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Using a combined hydrolysis factor to optimize high titer ethanol production from sulfite-pretreated poplar without detoxification ☆

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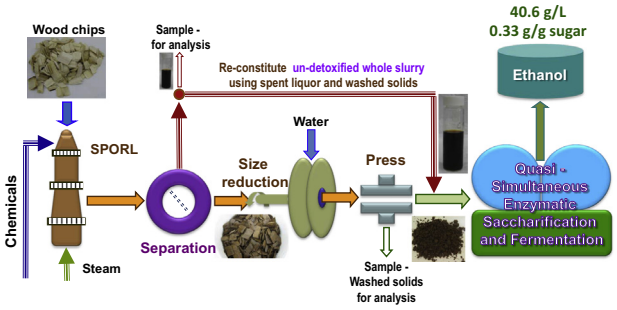
HIGHLIGHTS

- Combined hydrolysis factor for optimizing sulfite pretreatment.
- High ethanol titer at 41 g/L from poplar by sulfite without detoxification.
- Low cellulase loading of 15 FPU/g glucan (27 mL/kg wood).

GRAPHICAL ABSTRACT



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ABSTRACT

Sulfite pretreatment to overcome the recalcitrance of lignocelluloses (SPORL) was applied to poplar NE222 chips in a range of chemical loadings, temperatures, and times. The combined hydrolysis factor (*CHF*) as a pretreatment severity accurately predicted xylan dissolution by SPORL. Good correlations between *CHF* and pretreated solids enzymatic digestibility, sugar yield, and the formations of furfural and acetic acid were obtained. Therefore, *CHF* was used to balance sugar yield with the formation of fermentation inhibitors for high titer ethanol production without detoxification. The results indicated that optimal sugar yield can be achieved at *CHF* = 3.1, however, fermentation using un-detoxified whole slurries of NE222 pretreated at different severities by SPORL indicated *CHF* ≈ 2 produced best results. An ethanol titer of 41 g/L was achieved at total solids of approximately 20 wt% without detoxification with a low cellulase loading of 15 FPU/g glucan (27 mL/kg untreated wood).

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1. Introduction

Short rotation woody crops such as *Populus* spp. and their hybrids (i.e., hybrid poplars) are a significant component of the total biofuels and bioenergy feedstock resource in the USA and are, therefore, vital for growing a bioeconomy. An attractive aspect of growing hybrid poplars is their ability to grow on marginal lands to conserve water, recycle nutrients, and sequester carbon (Vance et al., 2010). However, production of these dedicated energy crops on such marginal and liability lands may lead to questions about their economic, logistic, and ecologic feasibility. In this context, conversion efficiencies at the back end of the energy supply chain are of utmost importance. Despite many research efforts have been made in bioconversion of hybrid poplars (Acker et al., 2014; Gupta

☆ This work was conducted when Zhang and Gu were visiting scientists at the USDA Forest Products Laboratory and on official government time of Zhu and Zalesny Jr.

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et al., 2014; Kim et al., 2013; Kundu et al., 2014; Wang et al., 2012; Wyman et al., 2009), achieving high titer and yield biofuel production through fermentation from poplars without detoxification remains a challenge.

As a woody biomass, poplars can be highly recalcitrant to enzymatic saccharification depending on its lignin content and structure (Studer et al., 2011; Wang et al., 2012). Severe pretreatments to remove this recalcitrance often result in substantial amount of sugar (especially xylose) degradation to furans (furfural) that can inhibit fermentation at high solid loadings for high titer biofuel production. Furthermore, unlike softwood species, poplar woods also contain a large amount of acetyl groups that can be easily converted into acetic acid by acidic pretreatments (Tian et al., 2011; Tunc and Van Heiningen, 2008) to inhibit fermentation (Casey et al., 2010; Helle et al., 2003). Acetic acid cannot be metabolized by yeasts (Wei et al., 2013). Furthermore, in-process reduction of acetic acid formation is difficult unless alkaline pretreatments were used. De-acetylation using hydroxide can reduce acetic acid formation (Chen et al., 2012; Kundu et al., 2014) but at the expense of additional processing. Post-pretreatment detoxification of acetic acid is also difficult as acetic acid cannot be easily neutralized or distillated (Xavier et al., 2010). The compounding effects of fermentation inhibition by furans and acetic acid along with aromatics (Palmqvist et al., 1999; Pampulha and Loureiro-Dias, 1989) make high titer biofuel production from poplar woods without detoxification difficult. Consequently, reported studies on biofuel production from poplar woods were limited to using washed solids alone or at low solids loadings when pretreatment spent liquor (hydrolysate) was used to avoid fermentation difficulties (Kim et al., 2013; Kundu et al., 2014; Zhu et al., 2011).

