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An irradiation-alkaline pretreatment of kenaf core for improving the sugar yield

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ABSTRACT

Kenaf (*Hibiscus cannabinus*) has received considerable attention as a renewable resource because of its fast growth and low lignin content. In the present study, a combination pretreatment of kenaf core using electron beam irradiation (EBI) and dilute alkali was investigated to improve the sugar yield in enzymatic hydrolysis. In the first step, EBI of the kenaf core was performed at various doses ranging from 100 to 500 kGy. The electron beam-irradiated kenaf core was then autoclaved with 3% dilute alkali at 120 °C for 5 h. The pretreated kenaf core was finally subjected to enzymatic hydrolysis at 50 °C for 24, 48, and 72 h by Celluclast 1.5 L (70 FPU/mL) and Novozyme-188 (40 CbU/mL). A total sugar yield of 72.4% was obtained with a 63.9% yield of glucose from pretreated kenaf core after 72 h of enzymatic hydrolysis. To further analyze effectiveness of EBI pretreatment, characterizations of kenaf core by a two-step process of EBI-alkaline pretreatment were also investigated.

1. Introduction

Lignocelluloses have been widely investigated as a potential candidate for producing fermentable sugars that can be converted to bioethanol [1–3]. Lignocelluloses are mainly composed of cellulose, hemicellulose, and lignin. Despite their chemical simplicity, carbohydrate polymers (cellulose and hemicellulose) are tightly packed by a lignin in the cell wall. The production of fermentable sugars from lignocelluloses is limited by structural resistance known as “biomass recalcitrance”.

A pretreatment process is essential to increase the yield of fermentable sugars and improve the rate of enzymatic hydrolysis [4]. A variety of pretreatment methods have been developed, including irradiation [5], steam explosion [6], acid hydrolysis [7], and alkali hydrolysis [8]. Acid and alkali hydrolysis pretreatments have commonly been used to facilitate enzymatic hydrolysis by the removal of lignin and hemicellulose [9,10]. However, the use of a high concentration of acid or alkali has several drawbacks, including the formation of by-products, the required neutralization, and corrosion [11–13]. Several combinational pretreatment methods have been introduced at mild conditions to enhance the enzymatic hydrolysis. Recently, acid and alkali pretreatments were combined with irradiation or steam explosion [14–16]. Several studies have demonstrated that (EBI) can disrupt the crystalline structure of lignocellulose. Moreover, pretreatment using an EBI method is known to be environmentally friendly, feasible, and controllable [17,18]. This research describes a dilute alkali pretreatment combined with EBI (EBI-dilute alkali pretreatment) to optimize the efficiency of enzymatic hydrolysis.

2. Material and methods

2.1. Materials

The kenaf core was supplied by the Korea Atomic Energy Research Institute (Jeongeup Province, Korea). The kenaf core was ground using a disintegrator. The kenaf core was sieved, and the powder that passed through a 500 μm sieve was collected. The kenaf core powder was then dried to remove moisture at 50 °C for 1 day in a vacuum oven. The main composition of the kenaf core was

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cellulose (45.1%), hemicellulose (23.4%), lignin (18.8%), and ash (10.0%). Two enzymes, Celluclast 1.5 L (Cellulase, Novo Co., 700 FPU/g-cellulase) and Novozyme-188 (β-glucosidase, Novo Co., 400 CBU/g-glucosidase) were supplied by Sigma–Aldrich and used for enzymatic hydrolysis.

2.2. Pretreatment

The dried kenaf core was prepared for EBI by sealing in polyethylene bags under ambient conditions. The kenaf core powder was irradiated using an ELV-8 type electron beam accelerator (EB tech Co., Korea). The EBI was conducted with an acceleration voltage of 2.5 MeV, a beam current of 25 mA and an absorbed dose rate of 25 kGy per scan. The experimental dosages were 100, 200, 300, and 500 kGy. Mixtures of electron beam-irradiated kenaf core samples (10 g) and 3% NaOH solution (190 g) were then autoclaved at 120 °C for 5 h. The resulting dark brown slurry was washed with deionized water and filtered several times (Whatman No. 1 filter paper) to remove the residue. The remaining solid material was dried at 50 °C for 1 day in a vacuum oven prior to enzymatic hydrolysis. The pretreated kenaf core samples were denoted E100A (100 kGy + 3% alkali), E200A (200 kGy + 3% alkali), E300A (300 kGy + 3% alkali), and E500A (500 kGy + 3% alkali), respectively.

2.3. Enzymatic hydrolysis

The effects of pretreatment on enzymatic hydrolysis of the kenaf core samples were analyzed according to the NREL's procedure [19]. Pretreated and untreated kenaf core samples (0.30 g) were mixed with 5.0 mL of a 0.1 M sodium citrate buffer solution (pH: 4.8) containing sodium azide as a chemical preservative. The average activities of the enzymes were 70 FPU/mL (Celluclast 1.5 L) and 40 CbU/mL (Novozyme-188). The enzymes were directly added to the sample mixture, and the enzymatic hydrolysis was performed at 50 °C in an incubator with agitation (150 rpm) for 24, 48, and 72 h.

