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Short Communication

Methods for mitigation of bio-oil extract toxicity

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ABSTRACT

Levoglucosan (1,6-anhydro-β-D-glucopyranose) and other anhydrosugars can be produced in significant quantities during fast pyrolysis of lignocellulosic material. Levoglucosan can be extracted and hydrolyzed to produce fermentable glucose, however co-extraction of fermentation inhibitors can reduce ethanol yields. This work was aimed at evaluating various methods for mitigating the toxicity of bio-oil aqueous extract. Among the detoxification techniques tested, it was found that overliming and solvent extraction were able to improve the fermentability of bio-oil hydrolyzates. Overliming was able to increase the yield of ethanol from bio-oil hydrolyzate by 0.19 ± 0.01 (g ethanol/g glucose) at 50% volume hydrolyzate and 0.45 ± 0.05 (g ethanol/g glucose) at 40% volume hydrolyzate. A number of extractants were examined and the best solvent was tri-*n*-octylamine with co-solvent 1-octanol. It was able to selectively (100% glucose retention) remove at least 90 ± 6.8% of acetic acid, which was the targeted inhibitor in bio-oil hydrolyzate. This increased the ethanol yield by 0.24 (g ethanol/g glucose) at 40% volume of hydrolyzate. In addition, a technique called adaptive evolution of yeasts was applied, which was capable of increasing the ethanol yield by up to 39% when compared with the unadapted parental strains.

1. Introduction

The technical feasibility of producing ethanol from bio-oil has been proven by previous researchers (Yu and Zhang, 2003, 2004; Zhang, 2004; Bennett et al., 2009) and involves four stages: extraction, hydrolysis, dilution or detoxification and fermentation. In the first stage, the anhydrosugars, mainly levoglucosan and cellobiosan, are extracted using water. In the second stage, anhydrosugars are hydrolyzed into glucose using dilute sulfuric acid. Co-extraction of fermentation inhibitors can reduce ethanol yields.

Overliming has long been regarded as a very effective, but impractical method for detoxification of hydrolyzates prior to fermentation. Overliming cotton-derived bio-oil hydrolyzate resulted in an ethanol yield of 0.39 g ethanol/g glucose, a yield which can be further improved when overliming is combined with other adsorbents such as diatomite, bentonite and zeolite (Yu and Zhang, 2004).

Liquid–liquid extraction is a technique routinely applied in chemical/biochemical processing. C8–C10 saturated aliphatic tertiary amines dissolved in organic solvents are effective extractants for carboxylic acids and polar diluents can improve performance (Senol, 2004; Yang et al., 1991). Extraction of hemicelluloses hydrolyzate with oleyl alcohol produced a twofold increase in ethanol productivity (Zausten et al., 2008).

Adaptive evolution is, by definition, a set of mutations that occur in response to a specific challenge or changes in the environment and is advantageous to the cells under these conditions (McBryde et al., 2006). This technique can potentially be used to develop an adapted yeast strain that can resist the inhibitory compounds in the fermentation broth.

The aim of this study was to evaluate methods to improve the fermentation of bio-oil hydrolyzate. The effective techniques evaluated included overliming and solvent extraction. In addition, a biological approach called adaptive evolution was used to aid the yeast to adapt to the inhibitory environment of bio-oil hydrolyzate in order to increase their resistance to inhibitors.

2. Methods

2.1. Storage and preparation of bio-oil

The bio-oil used in the project came from VTT the Technical Institute of Finland. It was stored at 4 °C. Before any experiment with the bio-oil, the oil was placed in an environmental shaker at 20 °C and 120 rpm for approximately 20 min.

2.2. Levoglucosan extraction from bio-oil

Bio-oil and water were weighed and measured according to the predetermined mass ratios of 10, 20, 40, 50, 100, 200, 400, 600, 800, 1000, and 2000 wt.% of water added to bio-oil. A handheld

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kitchen mixer (Braun, Multiquick MR405) with steel blades was used to mix the liquids and grind the tar-like organic phase formed in the separation of water and bio-oil. After 20 min of mixing, the aqueous phase samples were decanted into falcon tubes for storage and analysis. For hydrolysis, sulfuric acid was used as previously described (Bennett et al., 2009).

2.3. Techniques used to improve ethanol yield from fermentation of bio-oil hydrolyzate

2.3.1. Overliming

$Ca(OH)_2$ was added to the bio-oil hydrolyzate at 22 °C (with adequate mixing) until a pH of 10 was reached. The pH of the bio-oil hydrolyzate was measured with a pH meter (Thermo Orion, model 710 pH meter). The hydrolyzate was then mixed with a magnetic stirrer for 30 min. Afterward, the hydrolyzate was centrifuged for 15 min at 2285*g* (Damon/IEC division CU-5000). The supernatant was decanted to be used in subsequent fermentation experiments.

2.3.2. Extraction with organic solvents

The solvents used in the project were alamine 336, aliquat 336 (Cognis Inc., Tucson, AZ); tri-*n*-octylamine, tributyl phosphate (Acros Organics, New Jersey, USA); oleyl alcohol, oleic acid (Aldrich, USA) and primene-JMT (Rohm and Haas, Pennsylvania, USA). Alamine 336 and aliquat 336 are the trade name of chemicals, which are tri-octyl/decyl amines and had a structure of R_3N and R_3NCH_3, respectively. Primene-JMT is also a trade name of a primary aliphatic amine which had a structure of R_3–C–NH_2 (Senol, 2004). Each of the five amines was used in conjunction with two different co-solvents, 1-octanol (Fisher, USA) and kerosene (Acros Organics, New Jersey, USA). The solvent and co-solvent ratios were tested at 0%, 25%, 50%, and 75% v/v.

