The Viability of Tree Leaves for Cellulosic Ethanol.

Testing Select Quercus & Acer saccharum species for glucose content through acid & enzyme hydrolysis

Ryan Caudill
Grove High School
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Purpose

The purpose of this project is to measure the glucose levels in leaves collected from two varieties of sugar maple and two varieties of oak trees to see if they are viable sources for cellulosic ethanol production.

The varieties to be tested are:

Acer saccharum "Caddo" (Caddo sugar maple)
Acer saccharum "Commemoration" (Commemoration sugar maple)
Quercus rubra (red oak)
Quercus alba (white oak)

Hypothesis

A. It is hypothesized that the leaves of two sugar maple varieties, *Acer saccharum "Caddo"* and *Acer saccharum "Commemoration"* will contain higher levels of glucose than the leaves of the two varieties of Oak, *Quercus alba* and *Quercus rubra*.

B. It is also hypothesized that the leaves of all varieties chosen will contain enough glucose to be viable sources for cellulosic ethanol production.

Background Research

Carbon based fuels have been used in past generations and generations to come. Carbon based fuels are a great source of energy this is why they are used as fuels. When these fuels are burned for their energy to be released this will add carbon to the atmosphere, and that's not a good thing. Carbon is the primary greenhouse gas emitted by human activity. Also Carbon fuels are in a limited supply, they are nonrenewable and they are being used up at a very quick rate. It is important for the introduction of good reliable fuels that can be used on a very large scale.

Different sources that are being introduced that can help this issue are called Biofuels. Biofuels are fuels that are made from natural plant products such as corn and soybeans that can be grown and harvested.

Background Research (Continued)

Corn and soybeans are great resources for biofuels because they have large amounts of glucose contained within these plants. However, corn and soybeans are a major food resource. Using them both for fuel and food puts a higher demand on these products. Corn and soybeans are food that has always been in high demand. This is where other biofuels that contain large amounts of glucose or cellulose can be used in the place of food products. Some example would used cooking oils and grasses. Anything in abundance, made from plants might be a possible solution.

Tree leaves are renewable, and each fall we have a great abundance of leaves. Many people either bag them, adding to the land fill problem and some people burn them which would add carbon to the atmosphere. Why not check to see if leaves contain enough glucose for use in ethanol fermentation.

Background Research (Continued)

Glucose ($C_6H_{12}O_6$) is the main sugar that is used in the production of ethanol. Glucose is a high energy sugar that is burned in metabolism to release energy. Trees use glucose to make another polymer called Cellulose. Cellulose is a rigid and tough material used to make cell walls. Cellulosic Biomasses are great sources of energy to be used in fermentation ethanol. Cellulosic Biomasses can reduce back to glucose through a variety of hydrolysis methods.

In this project I did not make ethanol with the glucose from the tree leaves. I tested to see if the amount of glucose contained in the tree leaves will be viable to be used in fermentation ethanol.

Methods

Leaf Collection & Preparation

A) Contact Honey Creek Nursery for permission to collect leaves from maple and oak varieties.

B) Air dry or use dehydrator to completely dry leaf material

C) Grind leaf material using a coffee grinder.

Prepare Solutions (Using following formulas)

A) Dextrose Solutions

1) 1% (0.9g Dextrose + 500 ml Distilled Water)
2) 2.5% (2.25g Dextrose + 500 ml Distilled Water)
3) 5% (4.5g Dextrose + 500 ml Distilled Water)
4) 10% (9.0g Dextrose + 500 ml Distilled Water)

B) Dinitrosalicylic Acid

1) 10g Dinitrosalicylic Acid
2) .5g Sodium Sulfite
3) 10g Sodium Hydroxide
4) 1L Distilled Water
5) Mix well in a large beaker and let the solution sit overnight to reach equilibrium

Methods (Continued)

C) Rochelle Salt

1) 56.45g Potassium Sodium Tartrate

2) 500 ml of Distilled Water

D) Sulfuric Acid 1%

1) Add 25 ml of 18M sulfuric acid solution to 225 ml of distilled water, adding the water first and then adding the sulfuric acid for a 10% solution

2) Take 15 ml of the 10% solution and add 135 ml of distilled water, adding the water then the sulfuric acid. This will yield a 1% solution

E) Enzyme Solution

1) Call the company providing the enzyme and ask for the EGU/g of the batch

2) Take the EGU/g and with 1.2g/1ml find the units/ml

3) Add the right amount of water to the solution to create a 10 EGU/1ml solution

Methods (Continued)

DNS Glucose Assay : Glucose Standard

A) Add 2ml of DNS reagent to 2ml of glucose sample in a test tube (to avoid the loss of liquid due to evaporation, cover the test tube with a piece of paraffin film if a plain test tube is used.) Repeat for all concentrations of glucose stock solutions.

B) Heat the mixture at 90 C for 15 minutes to develop the red-brown color

C) Add 1 ml of a 40% Potassium Sodium Tartrate (Rochelle salt solution to stabilize the color

D) After cooling to room temperature in a cold water bath, record the absorbance with a spectrophotometer at 540 nm

Methods (Continued)

Acid Hydrolysis (Sulfuric Acid):

A) Add .5g of prepared leaf material to 10 ml of 5% H_2SO_4 solution in a test tube

B) Invert and mix well

C) Incubate the group of test tubes at 90 degrees Celsius for 2 hours

D) Cool and centrifuge

E) Remove 2ml of supernatant and move to smaller test tube.

