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Optimization of two-stage fractionation process for lignocellulosic biomass using response surface methodology (RSM)

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ABSTRACT

A two-stage process using aqueous ammonia and hot-water has been investigated to fractionate corn stover. To maximize hemicelluloses recovery and purity in the liquid hydrolyzate by optimizing the fractionation process, the experiments were carried out employing response surface methodology (RSM). A central composite design (CCD) was used to evaluate and confirm the effectiveness and interactions of factors. The optimal fractionation conditions were determined to be as follow: (1) First-stage reactor operated in batch mode using a 15% NH_4OH solution ($w_{\text{NH}_3} = 15\%$) at 1:10 solid:liquid ratio, 60 °C, and 24 h; (2) second stage percolation reactor operated using hot-water at 20 $\text{cm}^3 \text{min}^{-1}$, 200 °C, and 10 min.

The model predicted 51.5% xylan recovery yield and 82.4% xylan purity under these conditions. Experiments confirmed the maximum xylan recovery yield and purity were 54.7% and 83.9% respectively under the optimal reaction conditions.

With the solids resulting from the two-stage treatment, 87%–98% glucan digestibilities were obtained with 15 FPU of GC 220 per g-glucan and 30 CBU of Novo 188 per g-glucan enzyme loadings. Xylan digestibility of xylooligomer hydrolysates reached 76% with 8000 GXU per g-xylan of Multifect-Xylanase loading. In the simultaneous saccharification and fermentation (SSF) test using treated solids and *Saccharomyces cerevisiae* (D₅A), 86 % to 98% of ethanol yield was obtained on the basis of the glucan content in the treated solids.

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1. Introduction

Exploiting efficient processes for improving the utilization of lignocellulosic materials has been an issue of great interest in

biotechnology area during the last few decades. Recently, interest has been focused on the production of intermediates using fractionation processes [1–6].

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Fractionation of lignocellulosic materials can be one method to improve overall biomass utilization. When separated, the three main components in biomass: cellulose, hemicelluloses, and lignin, can be utilized in direct applications or precursors for industrial chemicals; for example, (1) fractionated lignin can be directly combusted as a fuel [7], converted to gasoline blending stock [8], used as resources for many value-added products including as activated carbon, binder, dispersant, emulsifier, and sequestrant [9–16]; (2) cellulose can be converted to cellulosic ethanol, or used in food and pharmaceutical applications; and (3) hemicellulose, mainly composed of xylose, can be converted to furfural [17,18], hydrogen [19], succinic acid [20], xylitol [21], and xylooligosaccharides [22].

In our laboratory, two-stage fractionation process using aqueous ammonia and hot-water has been developed to fractionate corn stover effectively into cellulose, hemicelluloses and lignin with high purity [23]. Ammonium hydroxide was used to separate lignin from lignocellulosic biomass in the first stage. We have reported elsewhere that this process effectively removed the lignin without significant loss of cellulose and hemicelluloses, and increase the enzymatic digestibility of the remaining solids [23–25]. The remaining solids after first stage treatment, which contained most of cellulose and hemicellulose, were treated using hot-water in the second stage [23].

In this study, response surface methodology (RSM) was applied to optimize the second stage reaction of the two-stage fractionation process hot-water to maximize xylan recovery yield and hydrolyzate purity. RSM is a statistical tool for designing experiments, building empirical models, and evaluating the effects of factors [26,27]. RSM can reduce the number of experimental trials needed to evaluate multiple parameters and their interactions [28–30]. Several variables of the fractionation process, including reaction temperature of hot-water treatment and flow rate of hot-water treatment, were selected as factors of experimental design by preliminary tests. Optimal reaction conditions of these two factors for maximal xylan recovery yield and purity were determined by central composite design (CCD). For further evaluation of the effectiveness of the optimized reaction conditions, enzymatic digestibility tests for glucan in residual solids and for xylan in xylooligomer hydrolysates were conducted. In addition, the production of ethanol from residual solid after fractionation was tested by simultaneous saccharification and fermentation (SSF) reactions.

