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Development of an efficient pretreatment process for enzymatic saccharification of Eastern redcedar

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HIGHLIGHTS

- Hemicellulose and lignin removal occurred during acid bisulfite pretreatments.
- Delignification was important for improving glucose-to-glucan yields.
- Delignification of redcedar was increased by increasing sodium bisulfite loadings.
- Highest overall theoretical yield of wood glucan-to-glucose was 87%.

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ABSTRACT

This study investigates the potential for extracting sugars from the polysaccharides of Eastern redcedar. Pretreatment temperature, time, sulfuric acid loading, sodium bisulfite loading and impregnation time were varied using factorial treatment design experiments for identifying near optimal overall wood glucan-to-glucose yields during acid bisulfite pretreatments. The highest overall wood glucan-to-glucose yield of 87% was achieved when redcedar was impregnated with pretreatment liquor containing 3.75 g of sulfuric acid/100 g of dry wood and 20 g of sodium bisulfite/100 g of dry wood at 90 °C for 3 h followed by increasing the temperature to 200 °C with a hold time of 10 min. Hemicellulose and lignin removal during pretreatments made the substrate amenable to enzymatic hydrolysis using 0.5 ml of Accelerase® 1500/g of glucan at 2% (w/w) solid loading. Preliminary mass balances showed 97% glucan recovery at pretreatment condition with 87% overall wood glucan-to-glucose yield and 59% delignification.

1. Introduction

Eastern redcedar (*Juniperus viriginiana* L.) is a member of the cypress family (*Cupressaceae*) and is one of the most widely distributed conifers in the US. It is commonly found in central and eastern US. Although generally referred to as cedar, it is actually one of 13 juniper species in the US (Hiziroglu et al., 2002). Eastern redcedar (hereafter referred to as redcedar) is considered a very invasive species as it adapts well to different soils, climactic conditions and topographies (Hiziroglu et al., 2002). Redcedar's encroachment in the Great Plains of the US is a very serious problem. Between 1985 and 2015, a 231% increase in redcedar acreage is estimated in Oklahoma (McKinley, 2012). Recent studies show that redcedars are spreading at a rate of 57 trees per hectare per year in the prairie lands of Kansas (Price et al., 2010) and at a rate of 121,000 hectares per year in the plains of Oklahoma (McKinley, 2012). According to an estimate made by McKinley (2012), 26% of the overall land base of Oklahoma will be covered with redcedars by 2015. The encroachment of redcedars has brought many ecological concerns to farmers, ranchers and wildlife species, reduced ground water yields and an increased risk of wildfires, which resulted in an estimated loss of $447 million in 2012 in Oklahoma (National Resources Conservation Service, 2012).

Common control strategies for the spread of redcedars in Oklahoma are prescribed fires, application of pesticides and mechanical clearing. Mechanical clearing of redcedar, although highly encouraged due to its selectivity, is cost intensive unless a valuable use for the wood can be identified that can use low quality wood not suitable for lumber. Processing units utilizing redcedar wood for oil extraction are available, but they have difficulties using the wood

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after oil extraction. Mulch application of oil extracted redcedar wood will be restricted as it will no longer have the ability to deter pests due to the loss of aromatic oil. A viable option that has not been focused on is the conversion of polysaccharides in redcedar into transportation fuels such as ethanol and butanol. Such a conversion process will provide the flexibility to use woody biomass of any quality and can make use of redcedar wood after oil extraction.

Redcedar encroachment has resulted in the availability of enormous amounts of redcedar wood across the Great Plains. A recently published report estimated the availability of 11.5 million dry metric tons of above ground redcedar biomass in just 17 counties in Northwest Oklahoma (Starks et al., 2011). Assuming, 80% of the above ground biomass is wood and 75% of the glucan in redcedar can be converted into ethanol (McKinley, 2012) 2 billion L (530 million gallons) of ethanol can be produced from existing redcedar in this small region. When the geographical distribution of redcedar across the US is taken into account, redcedars can easily become a promising source for cellulosic biofuels.

Redcedar is a softwood and generally softwood species are more difficult candidates for bioconversion processes to produce biofuels than hardwoods and agricultural residues because of their rigid structure and high lignin content (Ramos, 2003). Redcedar contains on a dry basis 40.3 ± 1.5% glucan, 8.5 ± 0.0% xylan, 2.0 ± 0.6% galactan, 1.4 ± 1.0% arabinan, 6.0 ± 1.2% mannan and 33.7 ± 0.6% lignin (mean ± 1 standard deviation) (Pasangulapati et al., 2012). The lignin content of redcedar is 5–25% higher than other softwoods investigated for ethanol production such as spruce, Douglas fir and pine (Zhu and Pan, 2010).

