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High temperature dilute phosphoric acid pretreatment of corn stover for furfural and ethanol production

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ABSTRACT

Furfural was produced from corn stover by one stage pretreatment process using dilute H_3PO_4 and solid residues following furfural production were used for ethanol production by *Saccharomyces cerevisiae* NRRL-Y2034. A series of experiments were conducted at varied temperatures (140–200 °C) and acid doses (0.0–2.0%, v/v) in order to determine optimal conditions. The effects of time (5–25 min) and substrate concentration (5–15%, w/w) on furfural production were determined at optimal temperature (200 °C) and acid dose (0.75%, v/v). Maximum furfural yield (10.8 ± 0.3 g/100 g stover) was achieved at 20–25 min duration with 5% (w/w) corn stover which corresponds to 61.6% of the potential yield. About 74% of the glucan content of corn stover was converted to glucose after enzymatic digestion of solid residues. *S. cerevisiae* NRRL-Y2034 fermented glucose from the solid residues efficiently to ethanol. It produced 0.47–0.50 g ethanol per g glucose which corresponds to 92–99% of the theoretical yield.

1. Introduction

Lignocellulosic biomass is the most abundant sustainable feedstock in the world and is considered as a promising source for production of fuels and chemicals to replace fossil fuels (Saha, 2003; Mamman et al., 2008). It consists of mainly cellulose (38–50%), hemicellulose (23–32%) and lignin (15–25%) (Saha, 2004). Due to its heterogeneous structure and recalcitrance, complex and expensive processes are required to convert lignocellulosic biomass to fuels and chemicals (Lange et al., 2012). For the last two decades, many efforts have focused on the hydrolysis of lignocellulosic feedstock into simple sugars for effective utilization of pentose and hexose sugars present in hemicellulose and cellulose, respectively (Dutta et al., 2012). Dilute acid hydrolysis of lignocellulosic biomass at high temperature helps to remove lignin from the structure, and solubilizes and converts hemicellulose into simple sugars (xylose, arabinose, galactose, mannose). The cellulose fraction remains unaltered during acid hydrolysis, however, accessibility of cellulase enzymes increases (Saha et al., 2005; Vazquez et al., 2007). The fractionation of hemicellulose and cellulose allows processing of each fraction separately into fuels and chemicals (Saha, 2003; Dutta et al., 2012).

Acid-catalyzed hydrolysis of the hemicellulosic pentosan fraction of biomass yields xylose (most predominant pentose in most feedstocks) and arabinose which are further converted to furfural (2-furaldehyde, $C_5H_4O_2$) by means cyclodehydration (Rong et al., 2012; Telleria et al., 2011). Antal et al. (1991) showed that dehydration of xylose to furfural is primarily catalyzed by Brønsted acids (donors of H^+), which catalyze formation of a 1,2-enodiol, and then it dehydrates to furfural. During acid treatment, both depolymerization and dehydration occur simultaneously. Furfural is a useful chemical solvent which has been used for separating saturated and unsaturated compounds in petroleum refining, gas, oil and diesel fuel (Mamman et al., 2008). The unsaturated bonds and aldehyde group in the structure make it highly versatile that can be involved in the production of a broad range of industrial chemicals such as plastics, pharmaceuticals and agrochemicals (Rong et al., 2012; Zhang et al., 2012). There is no synthetic route available for production of furfural; hence, it is exclusively produced from hemicellulose (Zhang et al., 2013). Any kind of lignocellulosic biomass containing hemicellulose such as corncobs, cotton seed hulls, oat hulls, bran, saw dust, bagasse, and rice hulls can be used for this purpose (Uppal et al., 2008). Two types of process technology are mainly used for furfural production. In single stage process, pentosans present in lignocellulose are hydrolyzed by acid into sugars (xylose and arabinose) and then

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dehydrated to furfural simultaneously. In two-stage process, the acid hydrolysis of pentosans to pentose sugars occurs under mild conditions, followed by dehydration of the sugars to furfural (Sanchez et al., 2013; Mansilla et al., 1998). This process has the advantage of preventing degradation of lignocellulosic residues. In industrial production, one stage process is preferred because of lower process cost. The remaining solid residue is burned for steam production (Mansilla et al., 1998; Sanchez et al., 2013).