2. Methods

2.1. Materials

Air-dried ground corn stover was supplied by the National Renewable Energy Laboratory (NREL, Golden, CO). The corn stover (*Zea mays*) which includes stalks, leaves, tassel, husks, and cobs from Pioneer 34M95 was harvested in Wray, north-eastern Colorado in 2002. The harvested corn stover was washed by distilled water and air-dried at ambient temperature, and then screened to a nominal size of 9–35 mesh. The

prepared corn stover was stored in the refrigerator at 4 °C. The composition of corn stover was determined by our lab following the chemical analysis and testing standard method developed by NREL [31]. The initial composition of the corn stover was 342 g kg⁻¹ glucan, 223 g kg⁻¹ xylan, 16 g kg⁻¹ galactan, 31 g kg⁻¹ arabinan, 122 g kg⁻¹ lignin (acid insoluble + acid soluble), 39 g kg⁻¹ acetate, 62 g kg⁻¹ sucrose, 16 g kg⁻¹ protein, 40 g kg⁻¹ uronic acid, 12 g kg⁻¹ ash, and 107 g kg⁻¹ other extractives.

Cellulase enzyme, GC-220 (Genencor International Inc., Lot No #301-04232-162) and Multifect-Xylanase (Genencor International Inc., Lot. #301-04021-015) were provided by Genencor International. The average activities of cellulase (GC-220) and xylanase (Multifect) were 45 FPU cm⁻³ and 8000 GXU cm⁻³, respectively. The β -glucosidase enzyme, Novozyme 188 (Novo Inc., lot no. 11K1088), was purchased from Sigma–Aldrich (St. Louis, MO). Activity of Novozyme 188 was 750 cellobiase unit (CBU) cm⁻³.

The microorganism used for SSF was *Saccharomyces cerevisiae* ATCC[®] 200062 (NREL-D₅A), which is a SERI strain genetically improved from Red Star baker's yeast. The growth media was YP medium. The mass fractions of yeast extract (Sigma cat. No. Y-0500) and peptone (Sigma cat. No. P-6588) in YP medium were 10 g kg⁻¹ and 20 g kg⁻¹ respectively.

2.2. Experimental setup and operation

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

2.2.1. First stage: aqueous ammonia treatment at low temperature

Corn stover was treated with 15% NH₄OH solution ($w_{\text{NH}_3} = 15\%$) in glass media bottles (Fischer Cat# 06-414-1C) at 60 °C for 24 h. Solid-to-liquid ratio was kept at 1:10. The source of ammonia was 29.5% of ammonium hydroxide (Fisher Cat# A669C). This was diluted to 15% NH₄OH solution ($w_{\text{NH}_3} = 15\%$) with deionized (DI) water and used for the treatment. After the completion of treatment, the solids and liquids were separated by fluted filter paper (Fisher Cat# 09-790-14F), and solids were washed with DI water using vacuum filter until the wash water had a neutral pH. Solid cakes were dried in the air until the moisture content of samples reached approximately 10% (drying conditions: ambient temperature and 48 h–72 h of drying time) and stored in the refrigerator for the second-stage hot water treatment.

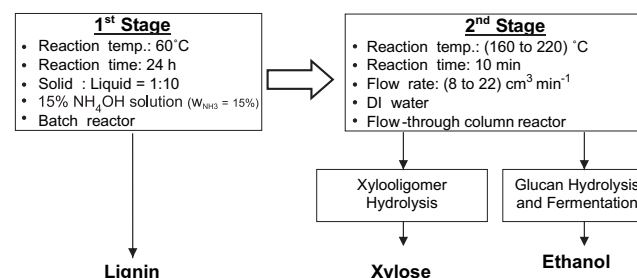


Fig. 1 – Experimental conditions.

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