Here we demonstrate high titer ethanol production from an undetoxified whole slurry of poplar wood pretreated by SPORL (sulfite pretreatment to overcome the recalcitrance of lignocelluloses). SPORL has demonstrated robust performance in pretreating softwoods for high titer and high yield bioethanol production without detoxification (Zhou et al., 2013; Zhu et al., 2015). The relatively lower furan formation by SPORL compared with dilute acid pretreatment (Shuai et al., 2010; Wang et al., 2009) can alleviate the problem of the compounding effect of fermentation inhibition. Therefore, optimization of SPORL with the aim to reduce this compounding effect on cell growth can be effective. Reducing pretreatment severity such as using a low pretreatment temperature or a low acid concentration can reduce xylan dissolution, acetic acid formation, and sugar degradation to furans. To address the deficiency in dissolving hemicelluloses at low temperatures or low acid conditions for efficient enzymatic saccharification of cellulose, we extended the pretreatment duration to maintain a sufficient pretreatment severity measured by a combined hydrolysis factor (*CHF*) that can accurately predict dissolution of hemicelluloses (Zhou et al., 2013; Zhu et al., 2012). This approach of pretreatment optimization differs from traditional statistical experimental designs. It also differs from other low temperature pretreatment studies that arbitrarily selected pretreatment duration (Chen et al., 2012). The objective of this study was to demonstrate pretreatment optimization using *CHF* to achieve in-process inhibitor reduction for high titer ethanol production from a poplar wood without post-pretreatment detoxification. The results can be used to enhance the sustainability of these conversion systems, especially with respect to the integration of optimization at the front end (i.e., during pretreatment) and the elimination of unnecessary steps thereafter (i.e., not needing post-pretreatment detoxification). Therefore this study is important for researchers and industrial representatives seeking to increase efficiencies in ethanol production from wood.

2. Methods

2.1. Materials

Wood logs of poplar NE222 (*Populus deltoides* Bartr. ex Marsh × *Populus nigra* L.) were harvested from Hugo Sauer Nursery in Rhinelander, WI, USA, and provided by the Institute for Applied Ecosystem Studies of the USDA Forest Service Northern Research Station. The logs were transported to the USDA Forest Products Lab, Madison, WI and chipped using a knife chipper (Carthage (CEM) Machine Co, Carthage, New York). The wood chips were screened to remove particles larger than 38 mm and less than 6 mm. The thicknesses of the accepted chips ranged from 1 to 5 mm. The moisture content of the accepted wood chips was 51.6%. The chips were kept frozen at −16 °C until use.

A commercial cellulase enzyme Cellic®CTec3 (abbreviated CTec3) was complimentarily provided by Novozymes North America (Franklinton, NC, USA). The cellulase activity was 217 FPU/mL calibrated using a literature method (Wood and Bhat, 1988). Sodium acetate, acetic acid, sulfuric acid, and sodium bisulfite were ACS reagent grade and were acquired from Sigma-Aldrich (St. Louis, MO, USA).

Saccharomyces cerevisiae YRH400, an engineered fungal strain (Hector et al., 2011), was provided by USDA Agriculture Research Service for fermentation of xylose and hexoses. The strain was grown at 30 °C for 2 days on YPD agar plates containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 20 g/L agar. A colony from the plate was transferred by loop to a liquid YPD medium and cultured in a flask overnight at 30 °C on a shaking bed incubator at 90 rpm (Thermo Fisher Scientific, Model 4450, Waltham, MA). The cultured medium was used as inoculant for fermentation.

2.2. Pretreatment

Poplar NE222 wood chips of 150 g in oven dried (OD) weight were placed in a 1-L reactor with a dilute sulfite solution at wood (OD) to liquor ratio W:L = 1:3 (kg/L) or total solids loading of 25 wt%. Three 1-L reactors were placed into a 23-L rotating wood pulping digester heated by a steam jacket in an autoclave configuration as described previously (Wang et al., 2012; Zhu et al., 2009). Dilute sulfite solutions were prepared using varied amounts of sodium bisulfite and sulfuric acid to adjust pH between 1.15 and 1.80. The sulfuric acid concentration in the sulfite solution varied in a range of 0.1–0.5% (v/v). The sodium bisulfite charge on OD wood ranged from 0 to 4 wt%. Sulfite charge at 0 wt% represented dilute acid pretreatment. Pretreatments were conducted at 135, 150, 160, and 170 °C with varied durations between 24 and 290 min, resulting in different pretreatment severities as listed in Table 1. Replicate pretreatments were conducted at two conditions in a time span of 3 weeks to ensure experimental repeatability. A total of 36 pretreatments were conducted.

At the end of each pretreatment, the steam jacket was flushed with cold tap water to cool down the digester. The 1-L reactor was further cooled with tap water before opening. The pretreated solids and freely drainable spent liquor were separated by a Büchner funnel using a nylon screen. The collected spent liquor was kept at 4 °C. The collected solids were disk-milled in a 30-cm laboratory disk refiner (Andritz Sprout-Bauer Atmospheric Refiner, Springfield, OH) with the addition of tap water (as washing) to a discharge solid dry matter (DM) consistency of approximately 10 wt% (Fig. 1). The disk plate gaps were 2, 0.76, and 0.76 mm for the three passes of milling. At the end of third pass of milling, the disk plates were washed using tap water to collect any remaining solids. The collected suspension was dewatered