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Electron beam irradiation enhances the digestibility and fermentation yield of water-soaked lignocellulosic biomass

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ABSTRACT

In order to overcome the limitation of commercial electron beam irradiation (EBI), lignocellulosic rice straw (RS) was pretreated using water soaking-based electron beam irradiation (WEBI). This environment-friendly pretreatment, without the formation (or release) of inhibitory compounds (especially hydroxymethylfurfural and furfural), significantly increased the enzymatic hydrolysis and fermentation yields of RS. Specifically, when water-soaked RS (solid:liquid ratio of 100%) was treated with WEBI doses of 1 MeV at 80 kGy, 0.12 mA, the glucose yield after 120 h of hydrolysis was 70.4% of the theoretical maximum. This value was predominantly higher than the 29.5% and 52.1% measured from untreated and EBI-treated RS, respectively. Furthermore, after simultaneous saccharification and fermentation for 48 h, the ethanol concentration, production yield, and productivity were 9.3 g/L, 57.0% of the theoretical maximum, and 0.19 g/L h, respectively. Finally, scanning electron microscopy images revealed that WEBI induced significant ultrastructural changes to the surface of lignocellulosic fibers.

1. Introduction

Second-generation biofuel production from renewable biomasses is being explored as an energy alternative because of the rising fuel costs, exhaustion of crude oil resources, and environmental problems, such as global warming, caused by the use of fossil fuels [9,16]. Lignocellulosic rice straw (RS) is estimated to account for the largest portion (7.31×10^{14} of dry matter per year) of renewable biomass in the world [12]. Therefore, RS is considered a powerful biomass for the production of monomeric sugars. However, RS is difficult to depolymerize using only hydrolases owing to its polymeric outer cell-wall membrane, which is surrounded by amorphous compounds (especially lignins). To commercialize the production of cellulosic bioethanol, the effective conversion of recalcitrant biomass, especially lignocellulose, into fermentable monomers appears to be necessary [1,18,8].

Irradiation technology (especially electron beam irradiation) has been widely used for changing the properties of polymers [7]. Such technology also extends the range of applications for the irradiated material. The main role of the irradiation program is to focus on the radiation-induced changes in the microstructural crystallinity of the substrates. Irradiation induces a chain–cleavage mechanism by depolymerizing the polymeric material. Recently, an environmentally friendly electron beam irradiation (EBI) pre-treatment, which produces less inhibitory byproducts than the conventional thermochemical methods, was developed using a linear electron accelerator, and was subsequently evaluated with various analytical methods [2]. Based on the mass balance of lignocellulolysis, the commercial value of the irradiation program is quite high due to the instantaneous processing. Furthermore, this program does not need a temperature control (e.g., a cooling process) or a neutralization step owing to the presence of stable downstream products and the absence of any byproducts. However, the exclusive use of EBI to enhance the enzymatic hydrolysis of lignocellulose has not been commercially successful.

Therefore, to address the disadvantages in the original EBI system, such as, low sugar yields, a water-soaked RS was used as part of the advanced system. I conducted this study to determine the feasibility and efficiency of the water soaking-based electron beam irradiation. Its impact was evaluated from the indices that measured the enzymatic hydrolysis and fermentation efficiencies.

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2. Materials and methods

2.1. Water soaking-based electron beam irradiation

Based on the condition (1 MeV and 80 kGy at 0.12 mA) for a systemized procedure [2], rice straw (RS) was irradiated with accelerated electrons by using a linear electron accelerator (Korea Atomic Energy Research Institute, Daejeon, Korea). Prior to the irradiation, RS was soaked in mineral water overnight in order to enhance the effects of the substrate pretreatment. The moisture contents (based on solid:liquid ratios) used were approximately 0% (0; control), 52% (2), 68% (1), 81% (0.5; saturation point), and over 81% (0.25 or 0.125; colloidal suspension), respectively. In order to prevent the loss of moisture from the treated RS, all samples were instantly packed in a polystyrene bag under vacuum (after the soaking) before the water soaking-based electron beam irradiation (WEBI) pretreatment. The WEBI was then uniformly applied to the surface area of the plate.

