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Using sulfite chemistry for robust bioconversion of Douglas-fir forest residue to bioethanol at high titer and lignosulfonate: A pilot-scale evaluation [☆]



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HIGHLIGHTS

- High ethanol yield of 284 L/tonne from forest residue at 42 g/L by SPORL.
- Enzymatic hydrolysis and fermentation of non-detoxified pretreated whole slurry.
- Low cellulase loading of 30 mL/kg forest residue.
- Lignosulfonate of equivalent properties of commercial product directly marketable.

GRAPHICAL ABSTRACT



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ABSTRACT

This study demonstrated at the pilot-scale (50 kg) use of Douglas-fir forest harvest residue, an underutilized forest biomass, for the production of high titer and high yield bioethanol using sulfite chemistry without solid–liquid separation and detoxification. Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses (SPORL) was directly applied to the ground forest harvest residue with no further mechanical size reduction, at a low temperature of 145 °C and calcium bisulfite or total SO₂ loadings of only 6.5 or 6.6 wt% on oven dry forest residue, respectively. The low temperature pretreatment facilitated high solids fermentation of the un-detoxified pretreated whole slurry. An ethanol yield of 282 L/tonne, equivalent to 70% theoretical, with a titer of 42 g/L was achieved. SPORL solubilized approximately 45% of the wood lignin as directly marketable lignosulfonate with properties equivalent to or better than a commercial lignosulfonate, important to improve the economics of biofuel production.

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1. Introduction

Forest residues from logging operations are underutilized and have significant advantages over herbaceous biomass for producing biofuel and bioproducts. Their relatively high bulk density reduces transportation cost, and flexible harvesting schedules

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eliminate long term storage needs (Zhu and Pan, 2010). Forest residues are one of the most affordable feedstocks (National Research Council, 2011) and can be sustainably produced in large quantities in various regions of the world (Gan and Smith, 2006; Perlack and Stokes, 2011). However, forest residues tend to have high lignin content due to the presence of bark and juvenile wood from the tree tops and branches (Yamamoto et al., 2014; Zhang et al., 2012). As a result, they have very strong recalcitrance to bioconversion to sugars (Leu et al., 2013; Yamamoto et al., 2014), especially those from softwoods such as the Douglas-fir (*Pseudotsuga menziesii*) forest harvest residue, as is studied here. Few processes are capable of removing this strong recalcitrance for efficient sugar/biofuel production despite that many research efforts have been devoted to woody biomass pretreatment (Iakovlev & van Heiningen, 2012; Monavari et al., 2010; Pan et al., 2007).

Sulfite chemistry is known for efficient depolymerization of carbohydrates and excellent delignification even under acidic conditions which further facilitates dissolution of hemicelluloses (Bryce, 1980). Both characteristics are very suitable for deconstruction of lignocelluloses (Leu and Zhu, 2013). The dissolved lignin can serve as a surfactant to substantially enhance enzymatic saccharification (Lou et al., 2014; Wang et al., 2013; Zhou et al., 2013a). Furthermore, the retained lignin on the solid substrate is sulfonated lowering its affinity to cellulase and substantially reducing nonproductive cellulase binding (Lou et al., 2013). Sulfite Pretreatment to Overcome the Recalcitrance of Lignocelluloses (SPORL) (Zhu et al., 2009) utilizes these advantages of sulfite chemistry and has achieved robust performance for woody biomass bioconversion to sugar and bioethanol (Wang et al., 2009; Zhou et al., 2013b; Zhu et al., 2011). Specifically, the elevated temperatures used in SPORL (Fig. 1), as compared to those used in sulfite pulping with nearly complete delignification, enhances hemicellulose dissolution, maintains a certain degree of lignin sulfonation without complete delignification, and requires a shorter reaction time to reduce cost. Building upon our previous success in efficient ethanol production from softwoods using SPORL, we demonstrated here high titer ethanol production from the un-detoxified whole slurry of Douglas-fir forest harvest residue pretreated in a pilot-scale wood pulping digester to pave the way for commercial-scale trial runs in a wood pulp mill. The technical issues addressed in this study are: (1) demonstrating SPORL process using commercial pulp mill chemistry (Gao et al., 2013), i.e., bubbling SO_2 into a hydroxide solution to produce the sulfite solution, rather than using H_2SO_4 and sodium bisulfite as practiced in all our previous studies for ease of pretreatment experiments in the laboratory (Luo et al., 2010; Wang et al., 2009; Zhou et al., 2013b; Zhu et al., 2009, 2011); (2) using a low pretreatment temperature of 145 °C to

accommodate facility limitations at pulp mills without reducing cellulose saccharification efficiency; (3) direct enzymatic saccharification and fermentation of the pretreated whole slurry at high solids without solids washing or slurry detoxification to simplify process integration.

