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Clean Pyrolytic Sugars Solution

Introduction

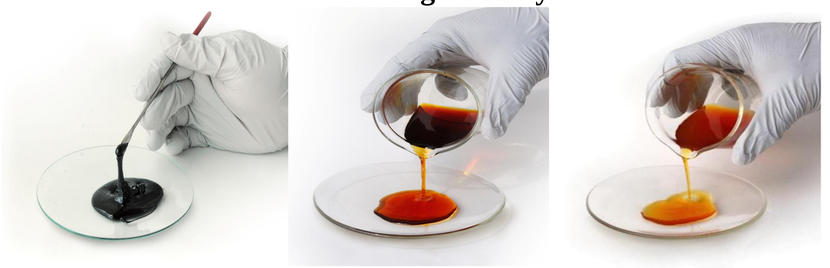
This study focuses on the effective removal of contaminants from pyrolytic sugar to produce a suitable fermentation substrate. Iowa State University utilizes a bio-oil recovery system from fast pyrolysis of lignocellulosic biomass as stage fractions (SF). The first two SFs collect "heavy ends" comprised of both sugars and phenolic oligomers. Exploiting differences in water solubility, we are able to recover a sugar-rich aqueous phase and a phenolic-rich raffinate. The sugar-rich aqueous phase contains small percentages of other water-soluble constituents such as low molecular weight acids, furans, and phenols that are possibly inhibitory to successful fermentation. Analyses of the sugar-rich aqueous phase by gas chromatography/flame ionization detector (GC/FID) indicated several compounds, acetol, guaiacol, and 5-hydroxymethylfurfural (5-HMF) known to be inhibitory to microbes/bacteria, were below the inhibitory wt% without additional detoxification. However, other compounds such as acetic acid, formic acid, and furfural require removal before fermentation. Current methods of detoxification were evaluated. These included overliming, liquid-liquid extraction, ionic liquid and ionic resin for removal of contaminants. Our research has shown the optimal candidate for detoxification of the pyrolytic sugars was sodium hydroxide overliming which showed maximum growth measurements utilizing ethanol-producing *Escherichia coli* (*E. coli*). We successfully removed the following percentages of compounds present in the initial sample utilizing sodium hydroxide overliming: 80% acetol, 80% furfural, 56% 2,6-dimethoxyphenol, 47% guaiacol, 74% vanillin, 91% phenol, and 82% 5-HMF with no degradation or loss of pyrolytic sugars.

Materials and Methods

1. Bio-oil was produced in a fast pyrolysis unit consisting of a fluidized bed operated at 450-500°C and a bio-oil recovery system that recovers bio-oil in distinct multiple stage fractions (SF) [1]. A sugar-rich aqueous phase and a phenolic-rich raffinate were separated from SF 1 and SF 2 [2].
2. Bio-oil constituents were evaluated and quantified using GC with a flame ionization detector (GC/FID), acid content was determined by ion-exchange chromatography, and the sugar content was determined using ultraviolet-visible range spectroscopy [3].
3. Liquid-liquid extraction detoxification method was evaluated. A solution of: 25% tri-n-octylamine in 1-octanol [4] was mixed in a 1 to 1 ratio by weight of the water-soluble sugars and placed on a shaker table for 2 h and centrifuged for 10 min at 2635 g.
4. The sugars and fermentation media were inoculated with ethanol-producing *Escherichia coli* (KO11 strain). The growth was presented by optical density (OD) at 550 nm. KO11 was cultured in 10 mL medium of Luria Broth and pyrolytic sugars in 50 mL centrifuge tubes at 37°C.
5. An ionic liquid:,1-methyl-3-octylimidazolium tetrafluoroborate [5], was mixed in a ratio of 1 part ionic liquid to 5 parts water-soluble sugars, vortex for 30 min and centrifuge for 20 min at 2635 g.
6. An ion-exchange resin: Dowex 66, was mixed in a ratio of 1 part to 5 parts water-soluble sugars, vortex for 30 min and centrifuge 20 min at 2635 g.
7. Three treatments by overliming were evaluated for detoxification of the sugar solutions. The solutions were brought to pH 7 after each treatment using H_2SO_4.
   - ✓ $Ca(OH)_2$: 30°C for 3 h at pH 11 [6]
   - ✓ NaOH: 80°C for 3 h at pH 9 [7]
   - ✓ NH_4OH: 55°C for 3 h at pH 9 [7]

Results

Are These Sugars Dirty?