Sulfuric acid is used as catalyst in the current commercial production of furfural (Telleria et al., 2011; Molina et al., 2012). Reaction temperature is maintained by steam injection to the system. Furfural formed during the process is continuously stripped out (Mao et al., 2012). However, this process is not economical due to degradation of furfural to organic acids, aldehydes and condensation products (Gairola and Smirnova, 2012). About 40–50% of cellulosic fraction is also lost by degradation of glucose to HMF, formic acid and levulinic acid. Hydrochloric acid, nitric acid (Yemis and Mazza, 2011), phosphoric (Vazquez et al., 2007) and formic acid (Yang et al., 2012) can also be used for the production of furfural. Phosphoric acid is environmentally friendly and the solid residues can be used as fertilizers. Moreover furfural is less degraded when phosphoric acid is used (Avci et al., 2013). Several other techniques have been proposed for production of furfural including addition of salts ($NaCl, KCl, CaCl_2, MgCl_2, FeCl_2, ZnCl_2$) to enhance conversion of xylose to furfural (Marcotullio and de Jong, 2011; Rong et al., 2012; Yoo et al., 2012; Molina et al., 2012) or for effective stripping of furfural with N_2 or supercritical CO_2 (Tellaria et al., 2011; Gairola and Smirnova, 2012). Some methods are based on prevention of furfural degradation during processing by using solvents such as toluene, n-butanol, methyl isobutyl ketone, dichloromethane and cyclopentylmethyl ether in biphasic system (Amiri et al., 2010; Rong et al., 2012; Lange et al., 2012).

Although there has been much efforts to increase furfural yield from lignocellulosic materials, research on the utilization of residues containing cellulose is limited (Mao et al., 2012). Production of ethanol from lignocellulosic biomass requires effective utilization of pentose and hexose sugars by microorganisms. Native ethanol producers (*Saccharomyces cerevisiae* and *Zymomonas mobilis*) are not able to utilize pentose sugars. Various strains of *Escherichia coli* and *S. cerevisiae* have been modified genetically to produce ethanol and metabolize pentoses to ethanol, respectively (Saha and Cotta, 2012), but challenges remain (Lange et al., 2012).

In this research paper, we report the production of furfural from corn stover in one stage process using dilute H_3PO_4. Our aim was to convert pentosans into furfural at the same time preventing the degradation of cellulose in order to use it for ethanol production. The effects of temperature, acid dose, time, solid concentration on the furfural production from corn stover were determined. To our knowledge it is the first report demonstrating that H_3PO_4 is a better catalyst than H_2SO_4 for furfural production. Further studies have been conducted to investigate fermentability of residual solids to ethanol by using conventional yeast strain (*S. cerevisiae* NRRL-Y2034).

2. Materials and methods

2.1. Materials

Corn stover (92.6 ± 0.2% dry matter) was harvested in the fall of 2011 (Peoria, IL). It was ground in a hammer mill so as to pass through a 1.27 mm screen and stored at ambient temperature in a tightly closed plastic bag. Composition of the corn stover (37.10 ± 0.03% glucan, 21.00 ± 0.10% xylan, 2.10 ± 0.01% galactan, 3.10 ± 0.4% arabinan, 1.80 ± 0.02% acid soluble lignin, 17.10 ± 0.02% acid insoluble lignin and 5.04 ± 0.02% ash on dry basis) was determined by using National Renewable Energy Laboratory Procedures (NREL), Golden, CO (Sluiter et al., 2008a,b).

