



High titer ethanol production from SPORL-pretreated lodgepole pine by simultaneous enzymatic saccharification and combined fermentation[☆]

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HIGHLIGHTS

- ▶ High titer of 47 g/L and high yield of 285 L/tonne wood ethanol production from lodgepole pine.
- ▶ Simultaneous enzymatic saccharification of pretreated solids and combined fermentation with pretreatment spent liquor.
- ▶ Fed-batch of unwashed solids in fermentation to manage inhibitor and to achieve high ethanol titer.

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ABSTRACT

Lodgepole wood chips were pretreated by sulfite pretreatment to overcome recalcitrance of lignocelluloses (SPORL) at 25% solids loading and 180 °C for 20 min with sulfuric acid and sodium bisulfite charges of 2.2 and 8 wt/wt% on an oven-dry wood basis, respectively. The pretreated wood chips were disk-milled with pretreatment spent liquor and water, and the solid fraction was separated from the liquor stream. The liquor was neutralized and concentrated through vacuum evaporation. Quasi-simultaneous enzymatic saccharification of the cellulosic solids and combined fermentation with the concentrated liquor was conducted at up to 20% total solids loading. Fed-batching of the solids facilitated liquefaction and saccharification, as well as managing instantaneous inhibitor concentrations. At a commercial cellulase (CTec2) loading of only 9 FPU or 0.06 mL/g untreated wood, a maximum ethanol titer of 47.4 g/L was achieved, resulting in a calculated yield of 285 L/tonne of wood using *Saccharomyces cerevisiae* YRH400 at 35 °C and pH 5.5.

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1. Introduction

Substituting biofuel from lignocellulosic biomass for petroleum based liquid fuel can help to mitigate climate change and sustainable economic development; however, barriers such as robust pretreatment, high solids enzymatic saccharification, efficient fermentation, and low energy separation need to be surmounted (Lynd et al., 2008; Zhu and Pan, 2010; Zhu and Zhuang, 2012). Despite significant progress in the last two decades, integration of biorefinery process technologies remains a challenge, partly because integration requires a coordinated effort, and optimization needs a systematic approach to examine overall process performance. For example, increasing pretreatment severity often results

in high total sugar yield; however, acid pretreatment will also increase the production of fermentation inhibitors in the hemicellulosic sugar stream (Larsson et al., 1999; Zhu et al., 2012). Washing pretreated solids can reduce enzyme inhibition by lignin and improve the efficiency of cellulose saccharification (Nagle et al., 2002; Tengborg et al., 2001), but washing consumes a significant amount of water (Liu and Zhu, 2010). While high solids processing can increase biofuel titer favorable to downstream separation, it requires high enzyme loading and energy costs for mixing (Liu et al., 2010; Zhang et al., 2010a).

Several integrated biorefinery studies have been reported that presented results from unprocessed biomass to biofuel production; however, most of these have been conducted at low solids levels with a final bioethanol concentration of less than 40 g/L (Hoyer et al., 2010; Jin et al., 2012; Monavari et al., 2010; Nieves et al., 2011; Tian et al., 2010; Zhu et al., 2010). Low solids processes can avoid some of the difficulties encountered when attempting enzymatic saccharification with high solids loadings. Likewise fermentation of dilute hydrolysate can avoid high concentrations of

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