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Enzymatic hydrolysis and ethanol yields of combined surfactant and dilute ammonia treated sugarcane bagasse

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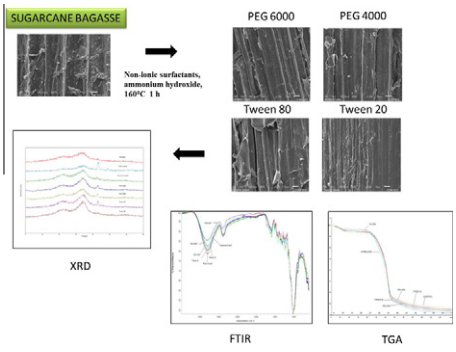
HIGHLIGHTS

- Pretreatment with combined surfactant and ammonia was effective.
- Tween 80–ammonia pretreatment was the most effective.
- Surfactant–ammonia pretreatment altered cellulose crystallinity.
- Significant lignin removal was observed.
- Significant sugar and ethanol yields were observed.

GRAPHICAL ABSTRACT



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ABSTRACT

Tween 80, Tween 20, PEG 4000 or PEG 6000 was used in combination with ammonium hydroxide for the pretreatment of sugarcane bagasse. Pretreatment was carried out by mixing sugarcane bagasse, ammonium hydroxide (28% v/v solution), and water at a ratio of 1:0.5:20, adding 3% (w/w) surfactant based on the weight of dry biomass, and heating the mixture to 160 °C for 1 h. Fibers were hydrolyzed using two concentrations of commercially available enzymes, Spezyme CP and Novozyme 188. The results indicated that PEG 4000 and Tween 80 gave the highest cellulose digestibilities (62%, 66%) and ethanol yields (73%, 69%) as compared to the use of only dilute ammonia (38%, 42%) or water (27%, 26%) as catalysts, respectively. The enhanced digestibilities of non-ionic surfactant–dilute ammonia treated biomass can be attributed to delignification and reduction of cellulose crystallinity as confirmed by FTIR, TGA and XRD analysis.

1. Introduction

Lignocellulosic biomass is considered as a potential resource for the production of second generation ethanol, which can provide enough transportation fuel without threatening the food supply and biodiversity. Cellulose, hemicellulose and lignin are the three main components linked into a carbohydrate complex highly resistant to biochemical conversion. Cellulose and hemicellulose must be broken down into monomeric sugars (fermentable sugars) before their fermentation into ethanol (Aita et al., 2011). This process can be summarized into four steps: (1) pretreatment, to break down the lignin–carbohydrate complex into cellulose, hemicellulose and lignin; (2) enzyme hydrolysis, to convert cellulose and hemicellulose into hexose and pentose sugars; (3) fermentation, to ferment hexose and/or pentose sugars to ethanol; and (4) ethanol recovery (Aita et al., 2011). Of these four steps, pretreatment is a key step because it helps breakdown the highly complex lignin–carbohydrate structure. An effective pretreatment can lower the downstream unit operation cost (Yang and Wyman, 2008).

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Pretreatment with caustic is applied to disrupt the structure of lignin and to increase the susceptibility of enzyme during hydrolysis. Caustic alters the lignin–carbohydrate complex in biomass by effectively disrupting the ester bonds between lignin and hemicellulose, commonly found in grasses, thus allowing for a great portion of hemicellulose to be selectively removed. Similarly, the lignin and cellulose complex is also altered causing the β-1,4-glucosidic bonds in cellulose to be more susceptible to enzymatic hydrolysis (Salvi et al., 2010). Several pretreatment methods have been developed (i.e., biological, acid, alkaline, ionic liquid), but most of these methods suffer from relatively low sugar yields, high process costs or great investment risks (Yang and Wyman, 2008). Dilute ammonia pretreatment has shown great success in delignification of grassy feedstocks by cleaving the C–O–C bonds and other ether and ester bonds present in the lignin–carbohydrate complex (Aita et al., 2011). Dilute ammonia is effective in lignin removal, exhibiting minor cellulose and hemicellulose solubilization than acid or hydrothermal processes (Carvalheiro et al., 2008). Alkaline pretreatment improves cellulose digestibility by increasing lignin removal thus improving enzyme hydrolysis and fermentation yields by decreasing the non-productive binding of lignin to enzymes (Eriksson et al., 2002; Qing et al., 2010). Aqueous ammonia has higher selectivity than other alkaline salts, it is non-polluting and non-corrosive, recoverable and widely use, therefore making it a valuable pretreatment additive (Kim et al., 2003).