F) Follow method describe above for DNS Glucose assay to test supernatant for glucose content.

G) Filter remaining acid from plant material, wash with distilled water, dry and freeze for second test to be done with enzyme hydrolysis.

Methods (Continued)

Enzyme Hydrolysis

A) Put 9.5 mL of cellulase enzyme solution into test tubes

B) Add 0.5g of plant material (as prepared after acid hydrolysis)

C) Repeat for other 3 leaf materials

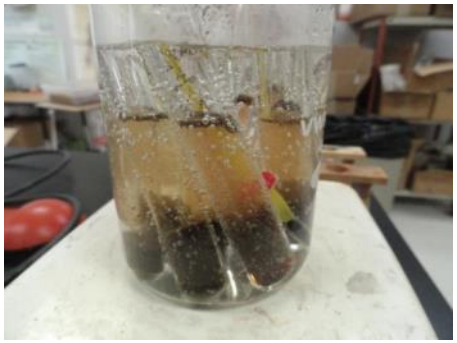
D) Incubate test tubes for 24 hours at 37 degrees Celsius

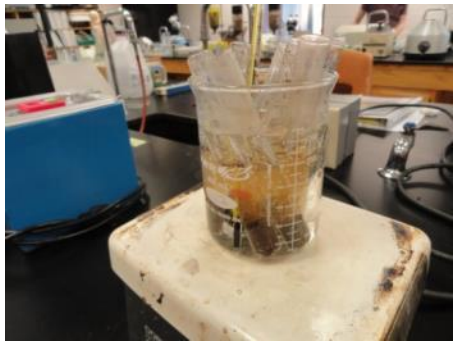
E) Place in hot water bath-90 degrees Celsius for 5-10 minutes to deactivate enzyme

F) Centrifuge Test tubes for 5 minutes

G) Test supernatant for glucose using DNS Glucose Assay

F) Repeat enzyme hydrolysis with untreated leaf material which has not gone through acid hydrolysis for the enzyme only test.





Results

It was hypothesized that leaves from two sugar maple varieties, *Acer saccharum* "Caddo" and *Acer saccharum* "Commemoration" would contain higher levels of glucose than leaves from two varieties of Oak, *Quercus rubra* and *Quercus alba*.

This hypothesis is rejected.

Enzyme Hydrolysis after acid hydrolysis showed that Oak leaves contained more glucose than leaves of sugar maples for leaf little. The same results were found with green leaves with enzyme hydrolysis after acid hydrolysis. Enzyme Hydrolysis only yielded the same results.

Results (Continued)

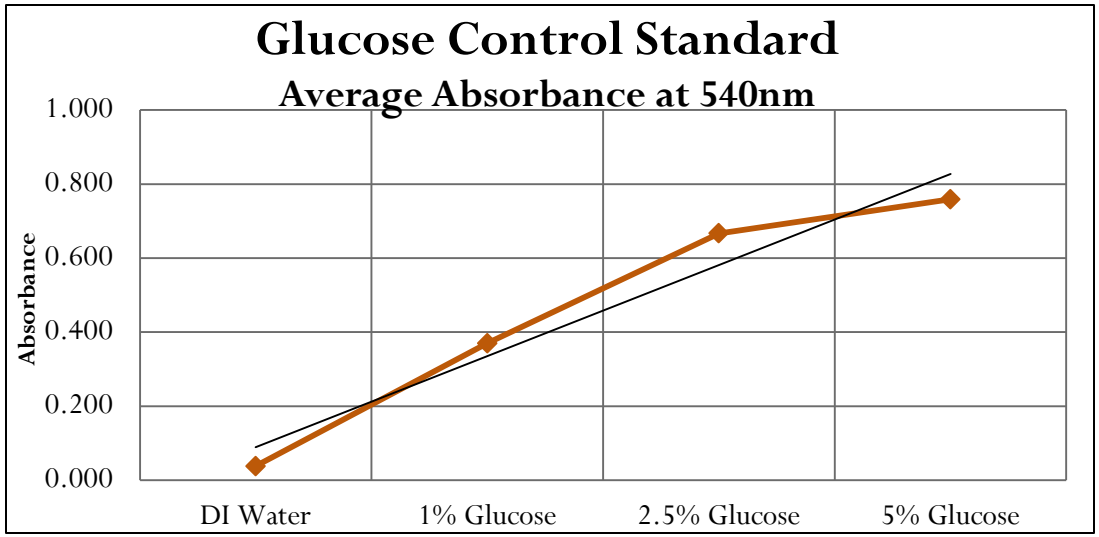
It was hypothesized that leaves of all varieties chosen will contain enough glucose to be viable sources for cellulosic ethanol production.

This hypothesis is accepted.

