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Effect of high dry solids loading on enzymatic hydrolysis of acid bisulfite pretreated Eastern redcedar

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HIGHLIGHTS

- Eastern redcedar was enzymatically hydrolyzed at 2–20% solids loading.
- More than 100 g/L fermentable glucose was achieved at 16% and 20% solids loading.
- Addition of metal balls during hydrolysis helped overcome rheological challenges.
- Separate high solids hydrolysis and fermentation produced 52 g/L of ethanol.
- An enzyme loading of 46 FPU/g glucan was required to produce high glucose titers.

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ABSTRACT

This study investigates hydrolysis of cellulose from Eastern redcedar to glucose at high solids loading. Enzymatic hydrolysis of pretreated redcedar was performed with 0.5 ml Accelerase® 1500/g glucan (46 FPU/g glucan) using dry solids loading from 2% to 20% (w/w). Rheological challenges observed at high solids loading were overcome by adding stainless steel balls to shake flask reactors. The highest glucose concentration, 126 g/L (84% glucan-to-glucose yield), was obtained using 20% solids loading with stainless steel balls as a mixing aid. This enzymatic hydrolyzate was fermented into ethanol using *Saccharomyces cerevisiae* D_5A to produce 52 g/L of ethanol (corresponding to 166 L/dry Mg of redcedar). Reducing enzyme dosage at 16% solids loading from 46 to 11.5 FPU/g glucan reduced glucan-to-glucose yields. This study has demonstrated the possibility of extracting sugars from the invasive species of Eastern redcedar with high solid loadings and their conversion into ethanol.

1. Introduction

Eastern redcedar (*Juniperus virginiana* L.) (hereafter referred to as redcedar) is an invasive softwood species spreading at an alarming rate in the central US plains. Approximately 2 billion L (530 million gallons) of ethanol could be produced from redcedar wood from just 17 counties in Northwest Oklahoma (Ramachandriya et al., 2013). A previous study identified near optimal pretreatment conditions for pretreating redcedar using sulfuric acid and sodium bisulfite and achieved 87% overall wood glucan-to-glucose yield (Ramachandriya et al., 2013); however, the enzymatic hydrolysis of pretreated redcedar was carried out at low (2% w/w dry basis) solids loading. Ideally, a bio-refinery utilizing redcedar as a feedstock would operate at high solids (substrate) loading to increase product concentrations and decrease capital and operating costs (Jørgensen et al., 2007; Kristensen et al., 2009). However, challenges such as increased viscosity resulting in mass transfer limitations and mixing difficulties and inhibition from toxic products such as fermentation inhibitors and lignin are common to operations at high solids loading (Alvira et al., 2013; Jørgensen et al., 2007; Roche et al., 2009).

In order to overcome the technical barriers for using lignocellulosic biomass at high solids loading, new bioreactor designs and strategies have been employed. Novel bioreactor designs such as laboratory-scale roller bottle reactors (RBRs) (Roche et al., 2009), bench scale helical stirring bioreactors (Zhang et al., 2010), horizontal five chambered liquefaction reactors (Jørgensen et al., 2007) and laboratory scale peg mixers (Zhang et al., 2009) have been developed and validated; however, shake flask studies are still the most common method of evaluating biomass digestion (Roche et al., 2009). Furthermore, novel strategies such as prehydrolysis and fed-batch operation of simultaneous saccharification

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and fermentations (SSFs) have also been demonstrated (Hoyer et al., 2013; Lan et al., 2013; Pessani et al., 2011). Prehydrolysis is carried out by liquefying lignocellulosic biomass at the optimum temperature for enzymatic hydrolysis for a defined time followed by addition of yeast or bacteria. In a fed-batch operation, fresh substrate is added after the viscosity of lignocellulosic biomass decreases. Most studies conducted with high solids loading are carried out in conjunction with fermentations (Hoyer et al., 2013; Zhang et al., 2010; Zhu et al., 2011) to avoid end-product inhibition of enzymes due to glucose and cellobiose (Xiao et al., 2004). However, a major drawback of SSF from a commercial standpoint is the inability to recirculate fermenting microorganisms for continuous operation since the organisms are mixed with biomass (Ishola et al., 2013; Olofsson et al., 2008). Recently, Ishola et al. (2013) showed continuous operation of a simultaneous saccharification, filtration and fermentation (SSFF) layout with 14.4% (w/w) suspended pretreated spruce for 4 weeks with 85% of theoretical ethanol yield. The development of continuous operating schemes such as SSFF will require enzymatic hydrolysis to be performed at high solids loading.

The objective of the present study was to determine the effect of solids loading on enzymatic hydrolysis of pretreated redcedar between 2% and 20% dry solids (herein all dry solids loading represented as % refers to dry solids on a w/w basis) as measured by glucose concentration produced and glucan-to-glucose yield of pretreated redcedar. Since technical challenges with mixing are common to hydrolysis of pretreated biomass above 15% dry solids (Alvira et al., 2013), enzymatic hydrolysis of pretreated redcedar above 15% were conducted both in the presence and absence of stainless steel balls that were used as a mixing aid. The effect of lowering enzyme dosage at high solids loading was also studied. Additionally, the fermentability of enzymatic hydrolyzate that was obtained at 20% dry solids loading was tested.