2.4. Analytical methods

2.4.1. Composition and sugar analyses

Contents of cellulose, hemicellulose, and lignin of kenaf core samples were determined using NREL's procedure [20], TAPPI TS-222 om-88 methods [21]. The amount of released sugars in the hydrosate was analyzed by high-performance liquid chromatography (HPLC, Shimazu Co., Japan) equipped with an RI detector (410 RI detector, Waters, USA) using an Aminex HPX-87P column (Bio-Rad, USA). The operating conditions of HPLC were a temperature of 65 °C with DI water (B&J HPLC grade, SK chemical, Korea) as the mobile phase and a flow rate of 0.6 mL/min. The sugar yields were calculated using the equation: Sugar yields (%) = [weight of released sugar (g)/weight of loaded kenaf core (g)] * 100.

2.4.2. Fourier transform infrared spectrometry

Fourier transform infrared spectrometry (FTIR) analysis was performed to detect changes in the chemical structure of the untreated and pretreated kenaf cores. Analysis was conducted using a using an FTIR spectrometer (Bruker Vertex 70 spectrometer, Germany) over the wavenumber range of 500 − 4000 cm^{-1}, with a resolution of 4 cm^{-1} and 64 scans per sample.

2.4.3. X-ray diffraction

The kenaf core samples were scanned on an X'Pert Powder diffratometer (PANalytical, Netherlands) from $2\theta = 5 - 50°$ with Cu Kα ($\lambda = 1.54$ Å) radiation at 40 kV and 30 mA and a scan step size of 0.03°. The crystallinity of each kenaf core was determined as the crystallinity index (CrI, %) using the peak height method of Segal [22]. Crystallinity Index (CrI, %) = $\{(I_{002} - I_{am})/I_{002}\}$ * 100 (I_{002} is the intensity of crystalline portion of cellulose at $2\theta = 22°$, and I_{am} is the peak intensity of the amorphous portion at $2\theta = 18°$).

2.4.4. Physical properties

Surface area and pore volume information of untreated and pretreated samples were measured by nitrogen adsorption measurements (ASAP 2020, Micromeritics, USA).

2.4.5. Scanning electron microscope

Surface morphology of the sample was obtained at magnification (×1000) for untreated and pretreated samples using a SEM (JEOL JSM-6390). All samples were coated by a sputtering of gold powder to enhance the contrast of the images.

3. Results and discussion

3.1. Effects of pretreatment on the composition of the kenaf core

The composition change of the kenaf core reflects the efficiency of pretreatment. Table 1 depicts the changes in the composition of untreated and pretreated kenaf core. The composition of the untreated kenaf core was 46.1% cellulose, 25.4% hemicellulose, 18.5% lignin, and 10.0% ash. When the kenaf core was pretreated by an alkali pretreatment only, the composition of carbohydrate (cellulose and hemicellulose) increased to 75.2%. However, for the EBI-dilute alkali pretreatment, the composition of carbohydrate increased to 79.6%, 83.2%, 87.3%, and 89.3% when the kenaf core was irradiated at 100, 200, 300, and 500 kGy, respectively. The kenaf core is more carbohydrate enriched because the percent compositions of ash and lignin decrease as the EBI dose increases. Moreover, the lignin content was found to be only 9.4%, which gradually decreased as the EBI dose increased. These results indicate that the pore size of the kenaf core was increased by EBI, allowing the dilute alkali solution to easily infiltrate the lignocellulose complex [23]. In addition, recent studies have shown changes in the composition for different pretreatment methods and are summarized in Table 2.

3.2. Sugar yields

The illustration in Fig. 1 shows the sugar yields resulting from untreated and pretreated kenaf core (dilute alkali, EBI, and EBI-dilute alkali pretreatment) after 72 h of enzymatic hydrolysis. A greater yield of released sugars was obtained from the electron beam-irradiated kenaf core than the kenaf core pretreated with dilute alkali. However, a sugar yield of 72% was obtained from the kenaf core subjected to the EBI-dilute alkali pretreatment process for an identical enzymatic hydrolysis time. Optimized removal of the lignin may be explained by the increased surface area between lignin and dilute alkali due to EBI pretreatment. The resulting sugar

Table 1
Compositional changes of the kenaf core using different pretreatment conditions.

Samples	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Ash (%)
Untreated	46.1 ± 0.05	25.4 ± 0.02	18.5 ± 0.02	10.0 ± 0.03
E500	46.1 ± 0.07	25.4 ± 0.09	18.5 ± 0.04	10.0 ± 0.05
3% NaOH	52.9 ± 0.06	22.3 ± 0.02	15.2 ± 0.01	9.6 ± 0.03
E100A	62.1 ± 0.05	17.5 ± 0.03	13.3 ± 0.02	7.1 ± 0.02
E200A	66.4 ± 0.02	16.8 ± 0.04	11.6 ± 0.03	5.2 ± 0.01
E300A	71.5 ± 0.03	15.8 ± 0.04	10.0 ± 0.04	2.7 ± 0.04
E500A	73.9 ± 0.04	15.4 ± 0.02	9.4 ± 0.02	1.3 ± 0.03

All the numbers are based on the average of three replicates of initial dried weight sample.