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Co-production of bioethanol and biodiesel from corn stover pretreated with nitric acid

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HIGHLIGHTS

- Corn stover pretreated with HNO_3 was used to co-produce bioethanol and biodiesel.
- The optimal reaction condition was 151.9 °C, 0.68% HNO_3 and 2.5 min.
- SSF of pretreated corn stover gave the maximum ethanol concentration of 22.4 g/L.
- *Cryptococcus curvatus* in corn stover hydrolysate showed a lipid yield of 1.04 g/L.

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ABSTRACT

This research explores the application of glucose and xylose from corn stover pretreated with nitric acid (HNO_3) for the co-production of bioethanol and biodiesel. Response surface methodology was employed to optimize HNO_3 pretreatment condition including HNO_3 concentration (0.2–1.0%), temperature (140–160 °C), and reaction time (1–10 min). The optimal reaction condition was 151.9 °C, 0.68% HNO_3 and 2.5 min, which resulted in the highest xylose yield of 77.8% and glucan content of 57.1%. Quasi-simultaneous saccharification and fermentation (Q-SSF) of pretreated corn stover with *Saccharomyces cerevisiae* gave an ethanol concentration of 22.4 g/L, corresponding to 69.1% theoretical ethanol yield based on initial cellulose weight. In addition, *Cryptococcus curvatus* in hydrolysate medium showed the lipid yield (final cell weight × lipid content) of 1.04 g/L, which was higher than those in defined medium using pure xylose. Our work demonstrates that the simultaneous production of ethanol and lipid can be one potentially promising option for lignocellulose-derived fuel production.

1. Introduction

The ever increasing environmental and energy stresses resulting from the extensive use of fossil fuels are major contributors to current efforts to develop non-petroleum solutions. Biofuels such as bioethanol and biodiesel are examples of such solutions, and even more advantageous than other renewable fuels in that they are liquid fuels having general compatibility with the current combustion engines [1]. Lignocellulosic biomass is a natural feedstock of choice, as it is the most abundant renewable resource and possesses potential energy value fit for biofuel production [2].

Lignocellulose is comprised of sugar polymer components (i.e., cellulose and hemicellulose) and non-sugar component (i.e., lignin), which are intertwined in a complex manner [3]. Generally, the polysaccharides of cellulose and hemicellulose are eventually hydrolyzed into monomers and subsequently converted into biofuels. To ensure as high biofuel yield as possible in particular from agricultural residues that contain a large amount of hemicellulose, therefore, it is important to utilize not only glucose from cellulose but also xylose from hemicellulose. Robust industrial fermenting yeasts currently used for bioethanol production are, however, unable to metabolize xylose, at least to a commercially meaningful level; it is more so in the co-presence of glucose [4]. One engineering way to overcome this critical barrier would be to separate both the sugar components in the process: for instance, stepwise isolation of dissolved xylose monomers from solid cellulose polymers in the pretreatment step. Each sugar portion can go through different downstream pathways: cellulose to ethanol fermentation and xylose to heterotrophic oil production. This fractionation is anticipated to lead to more complete use of sugar polymers.

Dilute acid treatment is the best method suited for this purpose: in this method, most of xylose fraction is recovered in the liquid fraction after pretreatment while glucan remains in the solid

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fraction [5–7]. After the acid pretreatment, the solid cellulose-rich fraction is processed via enzymatic saccharification and fermentation to producing ethanol. The xylose monomers in the hydrolysate, on the other hand, can take a separate pathway and serve as substrate for the cultivation of oil-producing yeasts, producing lipid and then ultimately biodiesel. Since the oleaginous microbes require carbon sources for the growth and lipid production, the xylose can contribute to the improvement of economic viability of the biodiesel production, particularly in heterotrophic cultivation [8].

In this study, nitric acid pretreatment, which exhibited exceptionally high efficiency for the hydrolysis of hemicellulose even in a short reaction time [9,10], was employed as a dilute acid hydrolysis method. A reasonable range of pretreatment conditions using corn stover was studied to efficiently produce both ethanol from the cellulose-rich pretreated solids with *Saccharomyces cerevisiae* and lipid from the xylose-rich pretreatment liquor using *Cryptococcus curvatus*.

2. Materials and methods

2.1. Corn stover

Corn stover was obtained from a local farm in Changwon, South Korea. The air-dired corn stover was ground using a laboratory blender (7012s, Waring Commercial Co. Ltd., USA), sieved for size to become less than 3 mm, and stored in a drying oven at 45 °C for further use. The main composition of raw corn stover was 40.6% glucan, 20.5% xylan, 2.6% arabinan, 1.7% galactan, 19.3% lignin, 7.6% ash, and 7.7% other non-identified compounds (e.g., protein, acetyl groups) on dry weight basis.