The solvent extraction experiments were initially conducted using a model solution containing 10 g/L glucose and 60 g/L acetic acid. Performance of the solvents was evaluated based on the amount of acetic acid removed and sugar retained after the extractions. Then, experiments were conducted to test if the solvents were inhibitory to fermentation. This was tested by extracting water with the proposed solvents. The water was then used to make up YPG medium for fermentation.

Solvent extractions were carried out in 50 mL falcon tubes at room temperature. Equal volumes of the solvents and hydrolyzates were used in the extraction experiment. They were mixed for approximately 2 h in an environmental shaker at room temperature (~20 °C). After 2 h, the solvents and the hydrolyzates were separated by centrifugation at 2285*g* for 10 min (Damon/IEC division CU-5000). The hydrolyzates were then transferred by pipette to serum vials for fermentation.

2.3.3. Adaptive evolution of yeast

A known percentage (either 10% or 35% v/v) of bio-oil hydrolyzates was diluted in YP medium. Yeast inocula were cultured and prepared in YPG medium. Then, the yeasts were inoculated into the YP medium that contained bio-oil hydrolyzate, in an effort to allow the yeast to adapt at a non-lethal level of inhibitors. The yeasts were cultured in shake flasks and transferred to new fermentation medium under the same stress conditions every day. In this work, the yeasts were adapted for a total of 36 days, approximately 121 generations for the aerobically-cultured yeast. Parameters investigated were concentration of hydrolyzate and the growth conditions of the yeasts (aerobic and micro-aerophilic conditions). The adapted yeast was evaluated using YPG containing 30% v/v extract.

2.3.4. Fermentation of bio-oil hydrolyzate

Saccharomyces cerevisiae T2 was used in this study. This is an industrial strain of yeast originally obtained from Tembec Inc. (Temiscaming, QC). The yeast was stored in a refrigerator at 4 °C on agar plates. When preparing the yeast for fermentation, a loop full of yeast from the agar plate was transferred aseptically to sterile YPG medium containing 1% (w/v) of yeast extract, 2% (w/v) of peptone and 2% (w/v) of glucose. The yeast was grown in the YPG medium for one full day in an environmental shaker at 30 °C and 150 rpm. Then, 2 mL of the starter culture broth was transferred to fresh YPG medium and allowed to grow for another 24 h. The yeast was harvested by centrifugation (2285*g*, 10 min). The pellet was washed once with sterile distilled water and then resuspended in 10 mL of sterile water. The yeast was inoculated to an approximate initial concentration of 2.5 g/L. Fermentation trials were carried out under micro-aerophilic and aerobic conditions as previously described (Helle et al., 2008).

3. Results and discussion

3.1. Optimal conditions for levoglucosan extraction

Phase separation was observed after addition of 9.86 wt.% of water, for a total water content of 27.3–31.0 wt.%, a range in agreement with previous work (Peacocke et al., 1994; Oasmaa et al., 2001; Bennett et al., 2009).

The optimal ratio for levoglucosan extraction with water was 100 wt.%. The maximum levoglucosan concentration obtained was approximately 4.98 wt.% (g levoglucosan/g bio-oil) (results not shown), a value that coincided closely with previously-published work (Bennett et al., 2009).

3.2. Effect of hydrolyzate concentration on fermentation

Due to the presence of inhibitors in bio-oil, it was necessary to dilute the bio-oil hydrolyzate with sterile water to lower the concentration of the inhibitors so that the yeasts were able to survive in the medium. Over the range tested, the ethanol yield was highest (0.49 g ethanol/g glucose) for 5% and 10% hydrolyzate. At 2% hydrolyzate, the ethanol yield was 0.20 ± 0.08 (g ethanol/g glucose), likely due to limited sugar availability. The yield of ethanol was decreased by 14% when the strength of hydrolyzate increased from 10% to 20%, and 58.4% upon increasing hydrolyzate strength to 25%. No ethanol was produced at 40% hydrolyzate.

3.3. Detoxification by overliming

Overliming improved the fermentability of bio-oil hydrolyzates. Ethanol yield increased from 0 to 0.19 ± 0.01 (g ethanol/g glucose) and 0.45 ± 0.05 (g ethanol/g glucose) at 50% and 40% v/v of bio-oil hydrolyzate, respectively. This was consistent with previously-published work which showed that the application of overliming to detoxify cotton-derived bio-oil hydrolyzate resulted in an ethanol yield of 0.39 g ethanol/g glucose (Yu and Zhang, 2004). This improved fermentability may be due to the removal of furans and phenolics (Martinez et al., 2001).

3.4. Detoxification by extraction with organic solvents

The artificial hydrolyzates used in the experiments contained 10 g/L of glucose and 60 g/L of acetic acid. The removal of acetic acid increased as the volume of 1-octanol in the system increased for alamine 336 and primene-JMT. There was no significant effect of TOA concentration over the range tested, and 25% was selected for further evaluation. The two best solvents for acetic acid