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ARTICLE

Electron Beam Irradiation and Dilute Alkali Pretreatment for Improving Saccharification of Rice Straw

Du-Yeong Kim · Byoung-Min Lee · Jin-Young Lee · Phil-Hyun Kang · Joon-Pyo Jeun

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Abstract Rice straw is one of the most abundant and low-cost biomasses available in the world. Thus, the rice straw as a potential candidate for future energy and chemical resource has been intensively studied in order to use as the current fossil fuels. However, the structure of rice straw makes it difficult to hydrolyze into fermentable sugars owing to the cellulose in rice straw being tightly surrounded by hemicellulose and lignin, thus pretreatment of rice straw is needed for this process. In the present study, an alkali pretreatment method assisted by electron beam irradiation was investigated to improve the saccharification in an enzymatic hydrolysis yield. After pretreatment, cellulose in rice straw was increased from 39.5 to 71.1%, and lignin decreased from 19.5 to 6.4%. The sugar yield of the pretreated rice straw increased with an increase in irradiation dose. The results of XRD and Fourier transform infrared spectroscopy analyses showed that the properties of the straw were changed by this pretreatment, which favored the following enzymatic hydrolysis.

Keywords bioethanol · electron beam · irradiation · pretreatment · rice straw

Introduction

Rice straw is one of the most abundant low-cost lignocellulosic crop residues. Its annual production is about 700–900 million tons (Jiang et al., 2011). Rice straw contains considerable amounts of cellulose and hemicellulose (Sarkar et al., 2012). Cellulose is a profibril linked by β-1,4-glucosidic bond of D-glucose and is composed of sturdy bonds in a linear form. These anhydrous glucose profibrils form microfibrils of a crystal structure through hemicellulose and the hydrogen bond between molecules (Taherzadeh and Karimi, 2008). These microfibrils are bundled and linked with the lignin, and form lignocellulose including an amorphous structure in the vertical direction. Hemicellulose is easily hydrolyzed by acid and forms xylose, galactose, arabinose, mannose, among others. Lignin, as a complex aromatic compound, cannot be fermented by microorganisms (Poornejad et al., 2013). Lignin limits the accessibility of rice straw to enzymes and requires several severe pretreatments (Chung et al., 2012; Hii et al., 2012). The crystalline structure of the cellulose surrounded by hemicellulose and the presence of the lignin makes rice straw difficult to hydrolyze into fermentable sugars to be utilized by microorganisms for biomass energy production (Parajó et al., 1994). Thus, breaking down the crystal structure by pretreating the lignocellulose or removing the lignin structure will make a condition in which enzymes can easily access and enable fast hydrolysis (Yang et al., 2013).

A number of different pretreatment technologies, such as steam treatment with diluted sulfuric acid, and organosolv extraction, have been developed to increase the removal efficiency of lignin and hemicellulose from lignocellulose (Silverstein et al., 2009; Binod et al., 2010). Alkali pretreatment is considered a promising chemical pretreatment method due to low operation cost, reduced degradation of holocellulose, and the subsequent formation of inhibitors for downstream processing (Agbor et al., 2011; Kang and Jeong, 2012; Mood et al., 2013). The main mechanisms of alkaline pretreatment are the degradation of ester bonds and the cleavage of glycosidic linkages in the lignocellulosic cell wall matrix, which lead to the alteration of the lignin structure, a reduction of the lignin–hemicellulose complex, cellulose swelling, and the partial decrystallization of cellulose (Talebnia et al., 2010; Mood et al., 2013). However, chemical pretreatments have serious disadvantages in terms of the requirement for specialized corrosion-resistant equipment, extensive washing, and proper disposal of the waste solution (Niu et al., 2009).