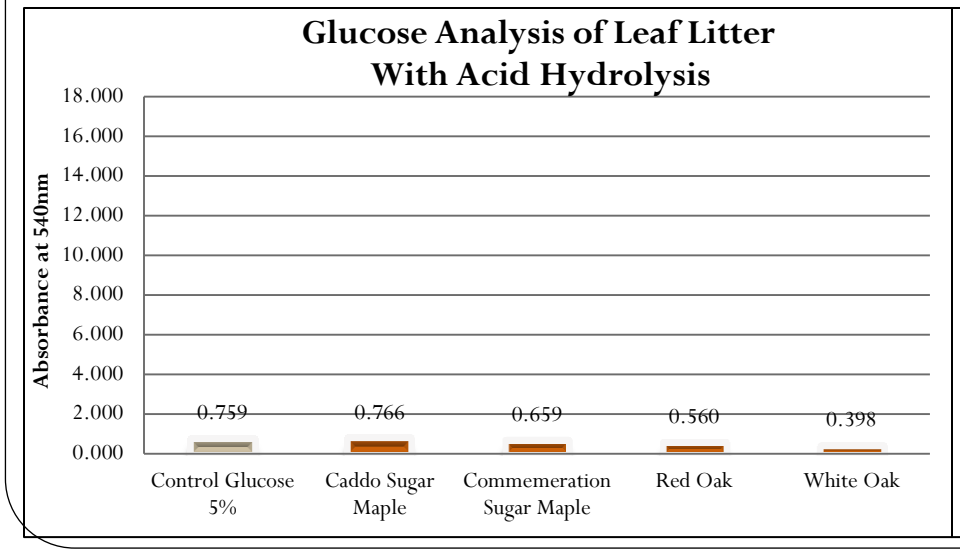
The percent absorbance increased over 5% glucose with enzyme only techniques with both leaf little and green leaves were over 500%. Leaf litter testing showed that *Acer saccharum* "Caddo" was over 760.34%, *Acer saccharum* "Commemoration" was over 554.49%, *Quercus rubra* was over 557.44%, and *Quercus alba* was over 826.22%. Green Leaf testing showed that *Acer saccharum* "Caddo" was over 1151.65%, *Acer saccharum* "Commemoration" was over 686.56%, *Quercus rubra* was over 1388.80%, and *Quercus alba* was over 2179.31% over the control of 5% glucose solution.

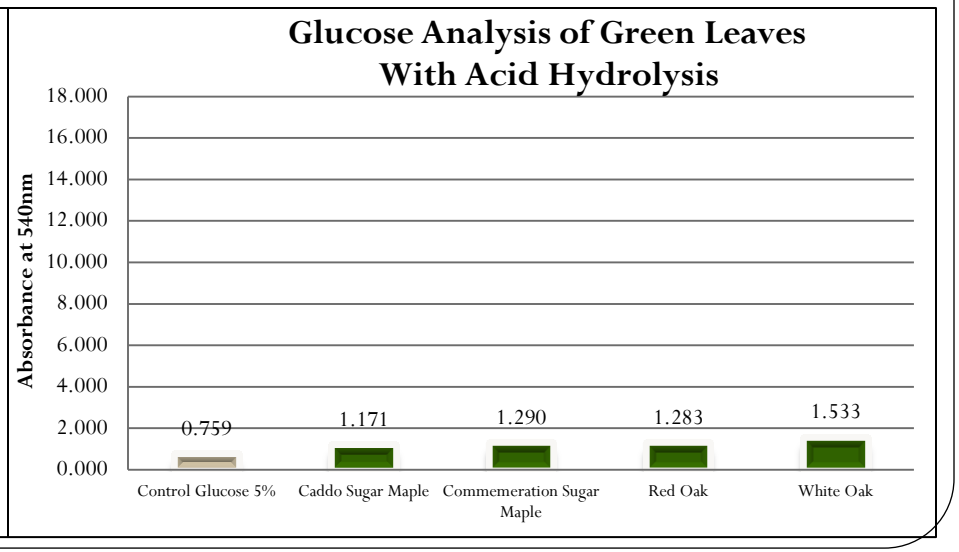
Graphs: Glucose Standard & Acid Hydrolysis

The data shown is compared to a 5% Glucose Standard as shown in the graph to the right.

