Not all Douglas fir Trees are Created Equally for Conversion to Biofuels

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Objectives

❑To determine the variation of biomass recalcitrance between Douglas-fir families.

❑ To understand the linkages between genetic traits and degree of recalcitrance of softwood to biochemical conversion.

❑ To provide insight to selection and genetic modification of softwood species for biofuel production.

Tasks

1. **Chemical composition** of the Douglas fir biomass. By measuring the total weight of each bag after each treatment, we can determine the total amount of extractives , carbohydrates(holocellulose), and lignin. Methods for chemical composition are as follow

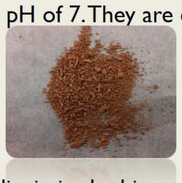
•Weigh empty bag

•Add wet biomass sample. Each bag contains 0.500 grams of wet sample (each sample duplicated; two bags for each treatment).

•Dry bag with biomass and weigh again, which gives moisture content of the biomass.

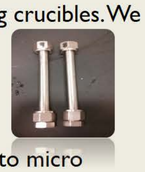
▪We ran samples through extractives process with acetone solvent. Air dry samples, then place in oven for one hour at 150^{0}C. Weigh the sample after oven, which gives amount extractives that is not part of chemical structure of the biomass.

▪Dilute acid treatment (72% Sulfuric acid) is used to break down the carbohydrates so that the remaining biomass content is lignin. We treated 15 bags with 260ml of Sulfuric acid at a time for two hours. After the two hour period, we removed the bags and place them into 6000ml flasks with 3.6l of DI water. They are placed in a autoclave for one hour. When finished, the bags are washed with hot water until a pH test strip reads a pH of 7. They are dried and are weighed to get total lignin content.



▪Sodium chlorite treatment is used to break down lignin in the biomass so that the left over material is carbohydrates (holocellulose). We used 2grams of Sodium chlorite powder, and 1.0ml of glacial acetic acid per 15 bags of biomass. We added the same amount of substance 5 times every hour in a water bath at 70^{0}C. After the 5 hour period, the samples were washed for one hour, dried, and weighed to measure total carbohydrate content.

2. **Pretreatment Yield** to help show high and low biomass recalcitrance. We did the following procedure:
   - Placed 0.500 grams of biomass inside reactor tubes as shown below.
   - Added 10ml of dilute acid and sealed the tube with the cap and soaked the tubes in water overnight.
   - We placed 15 tubes into a hot oil bath at 180^{0}C for 30 minutes.
   - We unsealed the tubes and drained the liquid through filtering crucibles. We collected the liquor and separated the solid waste.
   - Biomass and liquor were stored for further testing



3. **Enzymatic Hydrolysis** for sugar release—Ultimate Goal!!!!
   - With the pretreated biomass, we loaded 80-100 milligrams into micro centrifuge tubes.
   - To each tube we added 1.3ml of 50mM sodium acetate buffer and 0.609 ml of Ctec2 enzyme to help with reaction.
   - We loaded the tubes into a incubator set at 50°C for 72 hours with a RPM of 1400.
   - Glucose content of each was determined using a glucose detection kit and a spectrophotometer to measure UV absorption. As the kit was loaded, the color of the liquid turns from faint white to brilliant pink which indicates glucose. This is demonstrated in the picture below.

Results

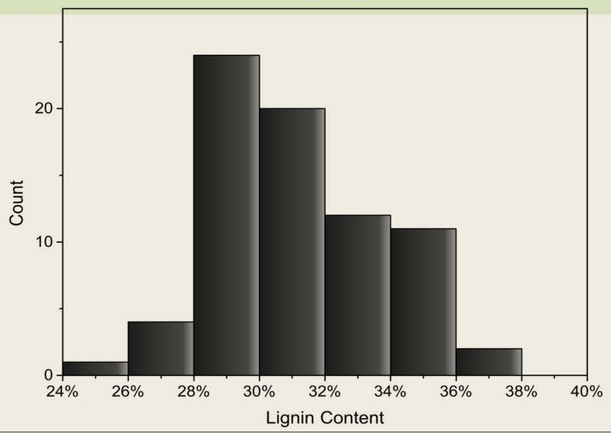


Figure1. Lignin content after dilute acid treatment. We treated over 160 samples of biomass. Each sample was duplicated to have more consistent outcome.

