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High-titer ethanol production from simultaneous saccharification and fermentation using a continuous feeding system

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HIGHLIGHTS

- SSF process with a continuous feeding system was used at high-solids loading.
- An angle-type impeller affected the SSF process at high solid loadings.
- Prehydrolysis and the SSF process yielded 63–70 g/L ethanol.
- SSF with an angle impeller yielded 69.2 g/L ethanol (87.2% yield), after 56 h.

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ABSTRACT

A high-solid loading of lignocellulosic biomass in hydrolysis and simultaneous saccharification and fermentation (SSF) processes would make biofuel production more economical. However, several bottlenecks are determined by biomass characteristics, such as absorptiveness. We investigated the enzymatic digestibility and fermentability of pretreated *Miscanthus* as a high-concentration biomass in an SSF process, using a continuous feeding system and a particular type of impeller. When prehydrolysis and SSF was performed with a 25% dry solid loading, containing 30 FPU/g cellulose, for 56 h, 63.4 ± 1.0 g/L ethanol was produced with a standard impeller, while 69.2 ± 1.6 g/L ethanol was produced with an angled-type of impeller. Ethanol was produced in a short time, and ethanol productivity was enhanced, by using the continuous feeding system and an impeller angled at 30°. These findings contribute to the development of a continuous-production process for obtaining bioethanol from lignocellulosic biomass.

1. Introduction

Since utilizing bioethanol as a substitute for fossil fuel could promote rural development, reduce greenhouse gases, and facilitate energy independence [1], bioethanol production from biomass, such as corn, sugarcane, and rice straw has attracted great interest. Typically, bioethanol is produced commercially by fermenting monomer sugar extracted from starch-based or sugar-based feedstock, but this has raised ethical concerns, as the rapid growth of such bioethanol production has increased the price of crops and foodstuffs. Thus, it is important to identify non-edible forms of biomass for biofuel production. Lignocellulosic biomasses, such as oil palm empty fruit bunches (EFB), *Miscanthus*, barley straw, and corn stover, are abundantly present worldwide, and can be used as renewable substrates for bioethanol production without competing with production of food or animal feed [2,3].

However, using lignocellulosic biomass poses multiple challenges, one of which is increasing the ethanol concentration in the fermentation broth in order reduce the cost of cellulose-derived ethanol, due to the great energy demand inherent to ethanol distillation [4,5]. A high concentration of biomass in the simultaneous saccharification and fermentation (SSF) process is essential for producing a higher ethanol titer. Solid loading of 30% (w/w) in starch-based ethanol fermentation is commonly used for obtaining an ethanol concentration above 8–10% (w/w) [6]. In lignocellulosic bioethanol production, a 25–30% loading of pretreated biomass is required for production of 5–10% (w/w) of ethanol. A high solid loading also reduces the water used for ethanol production [7].

Generally, in a batch-type SSF process, ethanol concentration is below 4% (w/w), requiring a significant increase in energy demand [4,8]. Obtaining anhydrous ethanol from a 3.7% fermented broth

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requires 2.4-fold more steam than from a 12% fermented broth [9]. Therefore, increasing the ethanol concentration is important to decrease the energy consumed during distillation and dehydration; this requires a high biomass loading. Nevertheless, a cellulosic substrate is always of low density, and has strong hygroscopicity; moreover, cellulosic slurries become progressively more paste-like and difficult to handle at solid concentrations exceeding 15 wt% [10]. This can be solved by using fed-batch SSF, in which a pretreated substrate is fed continuously or by scheduled additions in order to minimize the non-uniformity of the system [11]. A fed-batch process has been applied successfully for enzymatic saccharification and/or SSF of various pretreated substrates to obtain relatively high concentrations of sugar and ethanol [12–15]. Despite many advances in pretreatment technologies, achieving an ethanol concentration of >5 wt% is challenging, because the pretreated biomass loaded in the hydrolysis or SSF process is relatively low.

This study aimed to investigate the enzymatic digestibility and fermentability of pretreated *Miscanthus*, applied as a high-concentration biomass in SSF, using a continuous feeding system connected to the bottom of the bioreactor and a particular type of impeller.

2. Materials and methods

2.1. Materials

We used *Miscanthus sacchariflorus* genotype Geodaeuksae 1 obtained from the Bioenergy Crop Research Center in Muan, Korea. It was milled by cutter mill and sieved to a particle size of <6 mm, and then kept in a plastic container at room temperature. The *Miscanthus* contained 37.1% cellulose, 21.3% hemicellulose, 23.2% lignin, and 3.1% ash. The remainder included some organic compounds, such as uronic acid and acetyl groups, and other trace components, including minerals, waxes, fats, starches, resins, and gums [16]. Cellic® CTec 2 and Cellic® HTec 2 (Novo Inc., Hellerup, Denmark) were applied to SSF. All reagents used were of analytical grade, except for the sodium hydroxide (Duksan Chemical Co., Ltd., Ansan, Korea), which was of industrial grade. We used *Saccharomyces cerevisiae* CHY1011, isolated and identified by Changhae ethanol (Changhae Ethanol Co., Ltd., Jeonju, Korea) as an industrial strain [17] that uses glucose for producing ethanol.

