Biotechnology Reports 4 (2014) 30–33

Contents lists available at ScienceDirect

Biotechnology Reports

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

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

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ARTICLE INFO

Article history:
Received 19 March 2014
Received in revised form 16 July 2014
Accepted 18 July 2014
Available online 1 August 2014

Keywords:
Bioethanol
Biomass pretreatment
Water soaking-based electron beam irradiation

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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http://dx.doi.org/10.1016/j.btre.2014.07.006

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).

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

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

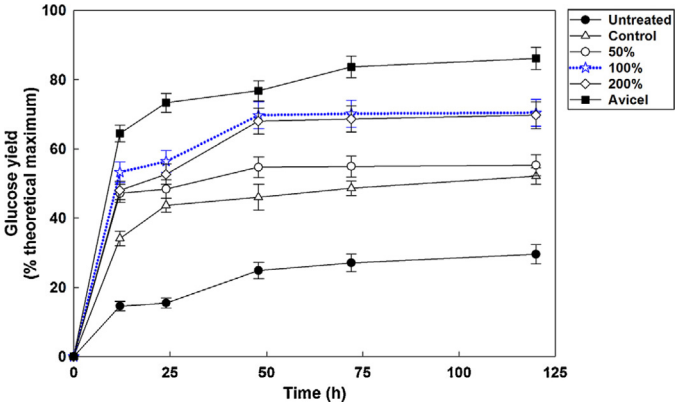

Fig. 1. Effects of the surface water soaking ratio (solid:liquid, 50–200%) on the enzyme digestibility of optimal electron beam irradiation-pretreated (1 MeV and 80 kGy at 0.12 mA) RS. Water (0%) was not added to the control treatment. Enzymatic hydrolysis was performed by subjecting samples to 30 CBU 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.