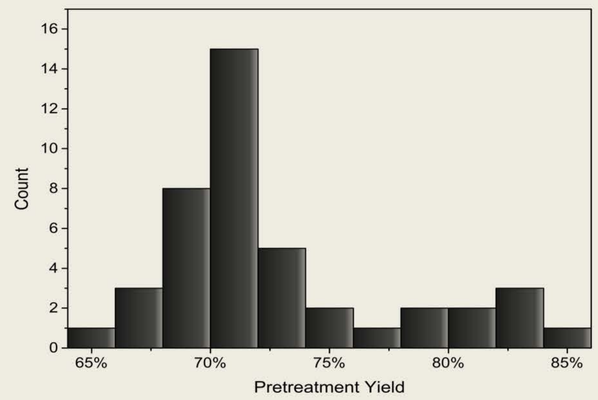


Figure 2. 46 total samples were run through the pretreatment process. These are the results after tube reaction and filtering process was completed. The yield represents the biomass that was weighed before treatment and the remaining biomass weighed after pretreatment.

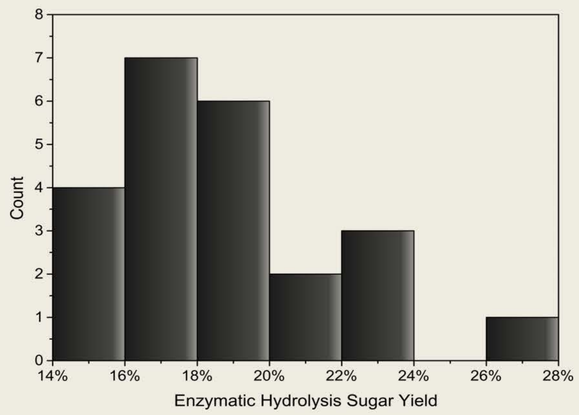


Figure 3: There was a total of 36 samples that we ran through the enzymatic hydrolysis treatment. Each sample was duplicated. These are the results of glucose content after 72 hours in the incubator.

Internship and Outreach

This internship has granted me a greater understanding of the biofuels project. I did not realize how big the picture really is when it comes to this particular field. It has given me an opportunity not only to do research, but to understand why there is a calling for this particular field. In figure 4, we can see the area in which my research was focused. It is one of many very important research areas within NARA that we focused on in my lab.

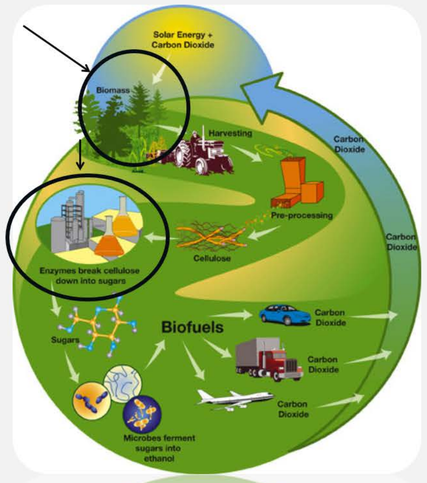


Figure 4. Picture courtesy of Brian from the webpage KEETSA: The Eco-Friendly and Green Blog Search Engine. "Biofuels Answer Fuel Issues, What About Food?" August, 15th.2007. Web.http://keetsa.com/blog/ecofriendly/biofuels-answer-fuel-issues-what-about-food/

MESA Day

One very good thing I wanted to mention about this internship is that I was able to take what I learned in the lab and use it to teach others. I know that NARA is a great leader of biofuels education. My mentor, Dr. Xiao Zhang, holds a teaching session every summer in our lab to help high school students from the Math Engineering Science Association understand basic concepts of chemistry. Four Ph.D Students and myself helped instruct the activities for the day. It was a great experience and I found the students were very grateful to learn also.

Conclusion

❑ Significant variations in the chemical composition and the degree of biomass recalcitrance are found among and within D. fir families.

❑ While the correlation between lignin/carbohydrate content and biomass recalcitrance is not apparent, the extractives content showed a strong negative impact.

❑ Chemical composition is not the only factor determining the recalcitrance of softwood for pretreatment and enzymatic hydrolysis.

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AND

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