2.2. Key physicochemical characteristics

The concentrations of the inhibitory byproducts, such as acetic acid, hydroxymethylfurfural, and furfural, and the theoretical maximum enzymatic hydrolysis of the WEBI-pretreated RS were analyzed by following the standard methods of the National Renewable Energy Laboratory (NREL) (http://www.nrel.gov/biomass/analytical_procedures.html). Based on the dry weight (w/w), the main components of RS were confirmed to be 36.0% glucan, 11.0% xylan, 20.0% lignin, along with negligible amounts of mannan (4.0%), galactan (3.0%), and arabinan (3.0%). After three replicates of the biochemical reactions, the hydrolysis reactions were carried out using the target substrates (untreated and pretreated RS samples). The hydrolysis yield was expressed as a percentage of the theoretical maximum of monomeric sugar (glucose) obtained from the cellulosic substrate. Filter paper (Whatman No. 1, Whatman, Brentford, UK) and Avicel (Sigma–Aldrich, St. Louis, MO, USA) were used as pure cellulose. In the presence of the water-soaked material, the change in the content of the reducing sugar was determined using a 3,5-dinitrosalicylic acid assay. In order to estimate the fermentation yield of the substrate, after three biological replicates of the cultures, simultaneous saccharification and fermentation were performed using the NREL-recommended methods. The ethanol yields from the fermentation tests were calculated using Eq. (1).

$$\text{Ethanol yield}(\%\ \text{theoretical maximum}) = \frac{\text{g of ethanol in broth}}{\text{g of theoretical maximal glucose from glucan in broth} \times 0.511} \times 100 \qquad (1)$$

2.3. Additional physicochemical characteristics

Scanning electron microscopy (SEM) was performed with a Hitachi S-4700 scanning electron microscope (Tokyo, Japan) at a voltage of 10 kV to observe the microstructural changes on the WEBI-pretreated substrates. Prior to SEM analysis, all samples were dried in a vacuum oven at 45 °C for 5 days and subsequently coated with gold–palladium. After WEBI pretreatment, the crystallinity index (CrI) of the substrates was determined using a powder X-ray diffractometer (Bruker D5005, Karlsruhe, Germany). As previously described [2], the diffraction spectra were analyzed using the θ–2θ method. Additionally, the crystalline portion of the substrate was identified based on the ratio of its crystalline intensity to the sum of its crystalline and amorphous intensities. Lastly, the generation of reactive oxygen species (hydrogen peroxide) was measured using the OxiSelect fluorometric assay STA-344 (Cell Biolabs, San Diego, CA, USA), which uses 10-acetyl-3,7-dihydroxyphenoxazine/horseradish peroxidase-based hydrogen peroxide detection according to the manufacturer's directions (http://www.cellbiolabs.com/). The mixtures were then incubated for 30 min in the dark, and the fluorescence was measured with an excitation at 530 nm and with an emission at 590 nm.

3. Results and discussion

3.1. Enzymatic hydrolysis of WEBI-pretreated RS

After the water-soaked rice straw (RS) was pretreated with the electron beam irradiation using previously determined optimal conditions (1 MeV and 80 kGy at 0.12 mA), the RS was hydrolyzed by the addition of both exo- and endocellulase for 120 h (Fig. 1). As the hydrolysis reaction progressed, the accumulated glucose yield (based on the % theoretical maximum), which indicates the enzymatic hydrolysis of lignocellulose, gradually increased. When the water soaking ratio (solid:liquid ratio) increased from 0% to 100%, the rate of glucose production and the extent of the reaction increased as WEBI levels were regulated in one direction. Glucose yields from the pretreated RS after 120 h of hydrolysis were 70.4% and 69.7%, with soaking ratios of 100% and 200%, respectively. Therefore, increasing the soaking ratio from 100% to 200% did not significantly increase the yield, indicating that the optimal dose for the effective pretreatment of lignocellulosic compounds is when a fixed ratio of 100% is used. However, pretreatment with a dose of over 200% resulted in a decreased yield, most likely due to substrate decomposition at higher doses. Additionally, unlike the high yields (Fig. 1), the enzymatic digestibility of the pretreated lignocellulose by the unsystematized EBI was just 14–37% of the maximum glucose yield after 1 day [10]. Interestingly, although the lignocellulolytic EBI system was systematically optimized for an improved hydrolysis yield, the product yield was <55% of the theoretical maximum after 5 days [2]. Based on these results, I speculated that certain parameters, especially the irradiation dose and the solid:liquid ratio, are either more important or less important than the lignocellulosic deconstruction. When a polymeric substrate (RS) is in contact with an adequate amount of solvent (mineral water; below 200% of the soaking ratio), it forms cross-linkages and swells