Optimal pretreatment conditions derived from conventional optimization studies based on sugar yield from very recalcitrant feedstock such as forest residue (Leu et al., 2013) may not be optimal for ethanol production due to elevated sugar degradation to inhibitors to cause difficulties in fermentation without detoxification. Furthermore, the maximal allowable operating temperature for commercial sulfite pulp mill digesters is well below the optimal temperature from previous optimization studies (Tian et al., 2011; Zhou et al., 2013b), which creates difficulties for process scale-up. Low temperature pretreatment has been used to reduce inhibitor formation but at the expense of additional processing steps to maintain enzymatic saccharification efficiency (Chen et al., 2012). To overcome these difficulties and balance sugar yield and sugar degradation, we used a combined hydrolysis factor (CHF) (Zhu et al., 2012) as a pretreatment severity measure to scale-up SPORL to a pilot scale of 50 kg at a low temperature of 145 °C used in this study. Utilizing a highly recalcitrant but low cost feedstock, forest harvest residue, avoids biofuel production competing for raw materials with the existing solid wood and pulp and paper industries. Therefore, this study has vital commercial importance.

2. Methods

2.1. The Douglas-fir forest residue

Douglas-fir forest residue was collected from a regeneration harvest in a primarily Douglas-fir stand on Mosby Creek southeast of Cottage Grove in Lane County, OR and owned by Weyerhaeuser Company. A horizontal drum fixed-hammer grinder (Model 4710B, Peterson Pacific Corporation, Eugene, OR) equipped with a combination of 76 and 102 mm grates was used to grind road piles of the residue. The ground residue was shipped to Weyerhaeuser Company at Federal Way, WA, by truck. The moisture content of the residue measured at arrival was 43.9%. A gyratory screen (Black-Clawson) equipped with a 44.5 mm (1.75 in.) diameter round-hole punched-plate top deck was used to remove oversized particles and a 3.2 mm (1/8 in.) clear-opening woven wire bottom screen (6 wires/inch mesh) to remove fines. The screening resulted in 7.6% fines and 9.8% oversize. The oversize fraction was further hammer milled, which resulted in near zero oversized particles and 14.9% fines from the 9.8% original screen oversize fractions. The total rejection of fines was 9.0%. The accept forest residue labeled as FS-10 was then air-dried to a moisture content of 15% before being shipped to the USDA Forest Products Laboratory, Madison, WI.

2.2. Enzymes, chemicals and yeast

Multiplex cellulase enzymes Cellic[®] CTec3 (abbreviated CTec3) was complementarily provided by Novozymes North America (Franklinton, NC). ACS reagent grade sodium acetate, acetic acid, and sulfuric acid were used as received from Sigma-Aldrich (St. Louis, MO). Calcium hydroxide (95% purity) was purchased from Alfa Aesar (Ward Hill, MA). Sulfur dioxide anhydrous was from Airgas USA (Madison, WI). Calcium bisulfite was produced onsite by bubbling sulfur dioxide to a calcium oxide slurry at the Forest Products Lab.

An engineered yeast strain of *Saccharomyces cerevisiae* YRH-400 was obtained from USDA Agriculture Research Service (Hector et al., 2011). Yeast extract peptone dextrose (YPD) agar plates

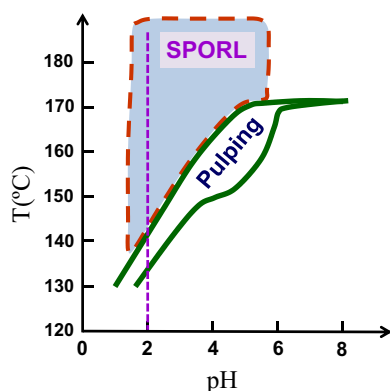


Fig. 1. Comparison of operating conditions between SPORL and sulfite pulping in the Temperature-pH diagram.

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