Extractive Inhibition of Cellulases in Saccharification

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Introduction

Due to the demand for alternative resources much research has been focused on alternative fuel sources such as the processing of biofuels. The Northwest Advanced Renewables Alliance (NARA) takes a holistic approach in building a supply chain to produce aviation biofuels and co-products from woody biomass, such as the tops of Douglas-fir tress.[1] My summer research was focused on the inhibitive effects of cellulases during the saccharification stage within the production of biofuels. I also examined if the extractives slowed down reaction rates of inhibition during saccharification.

Image 1: Douglas Fir

Objective

- To examine if the extractives Taxifolin, Abietic Acid, and α-Pinene, have inhibitive effects on enzymes during the saccharification process of biofuel production.
- This will determine the length of time for NARA's saccharification step.

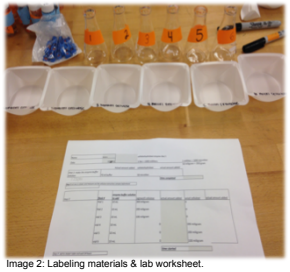
Image 2: Labeling materials & lab worksheet.

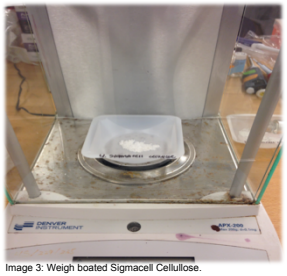
Image 3: Weigh boated Sigmacell Cellullose.

Methods

- Made enzyme-buffer solution(s), e.g. 70 ml buffer + 50 microliter of Endoglucanase.
- Placed buffer solution(s) in shaker table, e.g. 30 minutes or 24 hours.
- Weigh boated varying masses of Cellobiose Substrate, e.g. 300 milligram
- Added extractives to Cellobiose samples, e.g. 16 mg of Taxifolin.
- Solution(s) were distributed to labeled vials, e.g. Vial 1, Vail 2, etc.
- Vials were then placed on the shaker table, e.g. 30 minutes or 24 hours.


Image 4: Substrates ready to be transferred to vials.


Image 5: Aiden & Barbara filtering samples.

- Solution(s) were removed from shaker table.
- Samples were then filtered.
- Samples placed in boiling water for 30 minutes or 1 hour.
- Samples were removed.
- Placed in centrifuged for 6 minutes.
- Transferred samples from tubes to HPLC tubes & placed in refrigerator for later use.

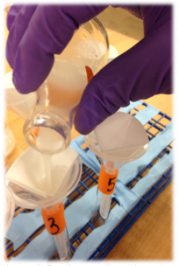
Image 6: Pouring samples to be filtered.


Image 7: Waiting for samples to filter through.

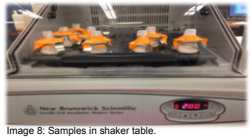
Image 8: Samples in shaker table.


Image 9: Samples were placed in boiling water.

Analysis

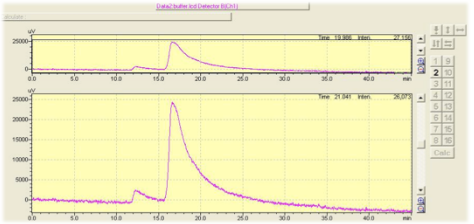
Image 10: Chromatogram of Buffer Solution.

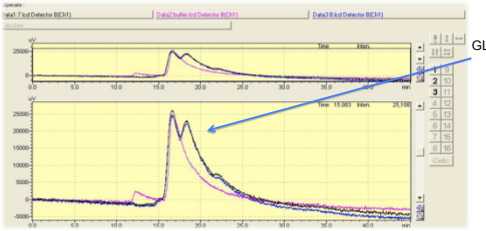

Image 11: Chromatogram of Buffer Solution & Enzyme-Abietic Solution.

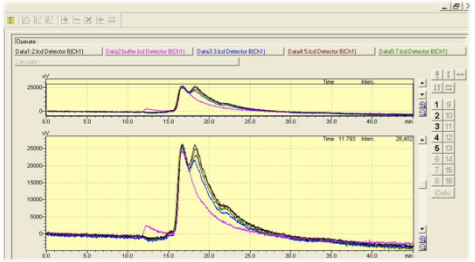
Image 12: Highest peaks of the Buffer, No Extractive, Taxifolin, α-Pinene, and Abietic Acid runs.

Conclusion

The data collected demonstrates:

-Taxifolin is a moderate strength inhibitor.

-Abietic Acid demonstrates to be a weaker inhibitor.

-α-Pinene seems to be unclear of its inhibition.

Of course the data runs have limitations such as time, varying volumes and masses as well as end yield goal(s).

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