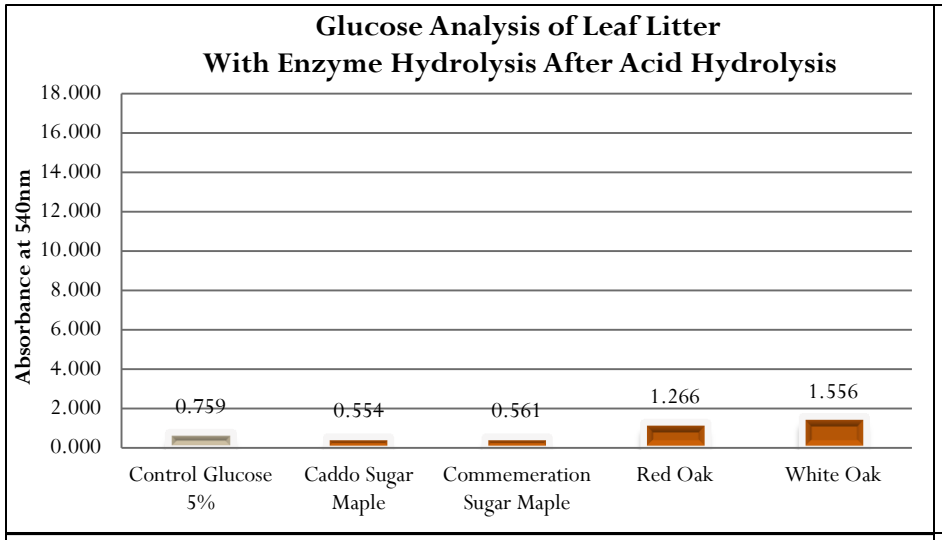


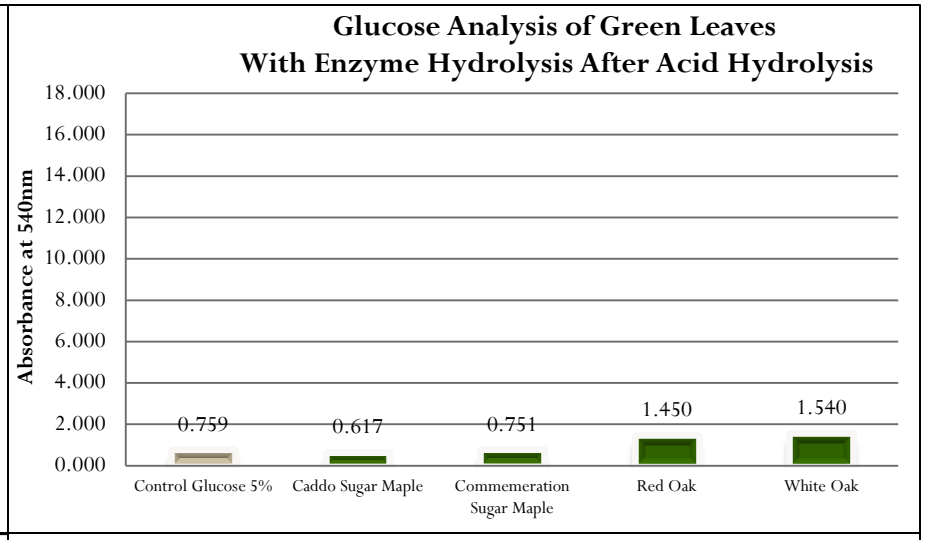
All Graphs compare the leaves to the 5% Standard.