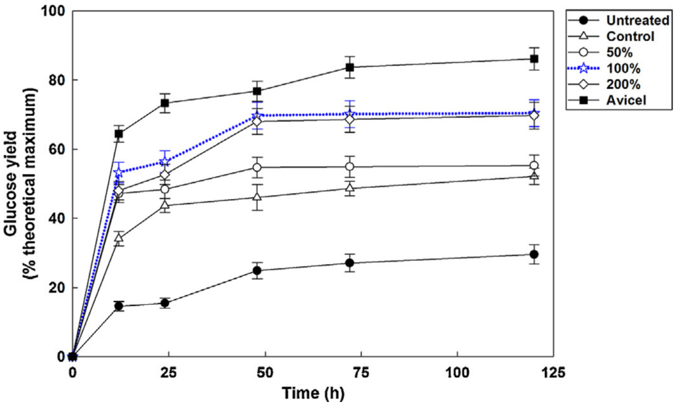

Fig. 1. Effects of the surface water soaking ratio (solid:liquid, 50–200%) on the enzyme digestibility of optimal electron beam irradiation-pretreated (1 MeV and 80 kGy at 0.12 mA) RS. Water (0%) was not added to the control treatment. Enzymatic hydrolysis was performed by subjecting samples to 30 CBU of beta-glucosidase (Novozyme 188) and 60 FPU of cellulase (Celluclast 1.5 L) per gram of glucan at pH 4.8 at 50 °C and 150 rpm for 120 h. All data shown are the mean ± standard deviation of observations conducted in triplicate.

spontaneously owing to the infiltration of the solvent. In other words, the adequate diffusion of the solvent may be useful to secure the internal peroxidative space for the interaction between electrons and target substrates in the RS substrate. Thus, these parameters together led to an aggressive attack on the recalcitrant surface of lignocellulose. However, too much water owing to the excessive swelling-capacity of the polymer can create a water barrier (e.g., a colloidal suspension) that blocks lignocellulosic peroxidation by producing radicals from the EBI electrons, mostly attributable to the surface water-soaking ratios (Fig. 1). Notably, when the water doses increase to >200%, the EBI-reduced depolymerization initiates an attack on the RS, thereby accelerating the process of aggregation.

Overall, the digestibility of the WEBI-treated RS, which is reflected in the monomeric sugar yields, was not higher than that of the lignocellulosic materials (71–99%) pretreated using conventional methods, such as dilute acid [11] and ammonia pretreatment [14,15,20]. However, I speculate that the environmentally friendly WEBI-based program for the disruption of recalcitrant materials is superior to others, especially because of the mild alkaline conditions (30–78%) and the fungal-based bioconversion (<50%). This process is also superior in terms of the % theoretical sugar maximum and cost/time effectiveness [5,17,21].

3.2. Simultaneous saccharification and fermentation of WEBI-RS

With the exception of the yield from overwork (over 96 h), Fig. 2 shows that the ethanol produced by fermenting WEBI-treated RS increased within 24 h of SSF and reached its maximum value after 48 h. After 48 h, the ethanol concentration, production yield, and productivity of the WEBI-treated straw were 9.3 g/L, 57.0% of theoretical maximum, and 0.19 g/L h, respectively. When the untreated straw was used in SSF, these values were 2.9 g/L (17.9% of theoretical maximum) and 0.06 g/L h, respectively. When only EBI was used, the maximal ethanol yield was determined to be 47.5% after 48 h. Interestingly, the ethanol yield from the WEBI system was approximately 3.2 times higher than that of untreated straw after 48 h of SSF, which is likely due to the acceleration of the cellulolytic process based on the enhanced digestibility of pretreated lignocellulose. In addition, regardless of whether the straw was treated or untreated, a low level of glucose (<0.3 g/L) was observed for a brief period during the SSF (Fig. 2). This value may have been higher during the release of glucose from the substrate than during the uptake of glucose by the fermentable yeast. Lastly, unlike the untreated straw (<0.1 g/L), the levels of acetic acid in the pretreated biomass were not detected with significant variance throughout the SSF period.