Pretreatment of lignocellulosic biomass is the first step in the biochemical production of ethanol where the biomass is converted to a form amenable to enzymatic hydrolysis and fermentation. The complex physical and chemical nature of softwoods limits the number of pretreatment options available. Therefore, the selection of a pretreatment process is critical due to the differences in the physical and chemical modes of action during different pretreatment technologies. Hot water pretreatment and alkaline pretreatment methods based on ammonia, such as ammonia recycle percolation (ARP), soaking in aqueous ammonia (SAA) and ammonia fiber expansion (AFEX), have not shown success in achieving high glucose yields after enzymatic hydrolysis of pretreated softwood (Ramos, 2003). Acidic pretreatments such as dilute acid pretreatments (Nguyen et al., 1998), steam explosion pretreatments assisted with acids (Kumar et al., 2010) and sulfite pretreatment to overcome the recalcitrance of lignocellulose (SPORL) (Shuai et al., 2010; Zhu and Pan, 2010) have been relatively more successful than hot water and ammonia pretreatments in achieving high glucose yields from softwood.

SPORL is a recently studied technology for softwood pretreatments (Zhu et al., 2009). It is a variant of sulfite pulping that was used to produce pulp and paper from woody biomass. Bisulfite salt (made of Na^+, NH^{4+}, Mg^{2+}, K^+ or Ca^{2+}) and sulfuric acid are the two chemicals required for this process. These chemicals play an important role in achieving delignification using sulfonation reactions resulting in a lignosulfonate rich prehydrolysate (Bryce, 1980). The presence of sulfuric acid also results in a significant removal of hemicellulose (Bryce, 1980; Zhu et al., 2009). Numerous studies have shown the effectiveness of SPORL with softwoods (Shuai et al., 2010; Zhu and Pan, 2010; Zhu et al., 2009).

The current study reports on a modified SPORL process for redcedar. Unlike previous studies using SPORL, finely ground biomass screened with a 2 mm screen was used in the present work as mechanical size reduction enhances biomass digestibility (Sun and Cheng, 2002). Pretreatment affects subsequent processes and hence its optimization is the first and most important step. Optimization experiments are generally sequential in nature and begin with screening experiments that aim to identify the more important factors affecting a process, while eliminating the less important ones (Myers and Montgomery, 1995). These screening studies are often referred as phase zero of optimization experiments (Myers and Montgomery, 1995). The next phase (phase I) of process optimization is referred to as the path of steepest ascent. During this phase, levels of factors are adjusted such that near optimum responses are obtained (Myers and Montgomery, 1995). The objective of this study was to determine the near optimal pretreatment conditions for the maximum wood glucan-to-glucose yield from redcedar. Factors were identified that affect the acid bisulfite process, which were pretreatment time, pretreatment temperature, sulfuric acid loading (g/100 g of dry wood), sodium bisulfite loading (g/100 g of dry wood) and impregnation time. These factors were varied sequentially using factorial treatment designs to identify the factor levels that result in the greatest yield of glucose from enzymatic hydrolysis of pretreated redcedar.

2. Methods

2.1. Biomass

Eastern redcedar (*Juniperus virginiana* L.) chips were acquired from a local manufacturer in Oklahoma. The chips contained both heartwood and softwood fractions of the trunk from redcedar trees that were 20–25 years. The biomass was ground using a Thomas-Wiley mill (Arthur H. Thomas Co., Philadelphia, PA) 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 zip-lock bags at room temperature prior to pretreatments and/or compositional analysis. The standard procedure developed by the National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008c) was used for compositional analysis of redcedar and is detailed in Pasangulapati et al. (2012).

2.2. Pretreatments

Acid bisulfite pretreatments were done in a 1-L bench top pressure reactor (Parr series 4250, Parr Instrument Company, Moline, IL) 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 resulting in a total mass of 600 g in the reactor. The pretreatment liquor was composed of deionized water, sulfuric acid and/or sodium bisulfite. The concentrations of these chemicals were varied for different factorial design experiments. The range of sulfuric acid loadings and sodium bisulfite loadings varied between 0.00 and 5.00 g/100 g of dry wood and 0 and 20 g/100 g of dry wood, respectively. The reactor was agitated at 150 rpm and biomass was soaked at 90 °C for 3 h for all studies except the preliminary screening study on chemical loading. This soaking process is commonly referred as impregnation, which is commonly used in the pulping process (Bryce, 1980). Impregnation allows sufficient time for the diffusion of chemicals to different parts of the wood for delignification (Bryce, 1980). At the end of 3 h, the reactor temperature was increased to a desired set point and held at that temperature for a desired time. In this study, different time–temperature combinations were investigated. Temperature was varied between 180 and 220 °C and hold time was varied between 5 and 40 min. At the end of pretreatment hold time, the reactor was cooled in an ice bath until the temperature was less than 55 °C. For the study on hold time, temperature and bleed-out (Section 3.2), steam and other vapors were bled-out through a check valve to reduce reactor pressure and then the reactor was cooled in an ice bath. After cooling the reactor, the solid and liquid fractions were separated using vacuum filtration through a Whatman #5 filter paper. About 5–6 g of sample