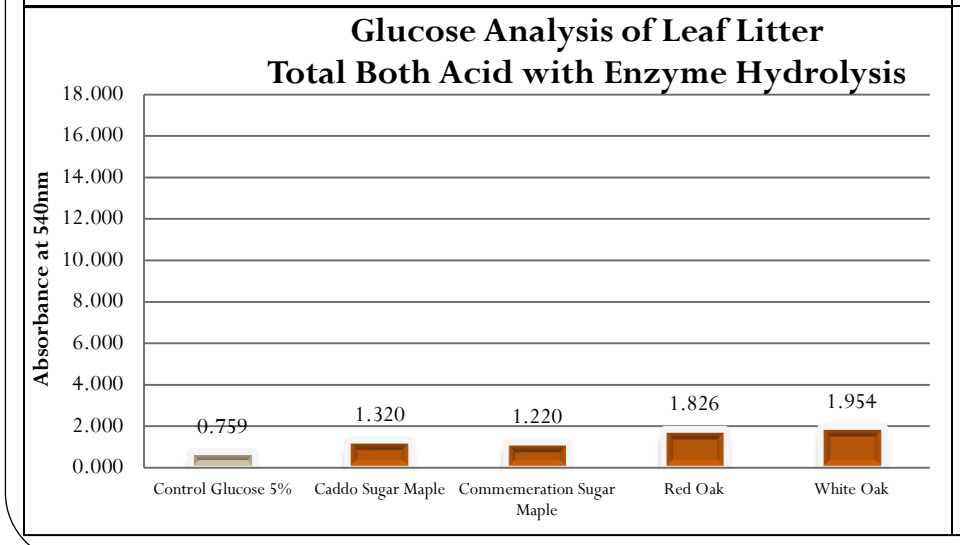


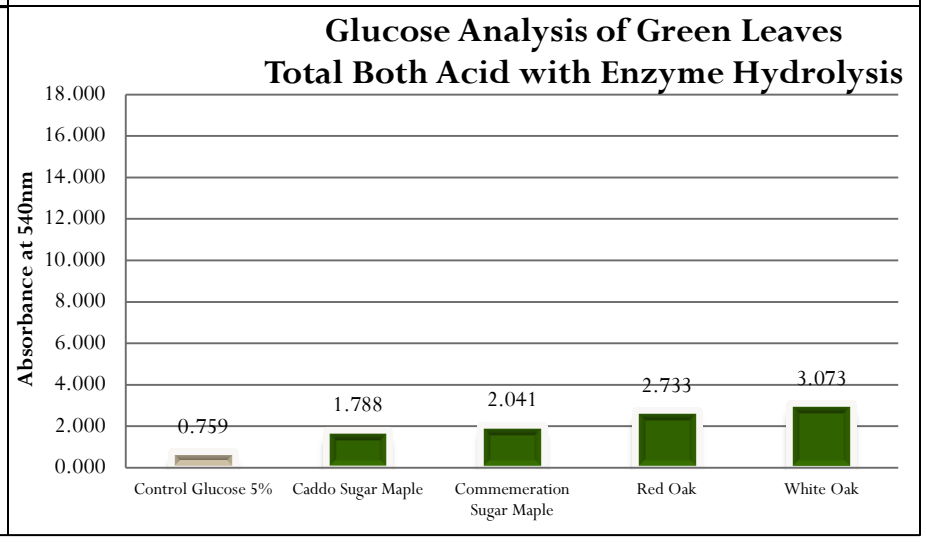


Graphs: Enzyme After Acid &Total for both Acid Plus enzyme Hydrolysis.