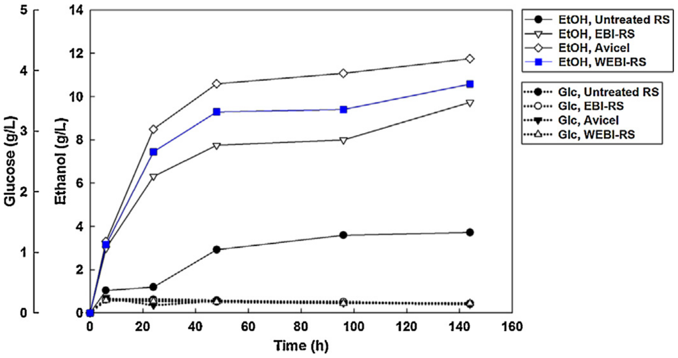

Fig. 2. Time course of SSF of optimal EBI-RS (1 MeV and 80 kGy at 0.12 mA). Mineral water (100%; S:L ratio = 1) was added for the WEBI pretreatment of RS. SSF using substrates with a glucan concentration of 3.1% (w/v) in 250 mL of medium was performed using *S. cerevisiae* D5A (ATCC 200062) as well as 30 CBU of beta-glucosidase (Novozyme 188) and 15 FPU of cellulase (Celluclast 1.5 L) per gram of glucan at an initial pH of 5.0. Samples were cultured at 38 °C and 150 rpm for 144 h. All data shown are the mean values of triplicate experiments.

In conventional pretreatment using an ammonia-soaking system, the production of ethanol via fermentation was 0.52 g/L h after 24 h and 0.26 g/L h after 48 h, respectively [13]. The fermentation yields during the above study are not greater than the yield (0.31 g/L h) observed after 24 h of SSF in the present study (Fig. 2). Furthermore, 9.8 g (62.0% of maximum) of ethanol in a statistical-based optimal biosystem was finally obtained after 144 h of SSF [3], which was not more than the WEBI-level (10.6 g; 67.1% of maximum; Fig. 2).

3.3. Changes in the microfibril structures

Unlike EBI pretreatments, WEBI-pretreated RS following the water soaking program revealed ultrastructural changes on the lignocellulosic surface (Fig. 3). The structures of the untreated surfaces were smooth and flat, whereas the pretreated surfaces had partially degraded face, scars, and cracks. Notably, the WEBI-pretreated rice straw had non-spherical protrusions, possibly due to reactive oxygen species (ROS), such as hydrogen peroxide, which induce oxidative cascades between electrons and water molecules (Fig. 3c). When compared to EBI pretreatment under optimal conditions (0.12 mA – 80 kGy – 1 MeV), changes in the crystalline portion were hard to distinguish by WEBI within the error range. Furthermore, based on a radiation-induced change in the crystallinity [2,7], a mechanism for the random-chain scission (occurring sequentially and uniformly on the substrate surfaces during the substrate disruption) was proposed. However, the exposure of the crystalline structures could be blocked by inducible aggregation or by the repolymerizing colonies, owing to the WEBI conditions (Fig. 3c).

3.4. Changes in the internal and external components using a WEBI-based program

The changes in the total mass following all pretreatments were negligible to within a reasonable error range, regardless of the conditions. For reference, the two major changeable components of the WEBI-based RS, xylan (approximately 12.5%) and lignin (approximately 8.3%), did not exhibit significant reductions of mass compared to those (12.1% and 7.7%, respectively) of the original EBI pretreatment. Furthermore, the extracellular portion of the reducing sugars (for the WEBI-based system or only for EBI) after the irradiation did not change with significant variance (below 0.8%), and thus it was actually similar to the percent yield of the theoretical glucose maximum. The formation of a water barrier may have prevented a direct attack to an external protective layer composed of hemicellulose and a lignin complex, thereby indirectly generating ROS or directly involving the oxidative degradation of the recalcitrant wall. Moreover, if water soaking helps to loosen the cell wall, then electrons have more space for extensive participation. However, the regulation of the substrate-specific or non-specific cascades via ROS in the WEBI system needs to be further investigated. Loss of the external layer components can also occur during the general conventional processes [19]. As for the pretreatment involving ammonia-soaking, the loss of lignin is significantly different during the removal of 50–85% of the initial content [14,13].