Celluclast 1.5 L (cellulase) and Novozym 188 (β-glucosidase) were purchased from Brentag Great Lakes (Milwaukee, WI, USA). Fiberzyme (hemicellulase) was provided by Dyadic Corp., Jupiter, FL. Glucose, xylose, arabinose, galactose, H_2SO_4, HNO_3, 2-furfuralaldehyde (furfural, 99%), 5-hydroxymethylfurfural (HMF, 99%) and KOH were purchased from Sigma-Aldrich, St. Louis, MO, USA. H_3PO_4 and $Ca(OH)_2$ were purchased from Fisher Scientific, Pennington, NJ, USA; yeast extract, peptone and agar were from Difco Laboratories, Detroit, MI, USA. The filter sterilization unit (0.2 mm) was purchased from Nalgene Company, Rochester, NY, USA. Aminex HPX 87P column (300 × 78 mm), Aminex HPX 87H column, De-ashing cartridge (30 × 4.6 mm), Carbo-P micro-guard cartridge (30 × 4.6 mm) and Cation H micro-guard cartridge (30 × 4.6 mm) were purchased from Bio-Rad Laboratories Inc., Hercules, CA, USA.

2.2. Dilute acid pretreatment of corn stover

Milled corn stover (5–15%, w/w) was slurried in dilute H_3PO_4 (0.0–2.0%, v/v) and pretreated in a rotating stainless steel reactor with infrared heating (Labomat BFA-12, Mathis USA, Inc., Concord, NC, USA) at 140–200 °C for 5–25 min. Twelve reactors (each 200 mL capacity) were used at one time. The heating and cooling times of the reactors were not considered part of the reported pretreatment time, even though the heat-up ramp took about 49 min (3.6 °C/min) to reach the final temperature at 200 °C. The reactors were water-cooled (6 °C/min) using tap water following pretreatment. The cooling time after pretreatment at 200 °C was about 28 min. The reactors were routinely rotated at 50 rpm with 60-s clockwise followed by 60-s counter clockwise rotations during pretreatment for proper mixing.

Effects of temperature (140, 160, 180 and 200 °C) and H_3PO_4 dose (0.00, 0.25, 0.5, 0.75, 1.0 and 2.0%; v/v) on the pretreatment of corn stover (10%, w/w) for 10 min duration were investigated. Pretreatment of corn stover (10%, w/w) was also performed at 200 °C for 10 min using H_2SO_4 at 200 °C with 0.25, 0.50, 0.75 and 1.0% (v/v) concentrations for comparison. Effects of corn stover solid loading (5–15%, w/w) and pretreatment time (5–25 min) on furfural production were investigated at 200 °C and 0.75% (v/v) H_3PO_4 dose which were determined as the optimum temperature and optimum acid dose, respectively. The pH was adjusted to 5.0 using solid $Ca(OH)_2$ just after each pretreatment. Samples were taken, centrifuged at 25,000 × *g* and analyzed for furfural, HMF, and released sugars.

The yield of furfural was calculated according to the following equation:

$$\text{Furfural}\,(\%) = \frac{\text{Furfural produced}\,(\text{g})}{\text{Xylose} + \text{arabinose in dry corn stover}\,(\text{g})} \times 100$$

2.3. Enzymatic hydrolysis

Enzymatic digestibility of dilute acid pretreated corn stover was performed at pH 5.0 and 45 °C for 72 h by gentle shaking at 135 rpm using a filter sterilized cocktail of 3 commercial enzyme (cellulase, β-glucosidase and hemicellulase) preparations. The enzyme cocktail contained 15 FPU cellulase (Celluclast 1.5 L) and 9 U β-glucosidase (Novozym 188) per g glucan content and 1578 U xylanase (Fiberzyme) per g hemicellulose content, unless otherwise specified. The solid residues were separated from the liquid portion by centrifugation at 25,000 × *g* for 10 min before using the liquid portion as enzymatically saccharified corn stover hydrolyzate. The commercial enzyme preparations contained small