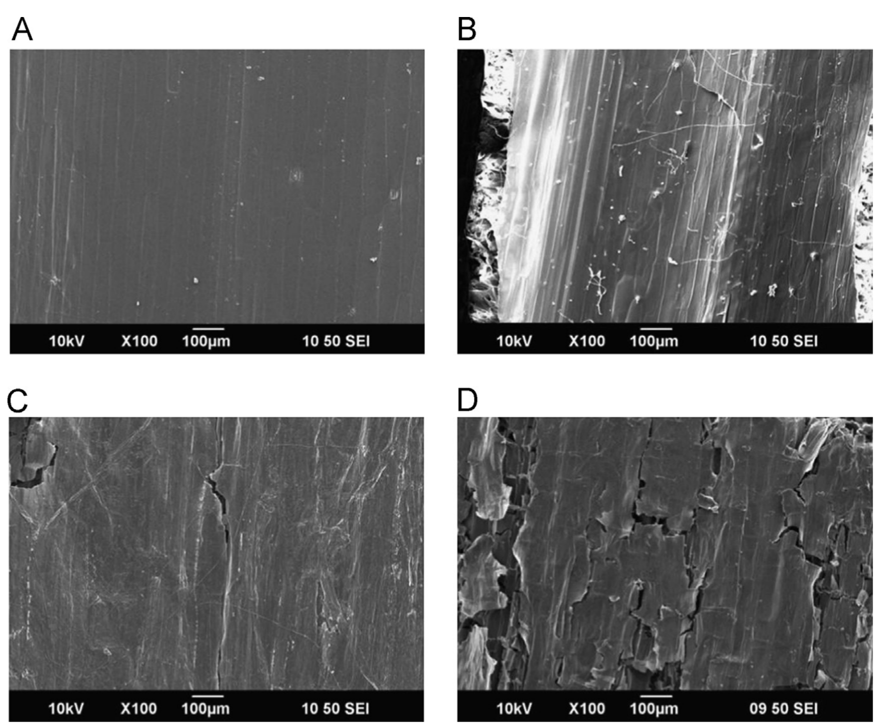

Fig. 1. Scanning electron micrographs of pretreated wheat straw: (A) control; (B) 1000 kGy of gamma irradiation; (C) 3% sulfuric acid and (D) combination of 3% sulfuric acid and 1000 kGy of gamma irradiation.