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Ethanol and furfural production from corn stover using a hybrid fractionation process with zinc chloride and simultaneous saccharification and fermentation (SSF)

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ABSTRACT

A two-stage hybrid fractionation process was investigated to produce cellulosic ethanol and furfural from corn stover. In the first stage, zinc chloride ($ZnCl_2$) was used to selectively solubilize hemicellulose. During the second stage, the remaining treated solids were converted into ethanol using commercial cellulase and *Saccharomyces cerevisiae* or recombinant *Escherichia coli*, KO11. This hybrid fractionation process recovered 93.8% of glucan, 89.7% of xylan, 71.1% of arabinan, and 74.9% of lignin under optimal reaction conditions (1st stage: 5% acidified $ZnCl_2$, 7.5 ml/min, 150 °C (10 min) and 170 °C (10 min); 2nd stage: simultaneous saccharification and fermentation (SSF) using *S. cerevisiae*). The furfural yield from the hemicellulose hydrolysates was 58%. The SSF of the treated solids resulted in 69–98% of the theoretical maximum ethanol yields based on the glucan content in the treated solids. After fermentation, the solid residues contained primarily lignin. Based on the total lignin in untreated corn stover, the lignin recovery yield was 74.9%.

1. Introduction

The interest in lignocellulosic biomass as a source of fuel ethanol has grown as conventional fuel prices rise [1,2]. Ethanol production from lignocellulosic materials requires the hydrolysis of carbohydrate polymers into monomeric sugars, which is typically performed with enzymes. In this bioconversion process, the enzymatic hydrolysis is one of the most costly unit operations [3]. Numerous efforts have been made to reduce the enzyme dosage and to improve the fermentability of the biomass. Pretreatments of lignocellulose using various alkaline or acidic reagents have been evaluated to improve the accessibility of the enzymes to the fiber [4]. Recently, the fermentation of both cellulose and hemicellulose using recombinant bacterial strains or recombinant yeast strains has been introduced to produce ethanol effectively [5,6]. However, the cost of ethanol production from biomass is still economically unfavorable because simultaneous conversion of both cellulose and hemicellulose using simultaneous saccharification and co-fermentation (SSCF) has turned out to be more problematic than conversion of cellulose using simultaneous saccharification and fermentation (SSF). The challenges in SSCF include the additional cost of enzymes needed for hemicellulose hydrolysis, the glucose inhibition of xylose uptake, and the low ethanol tolerance and poor stability of recombinant strains [7–11]. Therefore, the independent utilization of these two carbohydrates may be more desirable. If lignocellulosic biomass can be effectively fractionated into cellulose, hemicellulose, and lignin, it may be advantageous to improve the overall efficiency of biomass utilization. For example, cellulose-rich product stream can easily be fermented, which can result in a high ethanol concentration. Furthermore, hemicellulose and lignin can be used to produce value-added co-products. In addition, lignin can be burned as power plant or boiler fuel, feedstock for activated carbon [12], a hydrocarbon fuel additive to gasoline [13], and various other applications including a binding or emulsifying agent [14–16]. Cellulose and hemicellulose can be converted into ethanol, food, pharmaceutical products, and other chemicals [17–20].

In this study, a hybrid fractionation process using acidified zinc chloride ($ZnCl_2$) followed by SSF was used to achieve the fractionation of corn stover into cellulose, hemicellulose, and lignin. $ZnCl_2$ has many advantages for biomass fractionation and pretreatment; it is the most effective swelling inorganic chemical reagent for biomass [21]. Although it is not highly toxic to living cells, it is highly selective for hemicellulose hydrolysis [22]. Zinc ions can