2. Methods

2.1. Biomass characterization

Eastern redcedar (*J. virginiana* L.) chips were acquired from the Oklahoma State Forestry Services (Idabel, McCurtain County, OK, USA). The chips contained both heartwood and sapwood fractions of the trunk from redcedar trees. The biomass was ground using a Thomas-Wiley mill (Arthur H. Thomas Co., Philadelphia, PA, USA) equipped with a 2 mm screen. After grinding, the moisture content of the biomass was determined by a convection oven method (Sluiter et al., 2008a). Biomass was stored in resealable bags at room temperature prior to pretreatments and/or compositional analysis.

For compositional analysis, biomass was sieved through Tyler number +9/+60 sieve plates. The samples that were collected on the +60 sieve plate were used for compositional analysis. About 80% of the ground biomass was retained on the +60 sieve plate and the remaining portion was fines. Sieving of biomass was important because the NREL protocols for compositional analysis were developed for particle size between 180 and 850 μm (Hames et al., 2008). Ethanol extraction of sieved redcedar was then carried out using an accelerated solvent extractor (ASE) (Model 300, Dionex Corporation, Sunnyvale, CA, USA) to remove non-structural material using National Renewable Energy Laboratory (NREL) protocols (Sluiter et al., 2008d). The amount of extractives (on a percent dry weight basis) was calculated directly by evaporating ethanol at room temperature in a fume hood and measuring the residual mass.

Following extraction, the biomass was air dried and was analyzed for structural carbohydrates and lignin using a two-step acid hydrolysis procedure developed by NREL (Sluiter et al., 2008c). Structural carbohydrates were analyzed using HPLC (Model 1100, Agilent Technologies, Santa Clara, CA, USA) with a refractive index detector (RID) and a Bio-Rad Aminex HPX-87P column (Bio-Rad, Sunnyvale, CA, USA). Deionized water was used as an eluent at a flow rate of 0.6 ml/min and the column temperature was maintained at 85 °C. The total run time for each sample was 30 min. The HPLC with Chemstation software (Agilent Technologies) was calibrated at five levels using a known concentration of cellobiose, glucose, xylose, galactose, arabinose and mannose, before being used to quantitate the concentration of these compounds. Acid soluble lignin (ASL) content of biomass was determined using a UV–vis spectrophotometer (Cary 50 Bio, Varian Inc., Palo Alto, CA, USA) at a wavelength of 205 nm and an extinction coefficient of 110 L/g cm (Sluiter et al., 2008c). Acid insoluble lignin (AIL) was determined gravimetrically (Sluiter et al., 2008c).

2.2. Pretreatments

Acid bisulfite pretreatments were done in a 1-L bench top pressure reactor (Parr series 4250, Parr Instrument Company, Moline, IL, USA) equipped with an agitator, a heater and a control unit. The reactor was initially loaded with 100 g of dry biomass and then filled with a mass of pretreatment liquor to achieve a liquid-to-solid mass ratio of 5:1. The pretreatment liquor was composed of deionized water, sulfuric acid and sodium bisulfite. Sulfuric acid and sodium bisulfite loadings were 3.75 g/100 g dry wood and 20 g/100 g dry wood, respectively. The reactor was agitated at 150 rpm and biomass was soaked in the pretreatment liquor at 90 °C for 3 h. At the end of 3 h, the reactor temperature was increased to 200 °C and held for 10 min. These pretreatment conditions were identified as near-optimal in a previous study (Ramachandriya et al., 2013). At the end of the pretreatment hold time, the reactor was cooled in an ice bath until the temperature was less than 55 °C. After cooling the reactor, the solid and liquid fractions were separated using vacuum filtration through a Whatman #4 filter paper. About 5–6 g of sample were taken after filtration and dried in an oven at 105 °C to determine the moisture content of wet solids after pretreatment (Sluiter et al., 2008a). The remaining wet solids were then rinsed with 500 ml of deionized water at 60 °C four times to remove soluble sugars and fermentation inhibitors. The moisture content of washed pretreated solids was also determined using a standard NREL procedure (Sluiter et al., 2008a). Pretreated solids were then stored in plastic resealable bags at 4 °C until use for enzymatic hydrolysis. The compositions of pretreated solids and prehydrolyzate were determined using protocols developed by NREL (Sluiter et al., 2008b,c).

2.3. Effect of high solids loading

The first batch of redcedar with composition shown in Table 1 was used for this study. Accelerase® 1500 was generously provided by Genencor Inc. (Palo Alto, CA, USA) and it was the enzyme cocktail used for this study. An enzyme loading of 0.5 ml/g glucan was used for these studies. This loading was recommended by the manufacturer as a starting point for optimization. Enzymatic hydrolysis was done at pH 5 using 0.05 M sodium citrate buffer and 50 °C in an incubator shaker (MaxQ 4450, Thermo Scientific, Dubuque, IA, USA) at 250 rpm. The experiments were carried out in 250 ml baffled flasks containing a total mass of 100 g. The cellulase activity of Accelerase® 1500 was determined as 92 FPU/ml using a standard protocol developed by NREL (Adney and Baker, 2008). Analytical grade chemicals required for the enzyme assay were purchased from Sigma–Aldrich (St. Louis, MO, USA).

The different dry solids loading levels were 2%, 4%, 8%, 12%, 16% and 20%. Additionally, the effect of mixing aid was also determined at 16% and 20% dry solids loading. Twenty stainless steel metal