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High efficiency bioethanol production from barley straw using a continuous pretreatment reactor

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ABSTRACT

We developed a new pretreatment process for producing high-efficiency bioethanol from a lignocellulosic biomass. Barley straw was pretreated with sodium hydroxide in a twin-screw extruder for continuous pretreatment. The biomass to ethanol ratio (BTER) for optimal pretreatment conditions was evaluated by response surface methodology. Simultaneous saccharification and fermentation (SSF) was conducted to investigate the BTER with 30 FPU/g cellulose of enzyme and 7% (v/v) yeast (*Saccharomyces cerevisiae* CHY 1011) using 10% (w/v) pretreated biomass under various pretreatment conditions. The maximum BTER was 73.00% under optimal pretreatment conditions (86.61 °C, 0.58 M, and 84.79 mL/min for temperature, sodium hydroxide concentration, and solution flow rate, respectively) and the experimental BTER was 70.01 ± 0.59%. SSF was performed to investigate the optimal enzyme and biomass dosage. As a result, maximum ethanol concentration and ethanol yield were 46.00 g/L and 77.36% at a loading pretreated biomass of 20% with 30 FPU/g cellulose of the enzyme dosage for barley straw to bioethanol. These results are a significant contribution to the production of bioethanol from barley straw.

1. Introduction

Lignocellulose from sources such as corn stover, rice straw, *Miscanthus*, and barley straw is the most abundant renewable energy source on Earth. The annual global production of dry barley averages about 124 Tg. Europe (62%), Asia (15%), and North America (14%) are the major production regions. Barley yield ranges from 0.74 to 2.8 dry Mg/ha with a global average of 2.3 dry Mg/ha [1]. The grain and straw ratio is 1:0.76 of dry barley biomass [2]. About 94.24 Tg of dry barley straw are produced annually in the world [3]. Barley straw is very useful as a feedstock for bioethanol production, because almost all barley straw is burned or discarded [4]. Theoretically, up to 29.21 GL of bioethanol from barley straw can be produced [5,6].

Lignocellulose usually consists of cellulose, hemicellulose and lignin. Cellulose and hemicellulose can be changed to sugars by biological and chemical conversion, and the sugars can be fermented to ethanol or other valuable chemicals [7]. However, lignin is an inhibitor of the enzyme reaction, as it not only prevents cellulase from forming cellulose but also adsorbs enzyme, making it inactive for cellulose hydrolysis [8].

Thus, pretreatment is one of the key elements during bioconversion of lignocellulosic materials. The goal of any pretreatment processes is to remove structural and compositional obstacles to hydrolysis, to improve the rate of enzyme digestibility, and increase yields of fermentable sugars from substrates [9]. Pretreatment is required to modify the structure of the lignocellulosic biomass to make it more accessible to enzymes that convert carbohydrate polymers into fermentable sugars [10]. A successful pretreatment must satisfy the following: (i) improve sugar formation or the ability to subsequently form sugars by hydrolysis, (ii) avoid degradation of carbohydrates, (iii) avoid formation of fermentation by-products, and (iv) be cost effective [11].

Many studies have investigated pretreatments such as hydrothermal [12], dilute acid [13], ammonia fiber expansion [14], soaking in aqueous ammonia [15], and steam explosion [16]. Additionally, pretreatment methods using NaOH, which very effectively remove lignin, have been studied recently [17,18]. However, regardless of the pretreatment, different biomass materials show different hemicellulose and lignin removal results or enzyme digestibility even under the same conditions. Reducing the particle size of the lignocellulosic biomass through milling and grinding is one of the best pretreatments as a method to increase specific surface area. This pretreatment makes the enzyme more easily accessible and breaks down the substrate to sugars, but these kinds of pretreatments consume a large amount of energy. Thus, a new technology needs to be developed to cover the economic bottleneck of size reduction and to commercialize the bioethanol industry.

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Twin-screw extrusion is commonly used in the polymer and food industries and has many advantages as a highly cost-effective production process. A twin-screw extruder can be applied to a continuous process and is practical and useful for a large-scale production process. It can be easily controlled by temperature and provides high-efficiency pulverization by high-shearing force, high throughput, and adaptability to many different processes through modifications [19].