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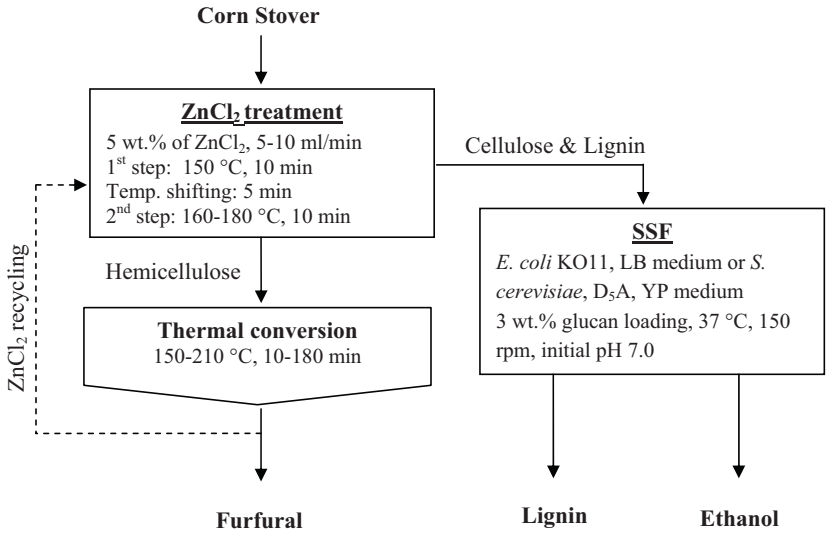

Fig. 1. Flow diagram of two-stage hybrid fractionation process.

react with carbohydrates to form zinc–cellulose complexes. These complexes are reactive to acid hydrolysis, which will hydrolyze cellulose to low-degree of polymerization (DP) compounds such as cellodextrin and hemicellulose to monosaccharides [23,24]. $ZnCl_2$ can expose the crystalline cellulose core by separating the hemicellulose from the biomass [22,23]. After $ZnCl_2$ treatment, most cellulose and lignin were retained in the solids, and the cellulose could then be converted into ethanol by fermentation. Additionally, solubilized hemicellulose in the first stage could then be converted to furfural at a high temperature.

Furfural, which is an acid degradation product of xylose, can be used as a chemical intermediate in herbicides, a solvent in lubricating oil refinement, and a solvent in the synthesis of pharmaceuticals, nylon, vegetable oil, plastic, and rubber products [25–27]. Furfural can be produced from hemicellulose-rich agricultural materials [26]. Hemicellulose can be hydrolyzed into monomeric C5 sugars, and furfural is formed by successive dehydration using acid-catalyzed reactions [28]. In this study, significant amounts of hemicellulose were hydrolyzed with an acidified $ZnCl_2$ solution. After this first fractionation stage, the acidified hemicellulose hydrolysates were converted into furfural without additional catalysts. After the furfural recovery by distillation, the remaining acidified $ZnCl_2$ solution can be reused in subsequent pretreatment steps.

In this article, the process conditions for effective fractionation and pretreatment are explored. Various effects on the compositional changes, enzyme digestibility, fermentability, recovery/utilization of each component, and other technical aspects related to development of the process were reported.

2. Materials and methods

2.1. Materials

The corn stover was harvested from central Iowa in 2009 and air-dried at ambient temperature. The corn stover was ground and screened to a nominal size of 9–35 mesh. The initial composition of the corn stover, as determined by the National Renewable Energy Laboratory (NREL; Golden, CO) laboratory analytical procedure (LAP; [29]), was 387 g/kg glucan, 233 g/kg xylan, 21 g/kg galactan, 45 g/kg arabinan, 171 g/kg lignin (acid insoluble + acid soluble), 15 g/kg sucrose, 12 g/kg ash, and 116 g/kg other extractives.

The cellulase enzyme, GC 220 (Lot # 301-04232-162), was provided by Genencor International. The average activity of the cellulase (GC-220) provided by the manufacturer was 45 filter paper unit (FPU)/ml and the protein content was 184 mg/ml. GC-220 is also known to contain β-glucosidase activity (196 cellobiase unit (CBU)/ml) and xylanase activity (1526 unit/ml) [30]. Novozyme 188, a β-glucosidase, was purchased from Sigma–Aldrich (Sigma Cat. #C-6150). The measured activity of the β-glucosidase [31] was 750 CBU/ml, and the protein content was 152 mg/ml.