D.-Y. Kim · B.-M. Lee · J.-Y. Lee · P.-H. Kang · J.-P. Jeun (✉)
Research Division for Industry and Environment, Korea Atomic Energy Research Institute, 29 Geumgu-gil, Jeongeup-si, Jeollabuk-do 580-185, Republic of Korea
E-mail: jpjeun@kaeri.re.kr

Electron beam pretreatment consumes less energy than conventional thermal processes, thus reducing the introduction of unwanted impurity, and is more effective, convenient, and environmental friendly than other radiation sources such as gamma-ray and UV radiation (Bak et al., 2009; Park et al., 2009). The reaction rate of degradation of a lignin-hemicellulose complex can be accelerated by electron beam irradiation during the alkali pretreatment. Moreover, it is common to increase the accessible surface area and pore size for enzyme and microbial attacks due to the irradiation effect on the disordering of the crystalline region in a cellulose structure (Janker-Obermeier et al., 2012).

In the present study, the efficiency of lignin removal from rice straw using alkali pretreatment assisted-electron beam irradiation was investigated. The objective was to develop the quantitative and structural changes in the components of pretreated rice straw and effect of the change on enzymatic hydrolysis rate of pretreated rice straw.

Materials and Methods

Materials. The rice straw used in this study was harvested from Korea Atomic Energy Research Institute (Korea) in 2012. It was dried at 40°C for 1 day. The rice straw was milled to produce a powder. The rice straw powders were separated until they could pass through a 500-micrometer scaled mesh and then dried at 40°C for 1day. The enzymes used for the saccharification of rice straw powders were Celluclast 1.5 L and novozyme-188 (β-glucosidase, Novo Co., Denmark).

Pretreatment. For electron beam irradiation, the dried rice straw powder was put into polystyrene cell culture. The electron beam irradiation was conducted using an ELV-3 accelerator installed at EB-Tech (Korea). The accelerating voltage was 2.5 MeV with a beam current of 25 mA. The absorbed dose ranged from 50 to 500 kGy. Irradiated and non-irradiated rice straw powders were pretreated by autoclaving with alkali before enzymatic hydrolysis. The rice straw powder was suspended in a solution of 3% NaOH in a flask at 120°C for 5 h under 1 bar pressure, which maintains a ratio of solid to liquid as 15 g:285 mL. After the autoclaving with dilute alkali, the rice straw powder was repeatedly washed and filtered using Whatman No.1 paper filters using deionized water until a neutral solution was achieved. The remaining solid materials were dried in a vacuum oven at 40°C to a constant weight for enzymatic hydrolysis.

Enzymatic hydrolysis. Pretreated rice straw samples were hydrolyzed to investigate the effects of pretreatment on the enzymatic hydrolysis in accordance with the National Renewable Energy Laboratory (NREL) standard procedure (2008). Pretreated and untreated rice straws were mixed with 0.1 M sodium citrate buffer solution (pH: 4.8) at a ratio of 1:19 (w/v). The average activities of the enzymes were 70 filter paper unit (FPU)/mL of Cellulast 1.5 L (cellulose, Novo Co.) and 40 cellobiose unit (CBU)/mL of Novogyme-188 (β-glucosidase, Novo Co.). The mixture was incubated at 50°C in a rotary shaker at 150 rpm for 24, 48, and 72 h.

Compositional analysis. The total solids, carbohydrates and lignin content of the non-irradiated rice straw and irradiated rice after enzymatic hydrolysis, were determined based on the Technical Association of the Pulp and Industry standard and suggested methods (2002) as well as Laboratory Analytical Procedures (LAP-002) from the NREL (2012).

Sugar yields. The reducing sugar released was analyzed by high performance liquid chromatography (HPLC, Shimazu Co., Japan) using a refractive index detector (410 RI detector, Waters, USA). The samples were separated using an Aminex HPX-87P column (Bio-Rad, USA) at 65°C with deionized water (B&J HPLC grade, SK chemical, Korea) as a mobile phase at a flow rate of 0.6 mL/min. The sugar yields were calculated using the following equation:

$$\text{Sugar Yields (\%)} = [\text{Released sugar amount (g)} / \text{Loaded untreated or pretreated rice straw amount (g)}]*100$$

Fourier transform infrared spectroscopy (FT-IR). The chemical changes were analyzed using FT-IR (Bruker Vertex 70 spectrometer, Germany). The FT-IR spectrum was recorded at a 4 cm^{-1} spectral resolution, with 64 scans and a wavelength range of 500–4,000 cm^{-1}.