2.2. Pretreatment process

Miscanthus was treated using the CHEMET (ChangHae Ethanol Multi ExTruder, Changhae Ethanol Co., Ltd., Jeonju, Korea) pretreatment process employing sodium hydroxide; this process used a twin-screw extruder. The CHEMET pretreatment conditions were optimized previously [18]: 95 °C, 0.4 M sodium hydroxide, 80 rpm twin-screw speed, and a flow rate of 120 mL/min (solid:liquid ratio = 1:8). The pretreated biomass contained 56.3% cellulose, 23.3% hemicellulose, 8.5% lignin, and 2.0% ash. After pretreatment, the samples were washed in tap water and dried at 50 °C for keeping the pretreated biomass with same condition during the experiment.

2.3. SSF with a continuous feeding system

SSF of the treated *Miscanthus* was conducted in a 5-L hydrolysis/fermentation tank (HF tank) equipped with a continuous feeding system and an impeller (Figs. 1 and 2). The reactor system was divided into three parts: biomass hopper, continuous feeder, and hydrolysis/fermentation tank. Crucially, the continuous feeding equipment was connected to the bottom of the HF tank. A single screw was applied to the continuous feeder, which prevented a back-flow from the HF tank. The tank included a pH control system, an enzyme supply system, and a water bath jacket for adjusting temperature. A paddle & anchor-type agitator was included in the tank, angled at 0–45° (Fig. 2), in order to stir the mixture well.

SSF involved two stages: the first stage entailed feeding and prehydrolyzing the pretreated biomass, and in the second stage SSF commenced. The first stage started with addition of 1500 mL of distilled water and 1500 g of biomass, which contained 45% moisture, to the HF tank, to which was added 30 FPU/g cellulose Cellic® CTec 2 and 15% Cellic® HTec 2 (based on the amount of loaded Cellic® CTec 2); this mixture was then allowed to react at 33 °C for 8 h. The large scale of hydrolysis and fermentation could not dramatically reduce the temperature and a lot of energy was needed to decrease the temperature, so prehydrolysis was conducted at 33 °C. The biomass feeding rate was 187.5 g/h in the prehydrolysis stage. After the first stage, a 7% seed culture (7.0 ± 0.2 g/L of cell density) was added to the HF tank content and the SSF process proceeded at 33 °C and pH 5.0 for 48 h. SSF samples were taken periodically to analyze the concentration of glucose, xylose, and ethanol. All experiments were conducted in duplicate, and the average value determined.

2.4. Analytical methods

Composition of pretreated and untreated materials was analyzed according to the NREL LAP procedure [19]. After the reaction was completed, glucose and ethanol concentration was determined using high-performance liquid chromatography (HPLC, Waters, Milford, MA, USA) with an Aminex HPX-87P column (Bio-Rad, Hercules, CA, USA) and a refractive index detector. The mobile phase was deionized water, and the flow rate was 0.6 mL/min at 85 °C.

2.5. Ethanol conversion yield

The ethanol yields were calculated according to the NREL LAP-008 (2001) equations [20]:

$$\text{Theoretical ethanol concentration} = 0.51 \times f[\text{Biomass}] \times 1.111$$

$$\text{Ethanol yield} = \frac{[EtOH]_f - [EtOH]_o}{\text{theoretical ethanol concentration}} \times 100\%$$

where [Biomass] is the dry biomass weight concentration at the start of fermentation (g/L); f is the cellulose fraction of dry biomass (g/g); 0.51 is the conversion factor for glucose to ethanol, based on the stoichiometric biochemistry of yeast; 1.111 is the conversion factor for cellulose to the equivalent glucose. $[EtOH]_f$ is the ethanol concentration at the end of the fermentation (g/L) minus any ethanol produced from the enzyme and medium; $[EtOH]_o$ is the ethanol concentration at the beginning of the fermentation (g/L), mainly due to seed inoculation. The volume in the unit of g/L only refers to the liquid fraction in the reaction system, not to the volume of the whole slurry. The liquid volume was calculated based on the water mass balance of the SSF operation. The ethanol concentration in the liquid phase was analyzed using HPLC as described above.

3. Results and discussion

3.1. Comparison of batch-type and continuous feeding system SSF processes

One of the major challenges for enzymatic hydrolysis or SSF at high-solids loading is the lack of available water, which is essential for efficient hydrolysis, for mass transfer and lubricity [21]. A high-water content enhances the effectiveness of the chemical and enzymatic reactions by providing a medium for solubilization