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# Inhibitors removal from bio-oil aqueous fraction for increased ethanol production

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## HIGHLIGHTS

- Bio-oil aqueous fraction was treated to remove inhibitors.
- Monomeric sugars were produced from levoglucosan by hydrolysis.
- Inhibitor removal allowed ethanol-producing microbes to metabolize monomeric sugars.
- Inhibitors were removed by solvent extraction, membrane filtration and freeze-drying.
- Ethanol yield from hydrolyzate fermentation by *Saccharomyces pastorianus* reached 98%.

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## ABSTRACT

Utilization of 1,6-anhydro-β-D-glucopyranose (levoglucosan) present (11% w/v) in the water fraction of bio-oil for ethanol production will facilitate improvement in comprehensive utilization of total carbon in biomass. One of the major challenges for conversion of anhydrous sugars from the bio-oil water fraction to bio-ethanol is the presence of inhibitory compounds that slow or impede the microbial fermentation process. Removal of inhibitory compounds was first approached by n-butanol extraction. Optimal ratio of n-butanol and bio-oil water fraction was 1.8:1. Removal of dissolved n-butanol was completed by evaporation. Concentration of sugars in the bio-oil water fraction was performed by membrane filtration and freeze drying. Fermentability of the pyrolytic sugars was tested by fermentation of hydrolyzed sugars with *Saccharomyces pastorianus* lager yeast. The yield of ethanol produced from pyrolytic sugars in the bio-oil water fraction reached a maximum of 98% of the theoretical yield.



## 1. Introduction

Production of fuel from lignocellulosic material is a subject of great interest because lignocellulosic materials are renewable, inexpensive, and readily available (Galbe and Zacchi, 2002; Mosier et al., 2005). One of the methods to convert lignocellulosic materials to fuel is fast pyrolysis. Fast pyrolysis is a thermal degradation process, in which lignocellulosic biomass is rapidly decomposed under high temperature (±500 °C) in the absence of oxygen (Bridgwater et al., 1999). Due to the high temperature the molecular bonds in lignocellulosic biomass are fractured to produce char, pyrolysis oil (bio-oil), and gas, each of which can be used as energy sources. During the pyrolysis process, up to 75% of initial dry biomass can be converted to bio-oil (Czernik and Bridgwater, 2004; Mohan et al., 2006). Until recently maximum reported percentage of 1,6-anhydro-β-D-glucopyranose, also known as levoglucosan (LG), in the bio-oil aqueous fraction has been reported to be 7.8 wt% (Bennett et al., 2009). LG can be separated and used as a carbon source for bio-ethanol production. On the other hand, removal of the LG-rich aqueous fraction from bio-oil can improve the bio-oil properties because a number of corrosive chemicals with low heating value such as water, 5-hydroxymethyl-2-furfuraldehyde (5-HMF), 2-furfuraldehyde (furfural) and acetic acids are removed along with the anhydrous sugars. Also, absence of the above compounds in the pyrolytic lignin fraction will reduce the cost of hydroprocessing by utilizing lower amounts of hydrogen and catalysts (Mercader et al., 2011). Upgraded pyrolytic lignin can be used for production of various grade fuels (Beauchet et al., 2012) and as a source of phenolic compounds for manufacturing chemicals (Mullen and Boateng, 2010). Ultimately, the utilization of anhydrous sugar for ethanol production will enhance total carbon use in biomass, thus allowing the biomass conversion to be more economically feasible.

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LG is a major degradation product of cellulose during fast pyrolysis. Previous studies have shown that LG yield can be increased by applying mild acid pretreatment to demineralize the feedstock prior to hydrolysis (Mourant et al., 2011; Patwardhan et al., 2010). Li et al. (2013b) developed a method to further increase LG yield during fast pyrolysis beyond simple mild acid pretreatment. The optimal bio-oil water extraction condition for maximizing levoglucosan yield was found to be 1.3:1 (water-to-bio-oil ratio), 25 °C, and 20 min, with a levoglucosan yield of 12.7 wt% of raw bio-oil or a 63 wt% increase over previous results (Li et al., 2013a). LG can be hydrolyzed to glucose and further fermented to biofuels and bioproducts (ethanol, butanol, lipids, etc.) (Bennett et al., 2009; Lian et al., 2010; Yu and Zhang, 2003a).

One of the key challenges for conversion of anhydrous sugars from bio-oil to bio-ethanol is the presence of inhibitory compounds in the bio-oil aqueous fraction. Chemicals such as 5-HMF, furfural, acetic acid, fractionated phenolic compounds and others impede or prevent the microbial fermentation process (Duarte et al., 2005; Klinke et al., 2004; Li and Chen, 2008). Among these inhibitory compounds, lignin-derived compounds have approximately 10 times the inhibitory ability compared to carbohydrate degradation products (Clark and Mackie, 1984). Thus, removal of the inhibitory compounds, especially lignin-derived compounds, is essential for successful ethanol production from pyrolytic sugars.

A study by Hassan et al. (2013) showed that n-butanol is an appropriate organic solvent for removal of lignin based compounds and other organic inhibitors such as acetic acid, 5-HMF and furfural from the bio-oil aqueous solution. The recyclability of n-butanol also reduces the cost for LG separation and purification.