X-ray diffraction. X-ray diffraction was carried out using an X'Pert Powder (PANalytical, Netherlands) diffraction instrument using Cu-K radiation (40 kV, 30 mA) with a 2 range of 5–50° to investigate the physical properties of the rice straw.

Results and Discussion

Rice straw composition. Breaking down hemicellulose and lignin that are resistant to biological degradation and disrupting the crystalline structure of cellulose is an important issue, because the digestibility of lignocellulose is limited by structural and compositional factors (Pal et al., 2013). The contents of cellulose, hemicelluloses, and lignin, solubilized during electron beam irradiation and the following autoclaving with alkali, along with ash are listed in Table 1. The compositional changes of untreated and pretreated rice straws, which were composed of lignin, hemicelluloses, and cellulose, provide the main carbon source for microbial enzyme production. (Table 1) Untreated rice straw contained 39.5 cellulose, 26.7, hemicellulose, 19.5 lignin, and 14.3% ash. However, the composition of non-irradiated rice straw after alkali pretreatment was 51.1 cellulose, 22.3 hemicellulose, 12.1 lignin, and 14.5% ash. In the case of alkali pretreated rice straw-assisted electron beam irradiation, the content of cellulose increased from 39.5% in the raw material to 71.1% after exposure to an irradiation dose of 500 kGy, depending on the irradiation dose, and the content of hemicellulose, lignin, and ash decreased from 26.7, 19.5, 14.3 to 17.0, 6.4, and 5.5%, respectively. Electron beam irradiation resulted in the depolymerization of lignin and a part of the hemicellulose, and accelerates changes in cellulosic

Table 1 Compositions of the untreated and pretreated rice straw after the two-stage pretreatment

Pretreatment condition		Cellulose (%)	Hemicellulose (%)	lignin (%	Ash (%)
Process	Irradiation dose (kGy)				
Non-pretreated	-	39.5±0.3	26.7±0.4	19.5±0.3	14.3±0.2
Alkali-only	-	51.1±0.2	22.3±0.2	12.1±0.2	14.5±0.3
Alkali with irradiation	100	60.0±0.4	18.6±0.3	11.0±0.2	10.4±0.2
	200	65.7±0.4	18.1±0.2	8.1±0.2	8.1±0.2
	300	69.4±0.3	17.4±0.3	7.0±0.3	6.2±0.3
	500	71.1±0.2	17.0±0.2	6.4±0.3	5.5±0.3

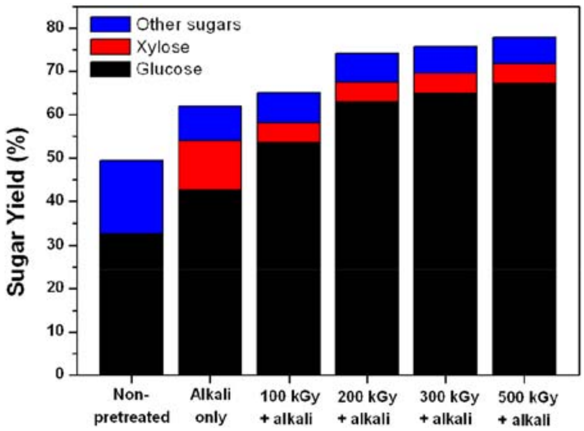

Fig. 1 Variations in the sugar yields of rice straw pretreated with electron beam irradiation doses of 0, 100, 200, 300, and 500 kGy and unpretreated rice straw after 72 h of hydrolysis.