2.2. Nitric acid pretreatment

Pretreatment was conducted in a stainless steel cylindrical reactor coated with Teflon on the inside with a volume of 70 mL. Before pretreatment, 5 g of corn stover and 50 mL of HNO_3 solution at 100 g/kg biomass loading were introduced into the reactor. The reactor was then heated to target temperatures using an oil bath. At the end of the pretreatment, the pretreated slurry was filtered by vacuum filtration using glass microfiber filters and the liquor retrieved for diesel production. The retained solid on the filter paper was then washed with DI water and collected for ethanol production (Fig. 1).

2.3. Quasi-simultaneous saccharification and fermentation (Q-SSF)

S. cerevisiae 7928 was obtained from Korean Collection for Type Culture (Daejeon, South Korea). Strains were cultivated in a 250-mL Erlenmeyer flask with 100 mL working volume of the inoculation medium containing 3.0 g/L yeast extract, 5.0 g/L peptone, 30.0 g/L glucose. After 24 h, the cells were harvested and used as an inoculum for ethanol production.

Quasi-simultaneous saccharification and fermentation (Q-SSF) tests consisted of pre-hydrolysis phase and SSF phase. The Q-SSF experiments were conducted in sterile 250-mL Erlenmeyer flasks with cap and needle to allow the escape of CO_2 in a shaking incubator at 170 rpm. The pretreated corn stover samples were pre-hydrolyzed in the flasks including 50 mM sodium citrate buffer (pH 4.8) and fermentation nutrients (5.0 g/L yeast extract, 5.0 g/L peptone, 5.0 g/L KH_2PO_4, 0.4 g/L $MgSO_4$ and trace element) with a working volume of 50 mL at a solid loading of 10%. The experiments were started by adding a commercial enzyme blend Cellic C-Tec 1 (Novozymes, Denmark) with an activity loading of 30 FPU/g cellulose and pre-hydrolyzed for 24 h at 50 °C. After pre-hydrolysis, an inoculum of *S. cerevisiae* was added into the medium (10%, v/v), and then SSF was run at reduced temperature to 38 °C for another 96 h. The samples were withdrawn periodically to determine the glucose and ethanol concentration. The ethanol yield was calculated assuming that 1 g glucose present in the liquid would theoretically give 0.511 g ethanol and 1 g cellulose when hydrolyzed will give 1.11 g glucose. Therefore, the ethanol yield was expressed using the following equation:

$$\text{Ethanol yield (\% theoretical yield)} = \frac{\text{Ethanol produced(g)}}{0.511 \times (\text{Cellulose(g)} \times 1.11)} \times 100 \quad (1)$$

2.4. Lipid production

C. curvatus, isolated from fermented seafood obtained in Suncheon, South Korea, was used for lipid production. After nitric acid pretreatment of corn stover, liquid hydrolysate (mainly xylose) was adjusted to pH 5.0 using sodium hydroxide, and the 1% (v/v) trace element was added. The medium was then filtered through 0.2 μm PES filter media (Whatman, UK) for use as a yeast substrate. A positive control experiment was conducted using the defined medium (0.5 g NH_4Cl, 2.7 g KH_2PO_4, 1.0 g Na_2HPO_4, 1.0 g $MgSO_4{\cdot}7H_2O$) and 10 mL trace element with 15 g commercial xylose in deionized water of 1 L. Yeast cultivation experiments were performed using a 100-mL Erlenmeyer flask with 50 mL working volume in a shaking incubator at 200 rpm and 30 °C.

2.5. Analytical methods

The components of corn stover were determined according to the National Renewable Energy Laboratory (NREL) analytical methods [11]. The released sugar monomers and inhibitors in

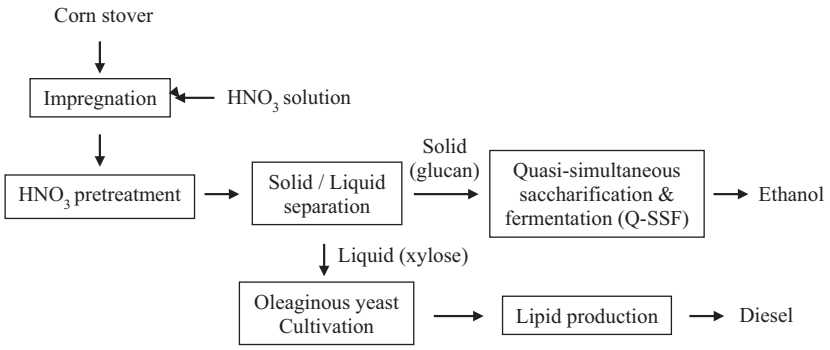

Fig. 1. The flow chart of the proposed integrated process for co-production of bioethanol and biodiesel.