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Short Communication

Effect of dilute acid pretreatment conditions and washing on the production of inhibitors and on recovery of sugars during wheat straw enzymatic hydrolysis

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ABSTRACT

Pretreatment is an essential process to break down recalcitrant biomass and dilute acid hydrolysis is one of the most efficient and cost effective pretreatment technologies available today. However there are potential disadvantages in using dilute acid as a pretreatment, such as the production of degradation products, which inhibits the ensuing processing chain and limits its adoption. In this work, wheat straw was pretreated under varying dilute acid conditions; the resulting degradation products were determined and the quality of sugar stream generated via enzymatic saccharification was monitored. The dilute acid pretreatment conditions were: temperatures of 140 and 160 °C, sulfuric acid concentrations of 5, 10 and 20 $dm^3\ m^{-3}$ and reaction times of 10, 20, 30, 45 and 60 min. Pretreated wheat straw was washed with six dilutions of water and hydrolyzed with commercial cellulase enzymes for 24–48 h. Optimal conditions for pretreating wheat straw were determined as: 140 °C, 10 $dm^3\ m^{-3}$ sulfuric acid concentration and a 30 min reaction time. At these conditions, the glucose yield from wheat straw was maximized at 89% of the theoretical maximum, while the concentrations of formic acid, furfural, acetic acid and 5-hydroxymethylfurfural were 32.37 ± 4.91, 12.08 ± 1.69, 7.98 ± 1.02 and 1.14 ± 0.22 $g\ kg^{-1}$, respectively. Increases in pretreatment severity led to increases in inhibitor generation, as well as a 27% reduction in monosaccharide yield. Rinsing with deionized water was effective in removing inhibitors, such as 86% of furfural. The formation of inhibitors was thus observed to depend on dilute acid pretreatment conditions.

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1. Introduction

The global consumption of crude oil and liquid fuels was 14.18 $hm^3\ day^{-1}$ in 2012, and is projected to grow at an increasing rate in the next two years [1]. Biofuels accounted for only 2% of the total global fuel consumption [2]. Biofuels can be produced from lignocellulosic biomass, such as agricultural residues [3]. Rice, corn, or wheat residues are potential feedstock for the commercial production of lignocellulosic

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biofuel, especially in the Non-OECD (Organization for Economic Cooperation and Development) Asian nations, which are projected to drive the global liquid fuel consumption rate [1]. Wheat occupies the largest cropping area harvested in the world and can supply 865 Tg of dry straw annually, on a sustainable basis [4]. Wheat straw has been used to produce cellulosic ethanol via dilute acid pretreatment and enzyme saccharification, at yields of 240 g kg^{-1} [5].

Briefly, cellulosic ethanol production through the biochemical platform involves pretreating the biomass to loosen plant cell walls, saccharifying pretreated biomass with enzymes, fermenting sugar stream and recovering ethanol [3]. Dilute acid pretreatment is a relatively low cost technology for the enhanced deconstruction of plant cell wall [6,7]. However, this method also leads to the production of inhibitors, like acetic acid, formic acid, levulinic acid, furfural, 5-hydroxymethylfurfural (HMF), which are inhibitory to enzymatic saccharification [8] and to fermentation microorganisms [9]. The produced inhibitors must be removed prior to saccharification and fermentation. Detoxification strategies include treatment with alkali or sulfite, evaporation of initial volume, anion exchange, enzymatic processing, or fungal co-cultivation [10]. Rinsing pretreated biomass with up to nine volumes of water has been shown to alleviate the effect of inhibitors [11]. However, adding this biomass-rinsing step increases water consumption in an already water intensive manufacturing process. Upcoming cellulosic biofuels plants are projected to consume 23–38 m^3 of water per m^3 of ethanol, of which one third will be used directly in processing [12].

In order to promote the manufacturing and consumption of second-generation biofuels, process sustainability needs to be increased, while the cost of production has to be reduced. Therefore water usage, necessary for inhibitor removal, needs to be reduced. Prior studies reported on the presence of inhibitors in biofuel-based wheat straw systems [5,13,14]. However, these studies did not identify dilute acid pretreatment conditions that maximized saccharification, as well as minimized inhibitor generation. In this study, wheat straw was pretreated at combinations of dilute acid concentrations, reaction time and temperature to produce a high quality fermentable sugar stream in which inhibitor concentrations were minimized.