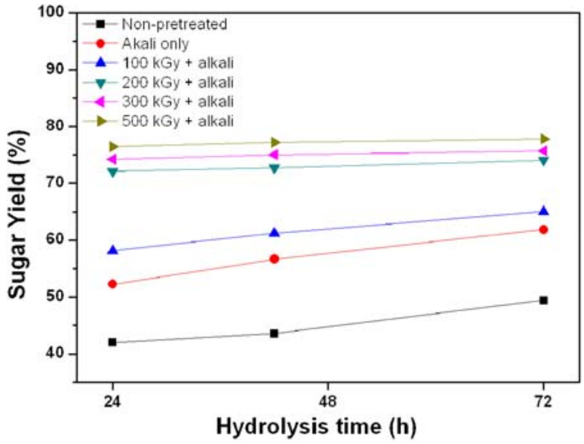

Fig. 2 Variations in the sugar yields based on the elapsed hydrolysis time in untreated and pretreated rice straw exposed to 0, 100, 200, 300, and 500 kGy of electron-beam irradiation.

biomass including an increase in the enzymatic accessibility during the alkali pretreatment. The depolymerization of lignin in the irradiation process made it easy to dissolve the lignin during the alkali-pretreatment. These changes in the components indicated that electron beam-assisted alkali pretreatment is more effective. The combination of electron beam irradiation and alkali pretreatment can markedly enhance the removal of lignin.

Sugar yields. The results of enzymatic hydrolysis of rice straw after pretreatment are shown in Fig. 1. The sugar yield of the rice straw before pretreatment based on 72 h was 49.4%. However, the sugar yield of the rice straw after pretreatment showed an increasing tendency proportional to the irradiation dose, and was 77% at 500 kGy. For glucose, a 106% improved sugar yield was shown relative to before the pretreatment of the rice straw. For xylose, although it was not extracted by enzymatic hydrolysis before the pretreatment of rice straw, the hydrolysis started in the pretreated specimen, and the sugar yield increased proportionally to the irradiation dose. It is proposed that the cellulose and hemicellulose chain are degraded by irradiation, lowering the molecular weight of cellulose and hemicellulose, and making the cellulose fraction more easily available for contact with the hydrolysis enzyme. The changes in chemical compositions and structures make rice straws more biodegradable, and the removal of lignin is believed to expose the cellulose surface, and thus is responsible for the enhancement of the enzymatic hydrolysis (Zhu et al., 2006).

The sugar yield according to enzymatic hydrolysis time and pretreatment condition is shown in Fig. 2. The sugar yield increased proportionally to the enzymatic hydrolysis time and the irradiation dose. The untreated rice straw showed 42.01 and 49.4% in 24 and 72 h of enzymatic hydrolysis, respectively. Additionally, it could be verified only in the alkali pretreated rice straw such that there was big difference in the sugar yield value according to the hydrolysis time by showing 52.2 and 61.8 % in 24 and 72 h of enzymatic hydrolysis, respectively. On the other hand, for the rice straw irradiated with 300 and 500 kGy, it could be verified that the difference in the sugar yield depending on time was approximately 1%, which this implies that the time needed for enzymatic hydrolysis is relatively short. The sugar yield based on only alkali pretreated rice straw, electron beam-assisted alkali pretreatment is more efficient than only alkali pretreatment, because increasing the irradiation dose significantly improves the degradation of crystalline cellulose and the removal of lignin and hemicellulose.

FT-IR Analysis. The FT-IR spectrum is frequently used to investigate the structure constituents and chemical bonds in lignocellulosic materials. Rice straw has cellulose, hemicelluloses, and lignin, which can be identified in distinct bands through FT-IR (Fig. 3). Bands between 1,200 and 1,000 cm^{-1} were typical of

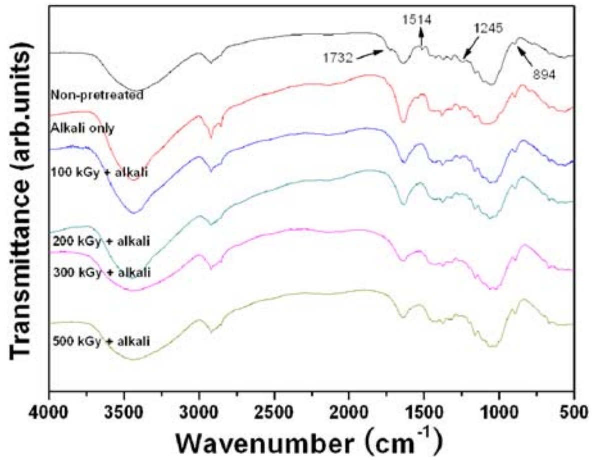

Fig. 3 Infrared spectra of un-pretreated and pretreated rice straw.