This study was conducted to investigate the possibility of enhancing ethanol production yield by applying a twin-screw extruder under low temperature and sodium hydroxide conditions for biofuel production. Barley straw was pretreated with sodium hydroxide in the twin-screw reactor and pretreated barley straw in optimal conditions was fermented using the simultaneous saccharification and fermentation (SSF) process. Since cellulase is inhibited by glucose as it is formed, rapid conversion of the glucose into ethanol by yeast results in faster rates, higher yields, and greater ethanol concentrations than possible for separate hydrolysis and fermentation [18,20]. Also, we applied response surface methodology (RSM) to delineate the effects of five level-three factors and their reciprocal interactions on pretreatment to optimize pretreatment conditions. We calculated the biomass to glucose ratio (BTGR) and the biomass to ethanol ratio (BTER) to evaluate the efficiency of bioethanol production from lignocellulosic biomass. The specific surface area of the pretreated biomass was investigated, as twin-screw extrusion has a good effect on dispersibility. Additionally, the optimum dosage of enzymes and pretreated biomass for the SSF process were determined for efficient bioethanol production.

2. Materials and methods

2.1. Materials

Barley straw was used as the lignocellulosic biomass and was obtained from the Jeollabuk-do Agricultural Research and Extension Service, Iksan, Korea. It was milled and fractionated to a particle size of <3 mm using sieves and was stored in a plastic container at room temperature. Moisture content (~5%) was maintained in the biomass through oven drying. Cellic® CTec II and Cellic® HTec II (Novo Inc., Bagsvaerd, Denmark) were applied for enzymatic hydrolysis and SSF. All reagents (except sodium hydroxide) were of analytical grade. Sodium hydroxide (Duksan Chemical Co. Ltd., Seoul, Korea) was of industrial grade.

2.2. Pretreatment using twin-screw reactor

The continuous twin-screw reactor was manufactured to pretreat lignocellulosic biomass by Changhae Ethanol Co. (Jeonju, Korea), and designated as a ChangHae Ethanol Multi ExTruder (CHEMET). The bench-scale twin-screw reactor had a co-rotating system, a screw diameter of 28 mm, and a L/D ratio of 36:1. The reactor was used for all pretreatment processes of the lignocellulosic biomass. The CHEMET was designed for operating at a maximum of 30 kg of biomass per day, 200 °C temperature, and 250 rpm of twin-screw speed. Fig. 1 shows a diagram and photograph of the CHEMET facility.

The CHEMET's screws were arrayed transfer, reversed transfer, agitation, retention, transfer, reversed transfer, transfer and discharge parts for barely straw pretreatment. The pretreatment solution penetrated into the biomass during agitation by the gear pump. The pretreatment temperature was controlled by heated oil, estimated transfer part (before discharge part), and was automatically maintained at an error range of ±2 °C. While the biomass feeding to discharge time varied slightly different depending on the situation, it typically took about four minutes.

To determine optimum pretreatment conditions, experiments were conducted with different concentrations of sodium hydroxide (0–0.8 M) at various temperatures (50–100 °C) and different flow rates of solution (72–120 mL/min) when biomass feeding rate and twin-screw rotation speed were fixed at 12 g/min and 100 rpm, respectively. After pretreating the biomass, samples were washed with tap water, dried for biomass composition analysis [21], and used in further experiments.

2.3. Enzymatic hydrolysis, and the SSF process for optimal conditions

The enzymatic hydrolysis and the SSF experiments were conducted for optimal pretreatment condition. The operating conditions of the enzymatic hydrolysis were 50 °C and pH 4.8 (0.05 M sodium citrate buffer) on a shaking incubator at 150 rpm for 48 h. Enzyme loading amount was 30 FPU/g cellulose (Cellic® CTec II), and 15% Cellic® HTec II (this value was based on the amount of loaded Cellic® CTec II). 10 g of pretreated biomass was loaded for enzymatic hydrolysis, and total liquid and solid was set to 100 mL. To investigate the effects of pretreatment on SSF, the same amount of enzyme as used for enzymatic hydrolysis and 7% (v/v) yeast (*Saccharomyces cerevisiae* CHY 1011) were added to each sample with a working volume of 100 mL. The operating conditions for SSF were at 32 °C and pH 4.8 on a shaking incubator at 150 rpm for 72 h. Samples were periodically analyzed for glucose and ethanol concentration using high performance liquid chromatography (HPLC; Waters, Milford, MA, USA) using a Bio-Rad Aminex HPX-87P column (Hercules, CA, USA), and a refractive index detector. The mobile phase was deionized water at a flow rate of 0.6 mL/min at 85 °C.