Lastly, regarding the use of external inhibitory compounds against either the hydrolysis or fermentation, although the theoretical yields of the WEBI-straw were not higher than those of lignocellulose pretreated using conventional methods, the generation of inhibitors, such as hydrogen peroxide, HMF, and furfural, was either negligible or not detected. In terms of the hydrolysis and fermentation yields, the intentional removal of

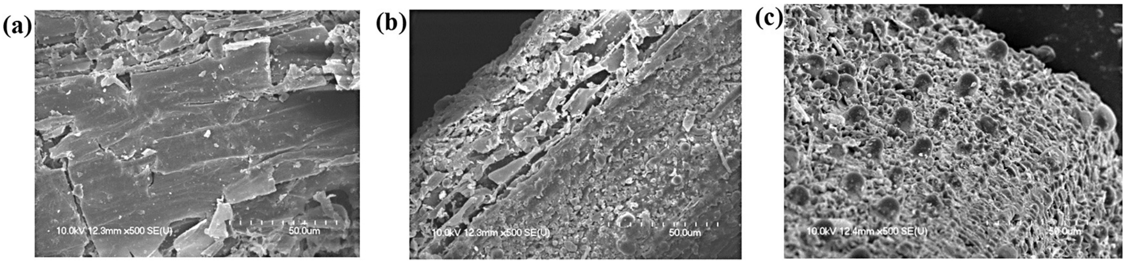

Fig. 3. Scanning electron micrographs of RS pretreated by EBI. (a) Untreated RS (500× magnified). (b) RS pretreated with optimal EBI at 0.12 mA, 1 MeV, and 80 kGy (500× magnified). (c) RS pretreated by optimal WEBI (100%; liquid:solid ratio = 1:1; 500× magnified).

the inhibitors was found to result in higher substrate conversion (% maximum) compared with substrate conversation on inhibitor accumulation [17]. Furthermore, in this system, I hypothesized that any accumulation of hydrogen peroxide would gradually be reduced to low levels (<0.01 mM) because of its utilization in the ligninolytic cascade. Therefore, although the accumulation of hydrogen peroxide has negative effects on the fermentable yeast [4] and carbon sources [6], SSF still functions under constant pH.

3.5. Mass balance after WEBI pretreatment

Using the same assumption for untreated samples, WEBI pretreatment and enzymatic digestibility steps resulted in a total of 22.4 g (untreated RS, 9.4 g) of glucose from 100 g of RS (Fig. 1). Furthermore, when 100 g of initial RS was consecutively subjected to WEBI pretreatment and then SSF, 10.6 g (untreated RS, 3.7 g; and EBI-RS, 9.7 g) of ethanol was produced after 144 h. The ethanol yield from fungal pretreated rice straw in SSF alone was 67.1% (untreated RS, 23.4%; and EBI-RS, 61.4%) of the theoretical maximum yield of ethanol (Fig. 2).

In addition, during the WEBI pretreatment, the loss of three main components (glucan, xylan, and lignin) and a total mass loss (w/w) in RS were negligible within the error range as they were <5% (i.e., <0.5 g) of the indices of evaluation (% glucose and % ethanol).

4. Conclusion

In order to upgrade traditional EBI, RS was pretreated to improve the hydrolysis yields by using a water-based electron beam at 0.12 mA – 80 kGy – 1 MeV. Based on the mass balance and the optimal WEBI (water soaking ratio of 100%) conditions, pretreated RS showed increases in the enzymatic hydrolysis (70.4% of the theoretical maximum) of cellulosic substrates as well as in ethanol production (67.1% of the theoretical maximum) in SSF, compared with those of the untreated RS. Structural composition analysis revealed that physical changes in lignocellulosic surfaces were most likely a result of WEBI. Quite importantly, the cost-effective yields resulting from the WEBI pretreatment were not lower than those resulting from the physicochemical programs, and inhibitors were rarely generated. However, no “physicochemical programs” (i.e., benchmark pretreatment runs) were included in the study.

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