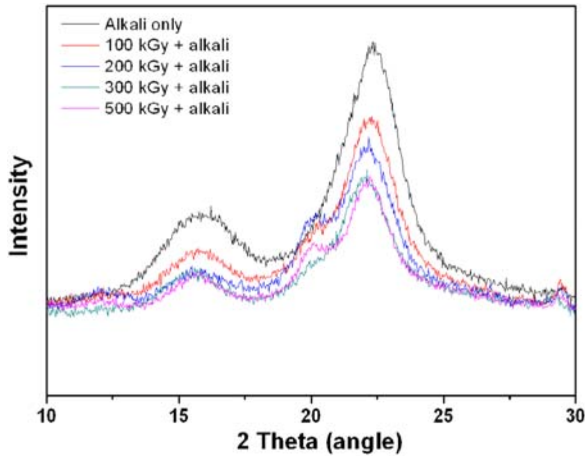

Fig. 4 Variations in the XRD result based on the electron beam dose in pretreated rice straw.

cellulose and hemicellulose. Distinct absorption bands appearing at 1,200–500 cm^{-1} were assigned to lignin (Yang et al., 2012). The peak at 1,514 cm^{-1} is attributed to aromatic skeletal from lignin. This band almost disappeared for electron beam-assisted alkali pretreated rice straw, indicating delignification. The shoulder at 1,732 cm^{-1}, which corresponds to acetyl and uronic ester groups of hemicelluloses or the ester linkage of carboxylic group from the lignin, disappeared after pretreatment assisted by electron beam irradiation. The disappearance of band at 1,732 cm^{-1} may be due to the destruction of ester linkages between lignin and hemicellulose. The band at 1,245 cm^{-1} may be attributed to C-O stretching of the syringyl units (Sun et al., 2000). After pretreatment, the corresponding peak disappeared, resulting in the degradation of hemicellulose. The band at 3,303 cm^{-1} corresponds to the OH stretching, which is shifted to a higher wave number by the degree of pretreatment. This can be explained by the increase in content of free hydroxyl groups in cellulose remaining even after alkali pretreatment assisted by electron beam irradiation. The band at approximately 2,920 cm^{-1} was probably due to C-H groups in cellulose. This band slightly increased in size with an increase in irradiation dose. The sharp band at 894 cm^{-1}, which corresponds to the C1 group frequency or ring frequency, is characteristic of β-glycosidic linkages between the sugar units (Poornejad et al., 2013). The absence of this band, which increased with pretreatment, implied that the cellulose content in pretreated rice straw increased.

X-ray diffraction analysis. The crystallinity and surface area of cellulose are important factors influencing enzymatic hydrolysis. Pretreated rice straw was investigated by XRD, as illustrated in Fig. 4. All the samples exhibit a typical cellulose I diffraction angle of around 14, 16, and 22^o (Li et al., 2010). These peak intensities decreased with increasing the irradiation dose but did not shift. In addition, the intensity of the amorphous region peaks at 18^o was decreased with increasing irradiation dose. The decrease of intensity at 18^o after pretreatment with increasing irradiation dose indicates that the pretreatment removed some amounts of amorphous components including hemicellulose and lignin, which increased the proportion of cellulose. The crystalline area of the cellulose decreased during the alkali pretreatment-assisted electron beam irradiation. The pretreatment of rice straw can destroy the cellulosic crystalline structure of cellulose in rice straw and enlarge the pore ratio and inner surface areas. It is widely accepted that high crystallinity cellulose will be more resistant to enzymatic hydrolysis (Binod et al., 2012; Liu et al., 2012). Decreasing the crystallinity will be beneficial for the digestibility of lignocelluloses.

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