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Research paper

Process yield and economic trade-offs for enzymatic hydrolysis of alkaline pretreated corn stover

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ABSTRACT

Response surface methodology was used to investigate the interaction of pH, temperature, and enzyme loadings on corn stover hydrolysis rates following soaking in aqueous ammonia pretreatment. Economic tradeoffs were estimated for cellulase and hemicellulase loadings under different hydrolysis conditions. Enzyme loadings had a more significant effect on rates than did pH or temperature. The effect of hydrolysis pH was independent of temperature and enzyme loadings, and the optimal pH for glucose and xylose yields were 4.5 and 4.3, respectively. Conducting hydrolysis at 50 °C rather than 37 °C enables either a 10% glucose yield increase, or a comparable yield with 40% and 65% reduction in cellulase and hemicellulase loadings, respectively. Although yield models showed that hydrolysis rates increase with higher enzyme loadings, economic models showed that optimal cellulase and hemicellulase loadings were as much as 47% and 23% lower, respectively, than the maximum loadings tested. Optimal enzyme loadings change with fluctuations in enzyme costs and ethanol price, but cellulase loadings were more sensitive to these changes than hemicellulase loadings. Enzyme loadings were also more sensitive to enzyme price at lower processing temperatures. Enzyme loadings can be adjusted to increase return based on enzyme costs, ethanol price, and process temperature.

1. Introduction

The enzymatic hydrolysis of cellulosic biomass for producing industrial sugars is a promising strategy for efficient utilization of renewable resources. Cellulase enzymes are the most important for effective enzymatic hydrolysis. Other supplementary enzymes may also be required to aid the hydrolysis depending on the pretreatment technology [1,2]. However, cost reduction by lowering enzyme loading will be critical to make bioindustries more competitive with petrochemical industries [3]. Though enzymes play a key role in the hydrolysis process, other factors such as pH and temperature also affect the overall yields. Therefore, developing a model to demonstrate the interactions of enzymatic hydrolysis factors to improve hydrolysis efficiency will have significant economic benefits.

Factors that affect enzymatic hydrolysis of cellulosic biomass includes the amount and type of substrate, enzyme loadings, and reaction conditions (e.g. pH and temperature). The range of pH for commercially available enzymes varies from 2.5 to 6.5, but enzymatic hydrolysis is typically carried out at a pH of 4.8–5.0 [4]. The optimal hydrolysis temperature varies from 45 to 70 °C depending on the enzyme mixture, but the most commonly used temperature is near 50 °C [4,5]. Enzymatic hydrolysis conditions vary with process configurations such as separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). The temperature for enzymatic hydrolysis can be optimized independently from the fermentation temperature in SHF, whereas compromise is needed for an optimal process temperature in SSF. The use of 37 °C is a favorable condition for SSF, since optimal temperatures for the yeast *Saccharomyces cerevisiae* are usually near 30 °C, and enzymes from *T. reesei* have highest activity at around 45–50 °C [6]. However, there have been few reports of SSF using other strains or organisms with temperature and pH optima closer to those of commercial cellulases. SSF at elevated temperatures were reported for ethanol production using *Candida acidothermophilium* and *Saccharomyces uvarum* [7]. Knowing how hydrolysis proceeds across the range of temperatures and pHs can allow mapping of those yields to microbial growth conditions for

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SSF processes with new organisms.

The primary objective of this study is to model how optimal cellulase and hemicellulase loadings change under a range of hydrolysis conditions (temperature and pH) after alkaline pretreatment. However, processes designed to optimize yield may not generate the greatest economic return. Consequently, we include an economic analysis that combines research-based yield models with select input and output market prices to estimate the most economically efficient processing conditions to compare with conditions based on yield optimization alone. The secondary objective was to determine potential economic tradeoffs between cellulase and hemicellulase enzyme loadings at different hydrolysis conditions.

2. Materials and methods

2.1. Raw material

Corn (*Zea mays* L.) stover (stalks and leaves) was collected from a USDA-ARS research field (46° 48′ 38.51″ N, 100° 54′ 52.53″ W) in Mandan, North Dakota, USA. Corn stover was air dried (10% moisture content, dry basis) and ground in a Wiley Mill with a 6 mm sieve. Sieved corn stover was stored in a sealed plastic bag at room temperature until use.

2.2. Soaking in aqueous ammonia (SAA) pretreatment

Biomass was pretreated by soaking in aqueous ammonia with 15% ammonium hydroxide mass fraction at 40 °C for 24 h. Ground corn stover was pretreated at a solid loading of 100 g L^{-1} in a 2-L, screw-capped Pyrex bottle. The pretreatment bottle was placed in a preheated incubator at 40 °C for 24 h after the mixture reached the desired temperature. The pretreated solids were separated by filtering through Whatman # 41 filter paper (20–25 μm pore size) using a vacuum filtration unit. The solids were washed with distilled water (~4 L), weighed, and stored in sealed plastic bags at 4 °C to use for subsequent enzymatic hydrolysis experiments. The moisture content and solid recovery of pretreated biomass were determined in triplicate by drying a small portion of wet solids (~2 g) overnight in a convection oven at 105 °C. A portion of pretreated wet solids (~20 g) was also dried at room temperature for compositional analysis.