2.4. Analysis of pretreatment efficiency

The component of raw and pretreated biomass was estimated for pretreatment efficiency. Recovery of glucan after pretreatment (R) was estimated with the following Eq. (1).

$$R\ [\%] = \frac{G_P\ [\%] \times S\ [\%]}{G_R\ [\%]} \times 100 \quad (1)$$

where S is the solid ratio after pretreatment which is weight of wash-pretreated and pretreated biomass after dry, G_P is the glucan concentration after pretreatment, and G_R is the glucan concentration of the raw biomass.

Also, production of glucose and ethanol from raw biomass was estimated to determine accurate pretreatment efficiency. The effectiveness of converted sugar from raw biomass is determined by multiplying the glucose recovery ratio by enzymatic digestibility after pretreatment and is designated as the biomass to glucose ratio (BTGR). The BTGR was calculated using the following Eq. (2):

$$\mathrm{BTGR}\ [\%] = \frac{S\ [\%] \times G_E\ [\%]}{\left(G_R\ [\%]/K_1\right) \times B_E\ [\%]} \times 100 \quad (2)$$

where S is the solid ratio after pretreatment, G_E is the glucose concentration after enzymatic hydrolysis, K_1 is glucan to glucose constant (0.9), and B_E is the biomass dosage at enzymatic hydrolysis.

The effectiveness of converted ethanol from raw biomass can also be determined by multiplying the glucose recovery ratio by ethanol production ratio after pretreatment. This is designated as the biomass to ethanol ratio (BTER). The BTER was calculated using the following Eq. (3):

$$\mathrm{BTER}\ [\%] = \frac{S\ [\%] \times E\ [\%]}{\left(G_R\ [\%]/K_1\right) \times B_S\ [\%] \times K_2} \times 100 \quad (3)$$

where E is the ethanol concentration after fermentation, B_S is the biomass dosage with SSF, and K_2 is an ethanol constant (0.5111).

2.5. RSM

An RSM central composite rotatable design (CCRD) was used to optimize the conditions. The parameters were pretreatment temperature, sodium hydroxide concentration, and flow rate of the solution. Table 1 shows the coded and uncoded levels of the independent factors (X_i) and the experimental design. Experimental data were analyzed via RSM, to fit the following second-order polynomial equation generated by Design-Expert 7 software (Stat-Ease Inc., Minneapolis, MN, USA). The quadratic response surface model was fitted to the following equation:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i x_i + \sum_{i=1}^{3} \beta_{ii} x_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} x_i x_j \quad (6)$$

where Y is the response factor (BTER), X_i is the ith independent factor, β_0 is the intercept, β_i is the first-order model coefficient, β_{ii} is the quadratic coefficient for the factor i, and β_{ij} is the linear model coefficient for the interaction between factors i and j [22].

2.6. Optimization of the SSF process

Pretreated barely straw was simultaneously saccharified and fermented using two kinds of enzyme complexes (Cellic® CTec II, and Cellic® HTec II) and yeast (*S. cerevisiae* CHY 1011). Barely straw that was pretreated with sodium hydroxide by the CHEMET under optimal conditions was added to a 250 mL Duran media bottle or a 500-mL flask containing 0.05 M citrate and autoclaved for 15 min. To investigate the effects of enzyme concentration on ethanol production, 5–40 FPU/g cellulose Cellic® CTec II and 15% Cellic® HTec II (this amount was based on Cellic® CTec II dosage) were loaded into each sample, which was a 100 mL working volume, and the solid caps were replaced with silistopper to exhaust the CO_2 released during fermentation. The operating conditions were 32 °C and 150 rpm for 100 h, and ethanol concentration was estimated by HPLC. After establishing the optimal enzyme dosage to efficiently produce ethanol, 1–30 w/v% biomass concentration was added to the SSF process in a working volume of 300 mL to determine suitable biomass dosage.