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Effect of anatomical fractionation on the enzymatic hydrolysis of acid and alkaline pretreated corn stover

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ABSTRACT

Due to concerns with biomass collection systems and soil sustainability there are opportunities to investigate the optimal plant fractions to collect for conversion. An ideal feedstock would require a low severity pretreatment to release a maximum amount of sugar during enzymatic hydrolysis. Corn stover fractions were separated manually and analyzed for glucan, xylan, acid soluble lignin, acid insoluble lignin, and ash composition. The stover fractions were also pretreated with either 0%, 0.4%, or 0.8% NaOH for 2 h at room temperature, washed, autoclaved and saccharified. In addition, dilute sulfuric acid pretreated samples underwent simultaneous saccharification and fermentation (SSF) to ethanol. In general, the two pretreatments produced similar trends with cobs, husks, and leaves responding best to the pretreatments, the tops of stalks responding slightly less, and the bottom of the stalks responding the least. For example, corn husks pretreated with 0.8% NaOH released over 90% (standard error of 3.8%) of the available glucan, while only 45% (standard error of 1.1%) of the glucan was produced from identically treated stalk bottoms. Estimates of the theoretical ethanol yield using acid pretreatment followed by SSF were 65% (standard error of 15.9%) for husks and 29% (standard error of 1.8%) for stalk bottoms. This suggests that integration of biomass collection systems to remove sustainable feedstocks could be integrated with the processes within a biorefinery to minimize overall ethanol production costs.

1. Introduction

Lignocellulosic materials represent an underutilized source of fermentable sugars. However, to convert lignocellulosic materials biologically, pretreatment is required to increase the digestibility of cellulose to glucose by enzymes (Mosier et al., 2005). Glucose can subsequently be fermented into ethanol or numerous other chemical building blocks (US Department of Energy, 2004). Hemicellulose can be converted to xylose with pretreatment and enzymatic hydrolysis and the resulting xylose fermented into valuable chemicals if the appropriate microorganism is available.

Pretreatment has been identified as one of the primary cost barriers in the processing of lignocellulosic materials to fermentable sugars (Aden et al., 2002; Lynd et al., 1996). Numerous factors affect the enzyme digestibility of cellulose such as crystallinity, accessible surface area, protection of cellulose by lignin, and numerous other factors (Mosier et al., 2005). It would be expected that biomass type and plant fraction could influence these factors. Hence, plant fractionation could influence the effectiveness of dilute acid or alkaline pretreatment.

Acid pretreatment of biomass has been extensively studied (Mosier et al., 2005). Dilute sulfuric acid hydrolysis has been shown to effectively hydrolyze the hemicellulose and increase the enzyme digestibility of the remaining cellulose (Nguyen et al., 2000; Varga et al., 2002). Dilute acid pretreatment (at pH values of 1.35–1.40) followed by enzymatic hydrolysis of corn stover and cobs has been investigated (Torget et al., 1991). Glucan conversion was as high as 90% (stalks) to 100% (cobs) at a temperature of 160 °C, while using a lower temperature of 140 °C reduced the glucan conversion to 79% (stalks) to 86% (cobs). Similar results were found with grasses and wheat straw.

Alkaline pretreatments result in less sugar degradation than acid pretreatments and yield a similar cellulose conversion level after enzymatic hydrolysis. Corn stover pretreated with 0.075 g $Ca(OH)_2$ per g biomass for 4 h at 120 °C resulted in conversion of 88% of the glucan during enzymatic hydrolysis (Karr and Holtzapple, 2000). Corn stover pretreated with 0.5% NaOH (0.05 g NaOH/g biomass) at 120 °C for 90 min followed by enzymatic hydrolysis resulted in a total sugar concentration of 0.479 g/g biomass (Varga et al., 2002).

The hydrolysis of pith and fiber from corn stalks using acid and alkaline pretreatment has been measured and yielded comparable results (Bootsma and Shanks, 2005). Based on mechanical

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separation techniques used in the pulping industry, they concluded that there were few differences between the pith and fiber. In addition, initial work has been conducted on the alkaline pretreatment and enzymatic hydrolysis of corn stover anatomical fractions (Crofcheck and Montross, 2004). Glucose produced from cobs, leaves and husks, stalks, and whole stover pretreated with 0.8% NaOH (0.06 g NaOH/g biomass) at room temperature for 2 h followed by enzymatic hydrolysis was 0.50, 0.36, 0.28, and 0.36 g glucose/g biomass, respectively. Interestingly, cobs with no pretreatment produced more glucose (0.32 g/g biomass) than pretreated stalks. This indicates that substantial differences existed between the corn stover fractions.

Stover collection systems need to be designed to maintain a balance between biomass production and maintenance of crop residues for sustainable soils. Recent research has indicated that only 20–30% of the crop residue can be removed without deleterious effects on soil carbon levels and increased risk of soil erosion (Wilhelm et al., 2004). Hence, it becomes important to remove the portion of corn stover with the highest fermentable sugar content. Differences in composition and susceptibility to pretreatment significantly impact the industrial suitability of feedstocks.

The objectives of this study were to determine the composition and estimate relative pretreatment requirements of corn stover anatomical fractions (cobs, leaves, husks, stalk below the ear, and stalk above the ear). The susceptibility of the stover fractions to alkaline pretreatment followed by enzymatic hydrolysis and to dilute acid pretreatment followed by simultaneous saccharification and fermentation (SSF) were investigated.