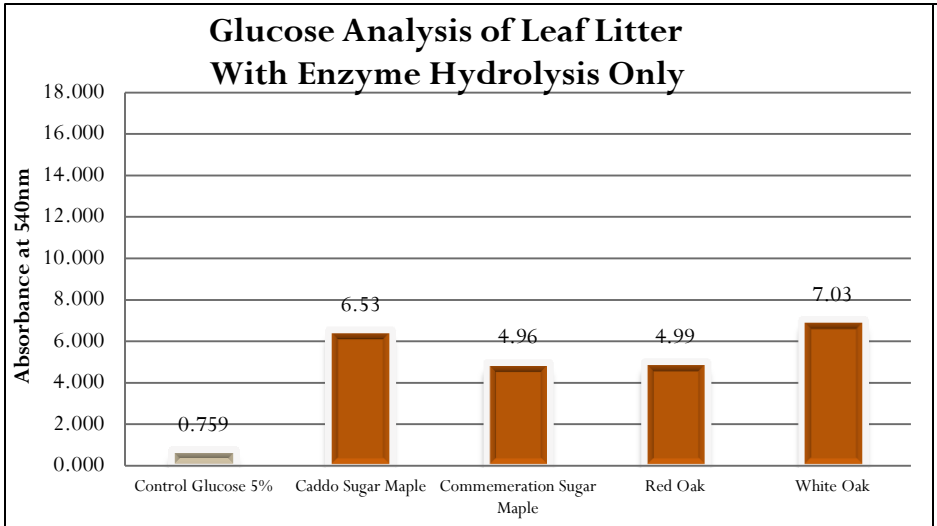


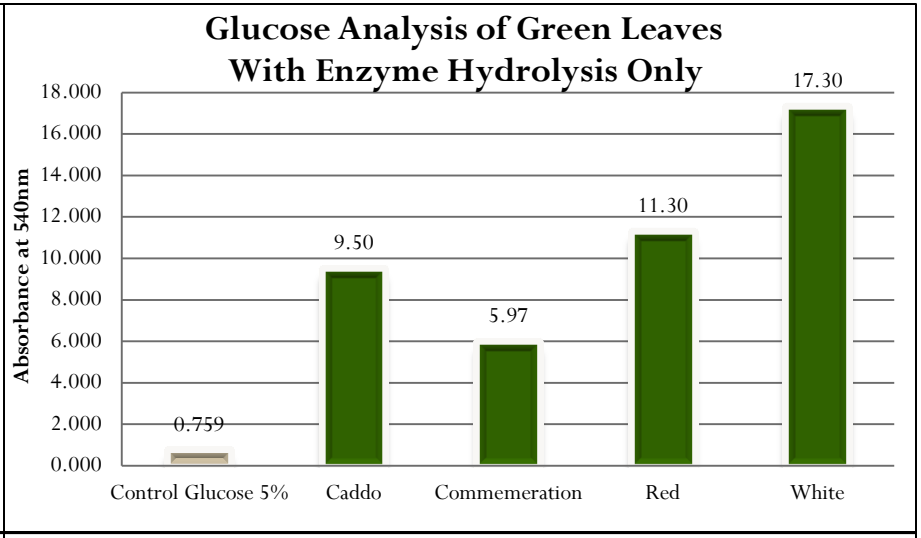


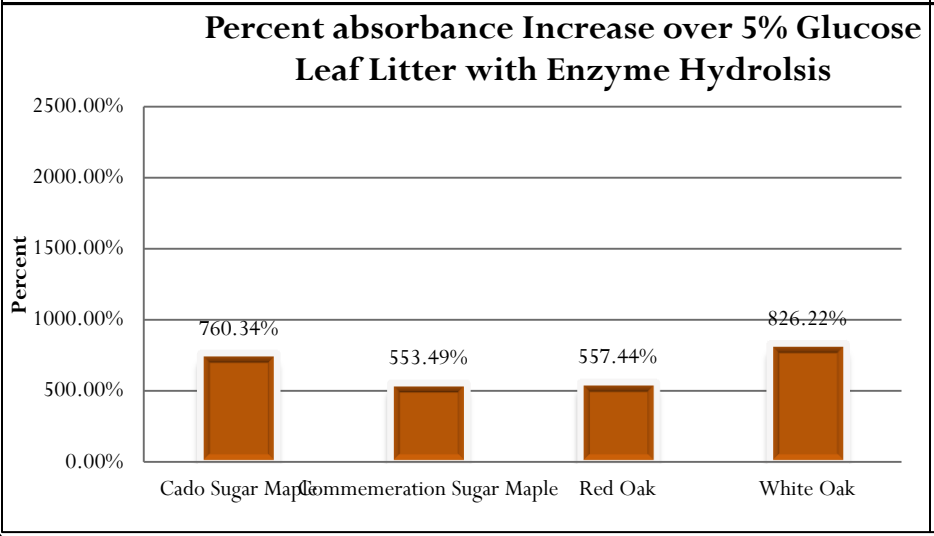


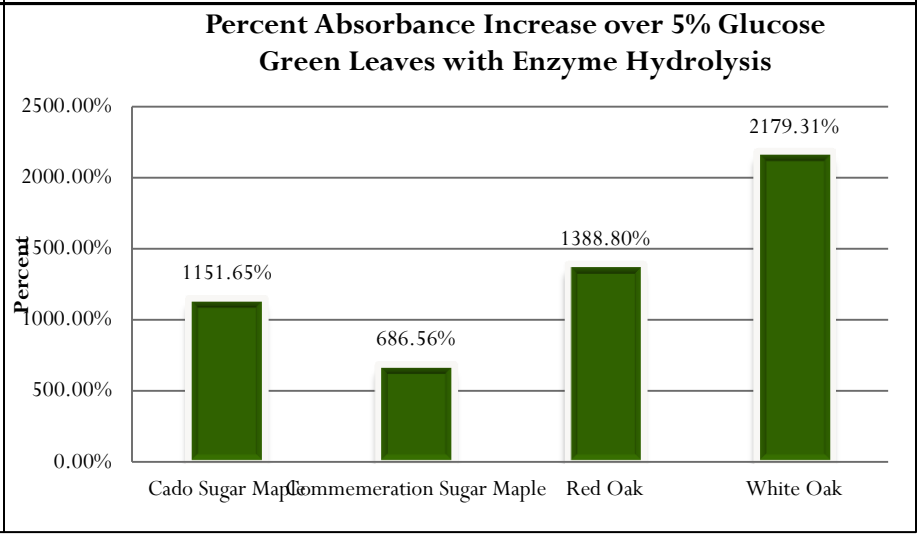


Graphs: Enzyme Hydrolysis Only







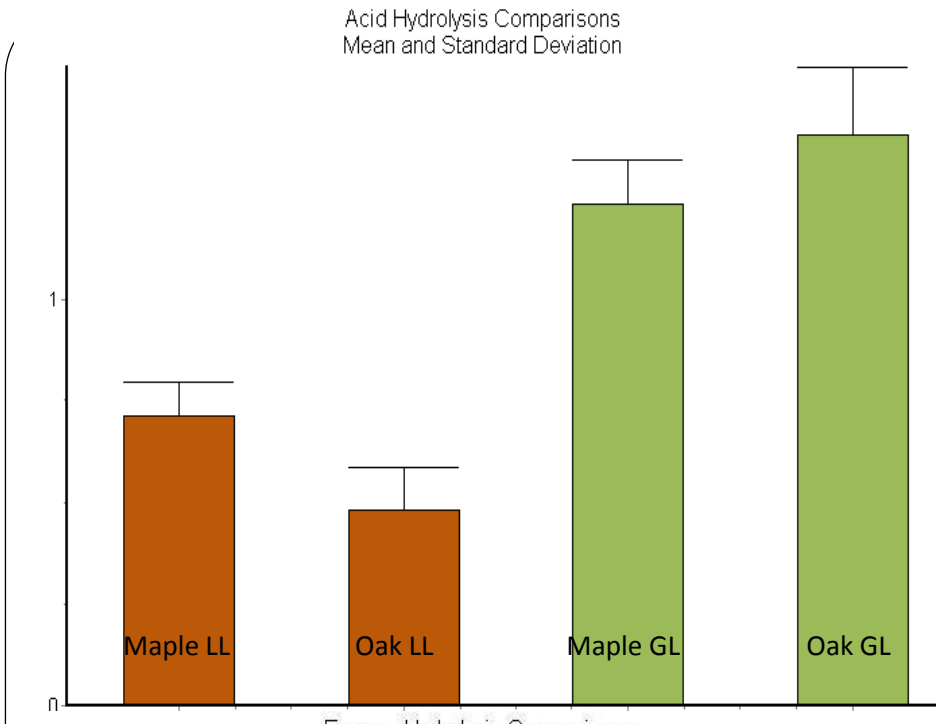


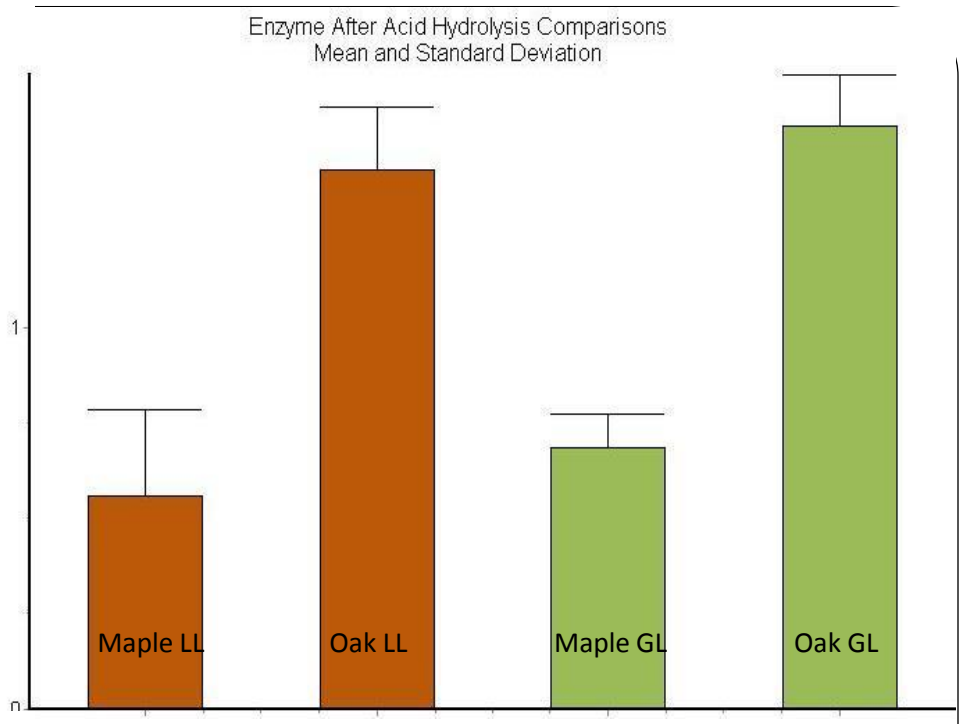
Statistical Analysis of Maple vs Oak Glucose Levels Using A One-Way Analysis of Variance (ANOVA)

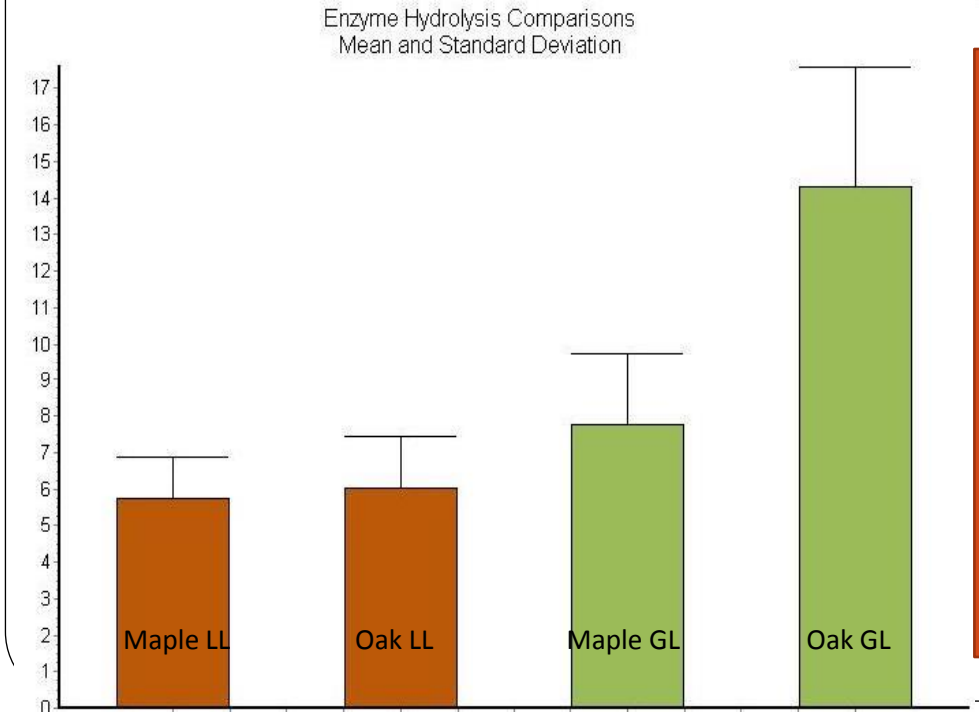
Measured by DNSA Glucose Assay Spectrophotometer Absorbency at 540nm

Test	Comparison	P Value	Significance
Acid Hydrolysis	Maple Leaf Litter vs Oak Leaf Litter	< 0.001	Extremely Significant
Acid Hydrolysis	Maple Green Leaves vs Oak Green Leaves	< 0.001	Extremely Significant
Enzyme After Acid Hydrolysis	Maple Leaf Litter vs Oak Leaf Litter	< 0.001	Extremely Significant
Enzyme After Acid Hydrolysis	Maple Green Leaves vs Oak Green Leaves	< 0.001	Extremely Significant
Enzyme Hydrolysis	Maple Leaf Litter vs Oak Leaf Litter	> 0.05	Not Significant
Enzyme Hydrolysis	Maple Green Leaves vs Oak Green Leaves	< 0.001	Extremely Significant

It is statistically shown that in Acid Hydrolysis with Leaf Litter, Maple had an extremely significance over oak leave litter. In enzyme hydrolysis only with leaf little, Oak had more but not a significance over the Maple.