The microorganism used for simultaneous saccharification and fermentation (SSF) was *Saccharomyces cerevisiae* American Type Culture Collection (ATCC)® 200062 (NREL-D_5A). The growth media was a YP medium, which contained 10 g/l yeast extract (Sigma Cat. #Y-0500) and 20 g/l peptone (Sigma Cat. #P-6588). For SSCF, recombinant *Escherichia coli* (KO11) ATCC® 55124 was purchased from the ATCC. *E. coli* KO11 is a recombinant cell strain that allows high expression levels of chromosomally integrated heterologous genes that enable it to metabolize both C5 and C6 sugars. LB medium (Sigma Cat. #L-3152) consisting of 10 g/l tryptone, 5 g/l yeast extract, and 10 g/l NaCl, supplemented with 40 mg/l chloramphenicol (Sigma Cat. #C-0378) was used for the growth of *E. coli* KO11. The fluted filter paper (medium pore) used for filtration was purchased from Fisher Scientific (Cat. #09-790-14F).

2.2. Experimental setup and operation

The reaction and operating conditions are summarized in Fig. 1.

2.2.1. First stage: $ZnCl_2$ treatment

The flow-through reactor system for $ZnCl_2$ treatment consisted of a SS-316 column reactor (2.3 cm internal diameter (ID) × 25.4 cm length (L); 104.3 cm^3 internal volume), a high performance liquid chromatography (HPLC) pump (Series II pump, Chrom Tech., Inc., MN), a temperature-programmable GC (gas chromatography) oven (Hewlett Packard 5890, HP Inc., Ontario, Canada), solution reservoirs, and a sample receiver tank (SS 304 cylinder; 1000 ml internal volume). A schematic diagram of the laboratory reactor set-up is shown in Fig. 2. For the $ZnCl_2$ treatment, a solution of 5.0% $ZnCl_2$ with 0.03% hydrochloric acid (HCl) was used. Ten grams (based on oven-dried weight) of air-dried corn stover was packed into the flow-through type reactor. The preheating coil was filled with $ZnCl_2$ solution using an HPLC pump without heating, and then the pump was turned off. To maintain a system pressure above the saturated pressure of the $ZnCl_2$ solution, the reactor system was filled with 300 psi of nitrogen backpressure. The reactor and preheating coil were preheated to the desired temperature for 15 min by a temperature-programmable GC oven, and the acidified $ZnCl_2$ solution was pumped into the reactor when it reached the target temperature. In this study, the preheating time was not included in the reaction time. The $ZnCl_2$ fractionation was performed in two-steps. First, 5% acidified $ZnCl_2$ at various flow rates (5.0, 7.5, and 10.0 ml/min) was heated to 150 °C for 10 min. Then, there was a separate heating step from 160 to 180 °C for 10 min while maintaining the same flow rate. After the completion of the first step, the temperature was shifted within a 5 min period while keeping the pump running. At the end of the run, the biomass in the flow-through reactor was washed with DI water to remove the residual sugars and $ZnCl_2$. After the completion of the treatment, both remaining solid and liquid hydrolysates were subjected to compositional analysis. Based on the results of this analysis, the furfural production tests were conducted with the fractionated liquid hydrolysates, and the enzymatic digestibility and fermentability were also tested with the residual solids.

2.2.2. Second stage: SSF/SSCF

SSF was conducted using *S. cerevisiae* ATCC® 200062 (NREL-D_5A), and SSCF was performed using recombinant *E. coli* ATCC® 55124 (KO11). Both were combined with the GC-220 cellulase enzyme, the Novo 188 β-glucosidase enzyme, and $ZnCl_2$-treated corn stover. A 250 ml Erlenmeyer flask capped with a rubber stopper and perforated with a syringe needle for venting CO_2 was used as a bioreactor. This