2. Methods

2.1. Sample collection

No-till corn (Pioneer 31R88, Dupont, Des Moines, IA) was planted in a randomized block experiment with five replications, six rows wide and 38 m long. Seeding rate was 66,720/ha, 47 l/ha of 28% liquid nitrogen (17 kg/ha) and 47 l/ha of 10-34-0 starter were applied at planting on May 5, 2003. Herbicides (Spirit (Syngenta, Greensboro, NC) and Basagran (BASF, Florham Park, NJ)) were applied at the label rates on May 23, 2003 for weed control. On June 10, 2003 an additional 373 l/ha of 28% liquid nitrogen (135 kg/ha) was side dressed on each plot.

On September 26, 2003 the plots were hand harvested to determine the quantity of available biomass. A 30 m rope with evenly spaced knots was used to select ten plants from each replication. The rope was placed between the middle two rows, five plants from each row were selected nearest the knot, and were cut above the crown roots. The plant population in the two rows was also determined at this time. The material was brought to the end of the field and sorted manually into husks, leaves, stalk above the ear, and stalk below the ear. The material was weighed and ground using a yard mulcher. Four replications were collected and blended together for further analysis.

2.2. Compositional analysis

All samples were dried at 45 °C and were further ground using a Wiley cutting mill (C.W. Brabender Instruments, South Hackensack, NJ) through a 2 mm circular screen and sealed in plastic bags. Total solids content and carbohydrates were measured following NREL protocols (US Department of Energy, 2006), however, spectroscopic assays using UV/Vis for glucose and xylose were used instead of HPLC due to the large quantity of samples analyzed. Neutralizing the samples with calcium carbonate was omitted because the UV/Vis assays were not affected by the acidity. Glucose and xylose concentrations were converted to the equivalent polymeric concentration (correction of 0.9 for glucose and 0.88 for xylose). Acid soluble lignin, acid insoluble lignin, and ash were measured (US Department of Energy, 2006) and all values are reported on a 0% moisture basis. In addition, samples in triplicate were analyzed for elemental content by the University of Kentucky College of Agriculture Regulatory Services using standard protocols (Peters et al., 2003).

After acid hydrolysis, glucose was measured using a UV/Vis assay (Russell and Baldwin, 1978). A standard curve was constructed with six standards with glucose concentrations between 0 and 25 mg/L. Samples (150 μL) were diluted to fall within the range of the standard curve using 1 mL of buffer (Russell and Baldwin, 1978). The absorbance for each sample was read at 340 nm (Thermo Electron, Spectronic Genesys 2, Waltham, MA) and compared to the standard curve to determine the amount of glucose.

After acid hydrolysis, xylose was measured based on the orcinol-sulfuric acid reaction (Brückner, 1955). A 10% orcinol solution was made by dissolving 0.75 g orcinol in 7.5 mL of ethanol. Six samples were prepared to produce a standard curve with concentrations between 0 and 1 g/L. Samples were diluted to fit within the calibration curve as needed. In a test tube, 25 μL of each sample was added to 150 μL of the 10% orcinol solution and gently mixed. Three milliliters of a ferrous chloride solution in hydrochloric acid (0.09 g $FeCl_3 \cdot 6H_2O$ in 75 mL of concentrated hydrochloric acid (95.5%) and 75 mL of deionized water) was added to each test tube. The test tubes were boiled for 21 min and allowed to cool to room temperature. The samples were read at 600 and 660 nm using the spectrometer and the difference in the readings was used to calculate the xylose concentration based on the standard curve.

2.3. Alkaline pretreatment and enzyme hydrolysis protocol

Each component (3 g (0.1 g ground through a 2 mm circular screen) was placed in a 50 mL centrifuge tube. Samples were pretreated by soaking at room temperature for 2 h in 20 mL of deionized water with 0%, 0.4%, or 0.8% NaOH (0, 0.029, or 0.058 g NaOH/g biomass). The samples were vacuum filtered and then washed with 60 mL of deionized water. The volume of the filtrate was measured to determine the total hydrolysis volume in order to later determine total sugar concentration. The pretreated samples were then transferred to 250 mL Erlenmeyer flasks and autoclaved for 15 min on solid cycle to prepare them for enzyme hydrolysis. The flasks were allowed to cool to room temperature before enzymes were added.

Enzyme hydrolysis was performed using a solution of sodium acetate (0.05 M) buffer that was autoclaved on liquid cycle for 15 min. After the solution cooled, sodium azide (0.35 g/L) was added to control microorganisms and the pH was adjusted to 4.8 using sodium hydroxide or hydrochloric acid. Enzyme loading of 0.75 g/100 mL was used for all experiments (Alltech, Inc., Nicholasville, KY) with a cellulase activity of 10,000 CMCU/g (carboxymethyl cellulose units/g measured at a pH of 4.8 and a temperature of 50 °C) and a xylanase activity of 150,000 XU/g (measured at a pH of 5.3 and a temperature of 50 °C). The cellulase activity in filter paper units was measured as 184 FPU/g (Adney and Baker, 1996). The samples were placed in a shaking incubator (New Brunswick, New Brunswick, NJ) at a temperature of 50 °C for 65 h. Samples (5 mL) were taken at 65 h, placed in a test tube and boiled for 5 min to inactivate the enzymes (Mandels et al., 1996). Samples (1 mL) were placed in micro-centrifuge tubes and centrifuged at 12,000 rpm for 10 min and stored at 4 °C until analysis.

A Thermo-Nicolet Nexus FT-IR 670 spectrometer (Waltham, MA) with a scanning range of 400–4000 cm^{-1}, a spectral resolution of 4 cm, and 128 scans was used to simultaneously measure glucose and xylose in the enzymatically hydrolyzed samples (Crof-