2. Materials and methods

2.1. Chemicals

Glucose, xylose and arabinose standards were purchased from Sigma–Aldrich (St. Louis, MO) and Alfa-Aesar (Ward Hill, MA). Sulfuric acid ACS reagent, 95.0–98.0%, was purchased from EMD Chemicals (Gibbstown, NJ). Calcium carbonate was obtained from Fisher Scientific (Fair Lawn, NJ). Water was prepared with a Direct-Q system (Millipore, Billerica, MA) that had resistivity of 18.2 MΩ. All solvents were of HPLC grade and filtered through 0.2 μm filter assembly (Thermo Fischer Scientific, Nashville, TN). Accellerase® 1500 enzyme, with an endoglucanase activity of 2200–2800 CMC U g^{-1} (Carboxymethylcellulose units) and β-glucosidase activity of 525–775 pNPG U g^{-1} (pNP-glucoside units) was graciously donated by Genencor (Rochester, NY).

2.2. Biomass and compositional analysis

Hard red winter wheat, *Triticum aestivum* L, was grown in Girard, Kansas (longitude 94° 50′ 16″ W and latitude 37° 30′ 40″ N) and harvested in June 2011. Residues, mainly stalks, were assembled into 0.61 m by 0.91 m square bales and kept in a covered outdoor storage facility. Once purchased in January 2012, the wheat straw bale was kept in a 4 °C walk-in refrigerator, until use in summer 2012. The biomass was ground in a Wiley mill and passed through a 20 mesh screen, such that the particle size was in the range of 0.80–0.91 mm [15]. The total solids, extractives, ash, structural carbohydrates, including glucose, xylose, arabinose, and the lignin content of wheat straw were determined as previously reported [16–20].

2.3. Dilute acid pretreatment

Dilute acid pretreatment of wheat straw was conducted at temperatures of 140 or 160 °C; sulfuric acid concentrations of 5, 10 and 20 dm^3 m^{-3} and reaction times of 10, 20, 30, 45 or 60 min. Wheat straw samples mixed with dilute acid solution, at 100 kg m^{-3} solid loading, were loaded in stainless steel tube reactors (length 10 cm, internal diameter 1.4065 cm, thickness 639 μm and total chamber volume of 16 cm^3) and submerged in an industrial fluidized sand bath (Techne Ltd., Burlington, NJ). The sand bath was heated at least one hour prior to the experiments, such that the pretreatments were efficiently conducted at the selected reaction temperatures [21]. After pretreatment, the volume of hydrolyzate was recorded and a portion of acid hydrolyzate was collected in 15 cm^3 centrifuge tubes. The hydrolyzed aliquots were centrifuged (Clinical 200 Large capacity centrifuges, VWR International, Houston, TX) at 2912 × *g* for 180 s in order to separate the solid and liquid fractions. The solid fraction was washed with deionized water, at 167 kg m^{-3} solid loading, for detoxification. Wash water was centrifuged and separated from the solids, at 2912 × *g*, for 600 s. Liquid and wash water fractions were analyzed for the presence of by-products, namely formic acid, acetic acid, furfural and HMF as well as for glucose and xylose.

2.4. Enzymatic hydrolysis

The solid fraction was washed and incubated with cellulase cocktail, Accellerase® 1500 (Genencor, Rochester, NY), at pH 4.8. For each experiment, 1.5 g of solid fraction was loaded in 50 cm^3 amber bottles with 5 cm^3 of sodium citrate buffer (100 mol m^{-3}), 0.5 cm^3 of the enzyme cocktail and 3 cm^3 of deionized water [22]. The mixture was heated to 55 °C in a reciprocating water bath (Thermo Scientific, Nashville, TN), agitated at 1.67 Hz for 48 h. Samples of 1 cm^3 were collected every 24 h and immediately immersed in an ice bath to inactivate the enzyme [23]. Afterward, the samples were centrifuged at 12,100 × *g*, for 60 s (Eppendorf MiniSpin® plus, Sigma–Aldrich, St. Louis, MO) and the liquid enzyme hydrolyzate was separated and stored at 4 °C prior to High Performance Liquid Chromatography (HPLC) analysis.

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