Original Bio-Oil | SF 1 Sugar Solution | SF 2 Sugar Solution

Known *Escherichia coli* Inhibitors [8]

Compound
5-Hydroxymethyl furfural (5-HMF)
Formic Acid
Valeric Acid
Butyric Acid
Acetic Acid
Furfural
Guaiacol
Acetol

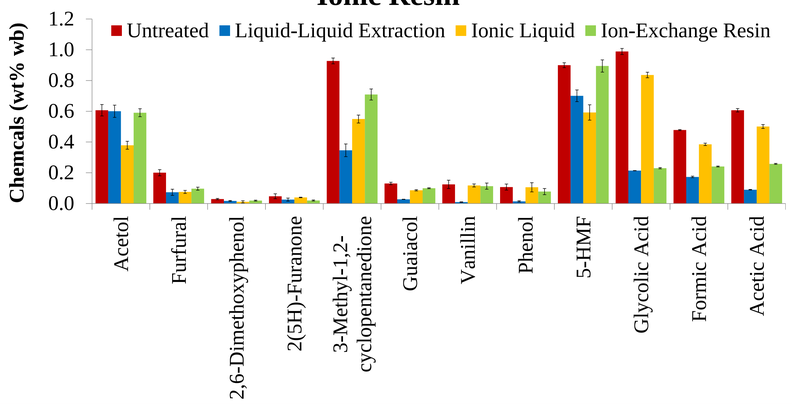
Compound Concentrations **Below** Inhibitory Wt%

- Acetol: (5.0, IC_{100}) [9]
- 5 HMF: (0.45, IC_{100}) [10]
- Guaiacol: (0.30, IC_{100}) [11]

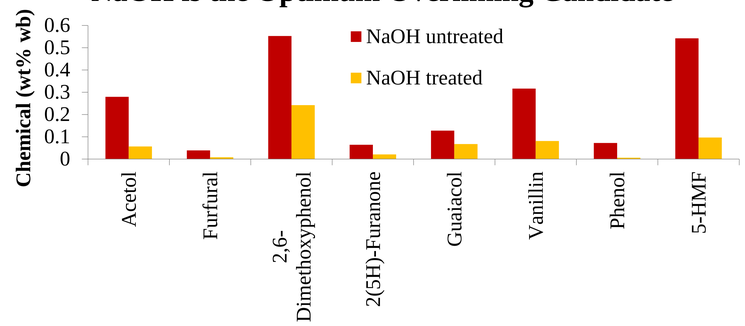
Compounds Remaining in Sugars

Compound	SF1 (wt% wb)	SF2 (wt% wb)
Acetol	0.55±0.07	0.31±0.01
Furfural	0.25±0.06	0.08±0.01
2,6-methoxyphenol	0.04±0.02	0
Furfuryl Alcohol	0.20±0.13	0.09±0.04
2(5H)-furanone	0.09±0.01	0.29±0.02
Guaiacol	0.10±0.04	0.05±0.01
Vanillin	0.06±0.01	0
Phenol	0	0.06±0.01
5-HMF	0.32±0.07	0.33±0.02
Acetic acid	0.89±0.01	0.56±0.02
Formic acid	0.57±0.002	0.34±0.03
Glycolic acid	0.84±0.01	0.43±0.04
Propionic acid	0.08±0.003	0.07±0.01

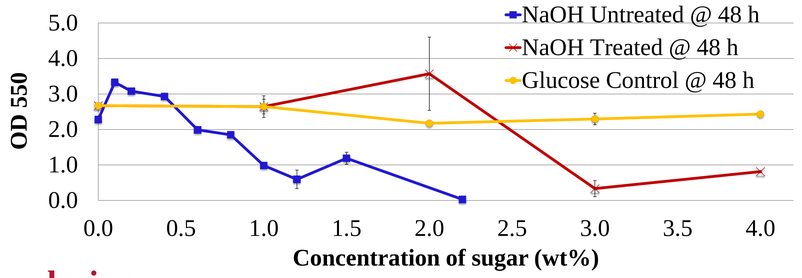
Comparison of Liquid-Liquid Extraction, Ionic Liquid, and Ionic Resin



NaOH is the Optimum Overliming Candidate



Utilization of Pyrolytic Sugars by Ethanologenic *E. coli*



Conclusions

1. **NaOH Overliming**
   - ✓ It was the most successful detoxification method with no loss of sugars.
   - ✓ Utilization of 1 and 2 wt% pyrolytic sugars was improved relative to the untreated sugars, but 3 wt% sugars was inhibitory.
2. **Other Overliming Treatments**
   - ✓ $Ca(OH)_2$ removed 7 wt% sugars and continued precipitating for several days.
   - ✓ NH_4OH did not perform as well as the NaOH and it showed no loss of sugars.
3. **Other Detoxification Methods**
   - **A. Liquid-Liquid Extraction**
     - ➢ It removed phenolics, acids, and reduced the furans without loss of sugar.
   - **B. Ionic Liquid**
     - ✓ Phenols were not removed as effectively however 5-HMF and acids were successfully removed with no loss of sugars.
   - **C. Ion-Exchange Resin**
     - ✓ Acids were successfully removed although phenolic compounds and furans were not, additionally 8 wt% sugars were lost.

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