Recently, several studies have indicated that the addition of non-ionic surfactants during pretreatment can enhance delignification and improve enzyme hydrolysis (Börjesson et al., 2007; Kurakake et al., 1994; Qi et al., 2010; Qing et al., 2010). Surfactants have both hydrophobic and hydrophilic properties that can decrease surface tension to help remove hydrophobic compounds (Escalante et al., 2005). It was reported that surfactants successively removed hydrophobic degradation products from lignin and hemicellulose thus enhancing delignification during pretreatment (Kurakake et al., 1994). Several mechanisms have been developed to explain the effect of surfactants on enzymes. Surfactants can enhance enzymatic digestibility by (1) changing the substrate structure to make it more accessible to enzymes (Kaar and Holtzapple, 1998); (2) stabilizing enzymes to prevent denaturation (Kaar and Holtzapple, 1998); (3) increasing positive interactions between substrates and enzymes (Eriksson et al., 2002; Kaar and Holtzapple, 1998); and (4) reducing enzyme non-productive binding to lignin and other molecules involved in cellulase activity (Eriksson et al., 2002; Qing et al., 2010). However, a mechanism that can consistently explain how surfactants improve enzymatic hydrolysis has yet to be developed. Non-ionic surfactants such as Tween 20, Tween 80, PEG 4000, and PEG 6000 have been found to be effective in reducing the amount of lignin remaining in the pretreated material and in accelerating the enzymatic hydrolysis by increasing cellulose accessibility (Börjesson et al., 2007). Kurakake et al. (1994) reported that surfactants having high HLB (hydrophile–lipophile balance) values were useful for the extraction of hydrophobic degradation products from lignin and hemicellulose (Kurakake et al., 1994). Tween 20 (HLB, 16.7), Tween 80 (HLB, 15.0), PEG 4000 (HLB, 18.5), and PEG 6000 (HLB, 19.0) are four non-ionic surfactants with high HLB values with potential use as catalysts during pretreatment and enzymatic hydrolysis.

The goal of this study was to investigate the combined effect of non-ionic surfactants (Tween 80, Tween 20, PEG 6000 or PEG 4000) and dilute ammonia on the pretreatment for sugarcane bagasse in terms of changes in biomass chemical composition, cellulose digestibility and ethanol yield as compared to pretreatment with dilute ammonia only.

2. Methods

2.1. Substrate

Sugarcane bagasse was collected from Louisiana sugar mills during the grinding season (September through December, 2011) and stored in 50 gal drums at −20 °C. Moisture content of sugarcane bagasse averaged 50% with particle size ranging from 0.050 to 1.5 cm.

2.2. Non-ionic surfactant–dilute ammonia pretreatment

Tween 80 (Sigma–Aldrich, St. Luis, MO, USA), Tween 20 (Sigma–Aldrich, St. Luis, MO, USA), PEG 4000 (Sigma–Aldrich, St. Luis, MO, USA) or PEG 6000 (Sigma–Aldrich, St. Luis, MO, USA) was used in combination with ammonium hydroxide for the pretreatment of sugarcane bagasse. The pretreatment was carried out by mixing sugarcane bagasse, ammonium hydroxide (28% v/v solution), and water at a ratio of 1:0.5:20; adding 3% (w/w) surfactant based on the weight of dry biomass, and heating the mixture to 160 °C for 1 h. The final ammonium hydroxide concentration was 0.65% w/w at 4.7% solids loading. The ratios and surfactant concentrations used in this study were determined based on previously published data (Aita et al., 2011; Salvi et al., 2010; Qing et al., 2010). Untreated, water treated and dilute ammonia treated sugarcane bagasse were used as controls. Liquid was drained from the reactor post pretreatment. The biomass was carefully collected and pressed to remove excess liquid. Pretreated materials were stored at −20 °C with a moisture content of less than 20%.

2.3. Chemical composition of sugarcane bagasse

Surfactant–dilute ammonia treated sugarcane bagasse and controls (untreated, water treated and dilute ammonia treated sugarcane bagasse) were analyzed for glucan, xylan, lignin, arabinan, mannan, ethanol extractives, and ash content using NREL's Laboratory Analytical Procedures (LAPs #42618, 42619, 42620, 42621, 42622). NREL reference material (8491 sugarcane bagasse) was analyzed as an internal sample to ensure the accuracy of the procedures.

2.4. Enzyme hydrolysis and fermentation

Surfactant–dilute ammonia treated, dilute ammonia treated, water treated, and untreated sugarcane bagasse at 10% solids loading were hydrolyzed using a combination of commercially available cellulose degrading enzymes, Spezyme CP (Genencor, Danisco US Inc., Rochester, NY, USA) and Novozyme 188 (Sigma–Aldrich, Inc., St. Luis, MO, USA). Spezyme CP (cellulases) and Novozyme 188 (cellobiases) were used at 15 FPU/g glucan, and at 15 CBU/g glucan (half strength), respectively. A second test at a higher enzyme loading of 30 FPU Spezyme CP and 30 CBU Novozyme 188/g glucan (full strength) was conducted to asses any improvements in both hydrolysis and fermentation yields. Approximately, 10 g dry weight (DW) of surfactant–dilute ammonia treated, dilute ammonia treated, water treated, and untreated bagasse were each loaded into 250 ml Erlenmeyer flasks following NREL's hydrolysis and fermentation protocols (LAP TP-510-42630). Additionally, 1 g yeast extract (Becton Dickinson and Company, Sparks, MD, USA), 2 g peptone (Becton Dickinson and Company, Sparks, MD, USA), 5 g citrate buffer (1 M stock solution, pH 4.8), and water were added to each flask to a final weight of 100 g. The pH of each mixture was adjusted to 4.8 with concentrated hydrochloric acid. All flasks were sterilized at 121 °C for 30 min. Enzymes were added