2.3. Compositional analysis for carbohydrate and lignin determination

Total solids content, structural carbohydrate, and lignin content of non-pretreated raw corn stover and SAA-pretreated corn stover were determined using the standard National Renewable Energy Laboratory Analytical Procedures [8]. It is necessary to remove the non-structural carbohydrates such as nitrites/nitrates, proteins, chlorophyll and waxes from the biomass prior to structural carbohydrate analysis to prevent interference with downstream processing. The extractives were removed from the non-pretreated biomass using accelerated solvent extraction following the National Renewable Energy Laboratory (NREL) Chemical Analysis and Testing Procedures [9]. All compositional analysis was done in duplicate.

2.4. Enzymes

In order to investigate the influence of enzyme concentration on the enzymatic hydrolysis of SAA-pretreated corn stover, commercial enzyme solutions, NS50013 (cellulase complex), and Cellic HTec (hemicellulase) were used. A supplementary cellobiase (β-glucosidase) enzyme, Novozyme 188, was also used as cellulase from *T. reesei* is deficient in cellobiase, restricting the conversion of cellobiose to glucose. All enzymes were provided by Novozymes North America, Inc. (Franklinton, NC, USA). According to the manufacturer information sheet, the optimum temperature for cellulase complex NS50013 is in the range of (45–50 °C), β-glucosidase Novozyme 188 in the range of 45–70 °C and xylanase Cellic HTec operating temperature in the range of 45–50 °C. Regarding operating pH, the range for NS50013 is from 4.5 to 6.5, Novozyme 188 is from 2.5 to 6.5, and Cellic HTec operating pH is from 4.5 to 6.0. The cellulase activity of NS50013 and β-glucosidase activity of Novozyme 188, were 77 filter paper units (FPU) cm^{-3} and 500 cellobiase units (CBU) cm^{-3}, respectively, as determined by Ghose [10]. Xylanase activity of Cellic HTec, as determined by Bailey et al. [11] was 10,600 xylanase units (XU) cm^{-3}. No detectable cellulase activity was identified for Novozyme 188 or Cellic HTec. Cellobiase activity of NS50013was 6 CBU cm^{-3}, which only contributed 0.07 CBU per FPU and thus considered negligible in calculations.

2.5. Enzymatic hydrolysis

Enzymatic hydrolysis was carried out with SAA-pretreated corn stover in 0.125 L Erlenmeyer flasks (working volume of 0.05 L) with a 10 g L^{-1} glucan loading. This low loading was chosen to minimize enzyme product inhibition following the National Renewable Energy Laboratory (NREL) Analytical Procedures [12]. Hydrolysis pH was adjusted between 4 and 5.4 with sodium citrate buffer (50 mol m^{-3}) according to the experimental design. Cellulase and hemicellulase were added on a glucan mass basis according to the experimental design. β-glucosidase was added at a constant ratio of cellulase (FPU):β-glucosidase (CBU) at 1:1 across all treatments. Sodium azide was added to the mixture at a concentration of 400 μg L^{-1} to prevent microbial contamination during enzymatic hydrolysis. Flasks were placed in a water bath shaker (MaxQ 7000, Thermo Scientific, Dubuque, IA, USA) and temperature was varied (37–50 °C) based on the experimental design. All flasks were continuously agitated at 2.17 Hz for 72 h. Aliquots (1 cm^3) were taken at 24 h intervals from each flask and immediately centrifuged at 13,226 × g for 5 min (Galaxy 16 Micro-centrifuge, VWR International, Bristol, CT, USA). After centrifugation, the supernatant was filtered through a 0.2 μm nylon filter (Pall Corporation; West Chester, PA, USA) and stored at −20 °C until sugar analysis.

2.6. High performance liquid chromatography (HPLC) analysis

Hydrolysis samples were analyzed by HPLC (Waters Corporation; Milford, MA) equipped with an autosampler, an isocratic pump, and a refractive index detector (model 2414, Waters Corporation). The sugars were analyzed using a Bio-Rad Aminex HPX-87P (300 × 7.8 mm) carbohydrate column (Bio-Rad Laboratories; Hercules, CA) and quantified with column and detector temperatures of 85 °C and 50 °C, respectively. The sugars from the injected sample (20 mm^3) were eluted with 18 mΩ NANOpure water at a flow rate of 0.6 cm^3 min^{-1}. Glucose and xylose were quantified using 4-point external standard curves with mixtures of cellobiose, glucose, xylose, galactose, and arabinose to quantify sugar concentrations in the sample.

2.7. Experimental design and statistical analysis

Response surface modeling (RSM) was used to model the effect of pH, temperature, cellulase and hemicellulase enzyme loadings on SAA-pretreated (15% ammonia at 40 °C for 24 h) corn stover hydrolysis yield using a Box-Behnken design of four variables and