According to Okolo et al. (1987), *Saccharomyces cerevisiae*, the parent yeast of *Saccharomyces pastorianus*, was inhibited by n-butanol. The concentration of n-butanol caused an exponential decrease in activity of *S. cerevisiae*. Research has been conducted to determine the effect of n-butanol on the growth *S. cerevisiae*. To allow this yeast to produce a n-butanol tolerant strain genetic engineering was performed on *S. cerevisiae* (González-Ramos et al., 2013). Specific research on the inhibitory influence of n-butanol on the growth of *S. pastorianus* is not available. However, it would not be surprising to find a similar effect of n-butanol in inhibiting the growth of *S. pastorianus*.

The objective of the current research was to develop a technically successful method to remove toxic compounds and increase the ethanol yield from pyrolytic anhydrosugars fermentation. This approach employed techniques combining n-butanol extraction and concentration of sugar while monitoring the fermentability of sugar solution at each step.

## 2. Methods

### 2.1. Bio-oil fractionation

Bio-oil used for this study was prepared utilizing clear pinewood particles of 1–3 mm at the Department of Sustainable Bioproducts, Mississippi State University. The method of LG increase described by Li et al. (2013b), which utilized water spray into the reactor pyrolysis vapor stream, was utilized to produce all study bio-oil. Characteristics of the bio-oil are shown in Table 1. Isolation of LG from bio-oil was based on its water solubility. Addition of water (2 L) into bio-oil (2 L) was in a 1:1 ratio because the water content in the raw bio-oil in this study was higher than that in the bio-oil used in the study by Li et al. (2013a). Shaking the mixture vigorously for 8–10 min and letting it settle resulted in an immiscible water fraction (at the top) and pyroligneous fraction (at the bottom). The bio-oil water fraction (BWF), rich in LG, was separated by simple decantation. Along with LG, a number of inhibitors such as furfural, 5-HMF, decomposed lignin and various acids were dissolved in the BWF. A schematic representation of the treatments applied to remove the above inhibitors from the BWF is shown in Fig. 1.

**Table 1**
Selected characteristics of the raw bio-oil for the current study.

| Bio-oil properties | Value | Elemental content (wt%) | Value |
|---|---|---|---|
| Water content (wt%) | 34.15 | Carbon | 33.45 |
| Density (g/L) | 1.1 | Nitrogen | 0.10 |
| pH | 2.32 | Hydrogen | 7.69 |
| Acetic acid (wt%) | 5.01 | Oxygen | 58.59 |
| Furfural (wt%) | 1.07 | | |
| 5-HMF (wt%) | 1.48 | | |
| Levoglucosan (wt%) | 11.30 | | |

### 2.2. Organic solvent extraction

Furfural, 5-HMF, weak acids, phenolics and other compounds toxic to ethanol producing microorganisms contained in the water fraction of bio-oil were extracted by n-butanol. For optimization of n-butanol to BWF ratio, 300 mL of n-butanol was divided into a single 90 mL and seven 30 mL (7 × 30 mL) portions. One hundred milliliter of BWF was then extracted with 90 mL of n-butanol in a separating funnel. After the n-butanol and the water fraction separated, the n-butanol fraction was removed. Next, the water fraction was again extracted with 30 mL of n-butanol. This step was repeated six more times in sequence with 30 mL of n-butanol each time (three replicates). The lignin content including phenolic monomers that existed in the extracted BWF (EBWF) was detected quantitatively by a Cary 100 Bio UV–Visible Spectrophotometer (Varian, Australia) at 278 nm (Fukushima and Hatfield, 2001; Yoshida et al., 2002). Inhibitor content in the EBWF was detected by high pressure liquid chromatography (HPLC).

### 2.3. Hydrolysis, membrane filtration and freeze drying

LG in BWF was hydrolyzed in the presence of sulfuric acid. The concentration of sulfuric acid in BWF was adjusted to 0.5 M and autoclaved at 125 °C for 44 min (Bennett et al., 2009). The hydrolyzate was then neutralized with 5 N sodium hydroxide to pH = 5; the addition of calcium carbonate raised the pH to ≈5.5–6 allowing further neutralization and settling of the excess calcium carbonate. The pH of the fraction after settling reached approximately 7. The neutralized hydrolyzate was then filtered twice through tight filter paper (3–5 μm Whatman) to remove excess calcium carbonate in order to prevent the membrane clogging during membrane filtration.

n-Butanol dissolved in BWF was removed by evaporation over a water bath at 85 °C. This sugar solution will be referred to as detoxified BWF (DBWF) in the succeeding steps. Sugars (mainly glucose) in the DBWF were concentrated on an Alfa-Laval M-20 Membrane filtration system (Alfa Laval Inc., Richmond, VA) using nano- (NF, 400 Da cut-off) and reverse osmosis (RO) membranes. First, the DBWF was hydrolyzed, neutralized and filtered, then introduced to the NF membrane. Both permeate and retentate were collected separately. Permeate of the NF membrane was used as a feed solution for the RO membrane. Retentates from each membrane were concentrated with a 4.5 Labconco freeze dryer system (Kansas City, Missouri).

### 2.4. Fermentation

*S. pastorianus* (ATCC 2345) was utilized for production of ethanol from pyrolytic sugars. Inoculum of *S. pastorianus* for fermentation was prepared according to NREL/TP-510-42630 Laboratory