Here you can see in the two cases of when Maple Leaf Litter in Acid Hydrolysis was extremely significant over Oak Leaf litter. In the Enzyme only testing you can see where the oak was higher but not significantly higher than the maple.

Impact

The results of this project are important to anyone who owns trees. Leaves from oak trees are high in glucose making them a promising source in the production of ethanol. In the South East United States, oak trees are plentiful. Knowing this, people of urban and rural areas can benefit from the leaves that they would otherwise put into landfills or burn releasing carbon into the atmosphere. Using the leaves to produce ethanol does not increase the carbon load in the atmosphere. People could sell their leaves and or recycle them to be used in ethanol production. This would be one more step in helping to reduce the rate of global warming and to reduce our human carbon footprint on the world.

Impact (Continued)

The information gained during this project could help researchers working with other cellulosic sources to be used as bio-fuels. Before now, starchy food products like corn are being used for ethanol production because starch is easier to break down than cellulose. As technology and methods become easier for the breakdown of cellulose, cellulose will increase as a viable source for ethanol. Hopefully the information about glucose levels in leaf litter will help advance the methods and spark someone's interest in using a renewable non-food source for ethanol.

Conclusion

The purpose of this project for measuring the glucose levels in leaves to see if they are a viable source for cellulosic ethanol production was achieved. All leaves tested had absorbance levels above the absorbance of the five percent glucose solution. Since the absorbance levels of the leaves were all over 500% higher than the control glucose solution, it could be assumed that the glucose levels were much higher than 5% glucose.

This project was done in a high school lab which did not have access to the precise measuring systems needed to make glucose standards high enough to compare to the glucose levels in the leaves. This did not allow me to convert the absorbance to exact glucose levels.

Conclusion (Continued)

Another problem I found when measuring the solutions from the enzyme only test, the glucose levels were so high the solutions had to be diluted so an accurate measurement could be taken.

A better glucose standard could be done to help find the precise glucose levels in the leaves. I would also like to alter the enzyme concentration to find the maximum amount of glucose in the leaves.

Bibliography

"BioFuels." *BioFuels: Cellulose Lab Teacher Guide*. LEHIGH Universtity, n.d. Web. 24 Oct. 2013. <http://www.ei.lehigh.edu/eli/energy/resources/handouts/labs/biofuel_cellulose_lab_teacher.pdf>.

"Caddo Sugar Maple (Acer Saccharum "Caddo") in Saskatoon, Saskatchewan (SK) at Lakeshore Garden Centres." *Lakeshore Garden Centres Plant Finder Tool*. Lakeshore Garden Centre, n.d. Web. 21 Oct. 2013. <http://qscaping.com/20000020/Plant/5903/Caddo_Sugar_Maple>.

"Carbohydrates - Cellulose." *Carbohydrates - Cellulose*. Elmhurst College, n.d. Web. 24 Oct. 2013. <http://www.elmhurst.edu/~chm/vchembook/547cellulose.html>.

"Commemoration Sugar Maple (Acer Saccharum "Commemoration")." *Oakland Nurseries Plant Finder*. Oakland Nurseries, n.d. Web. 21 Oct. 2013. <http://plants.oaklandnursery.com/12130001/Plant/5904/Commemoration_Sugar_Maple>.

"Glucose." *Sugars*. Hyper Physics, n.d. Web. 23 Oct. 2013. <http://hyperphysics.phy-astr.gsu.edu/hbase/organic/sugar.html>.

Bibliography (Continued)

"Quercus Alba - Plant Finder." *Quercus Alba - Plant Finder*. Missouri Botanical Gardens, n.d. Web. 20 Oct. 2013. <http://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kempercode=l930>.

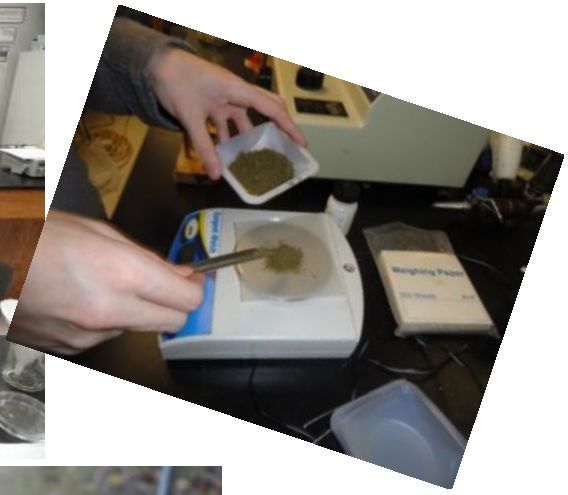
"Quercus Rubra - Plant Finder." *Quercus Rubra - Plant Finder*. Missouri Botanical Gardens, n.d. Web. 20 Oct. 2013. <http://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kempercode=i760>.

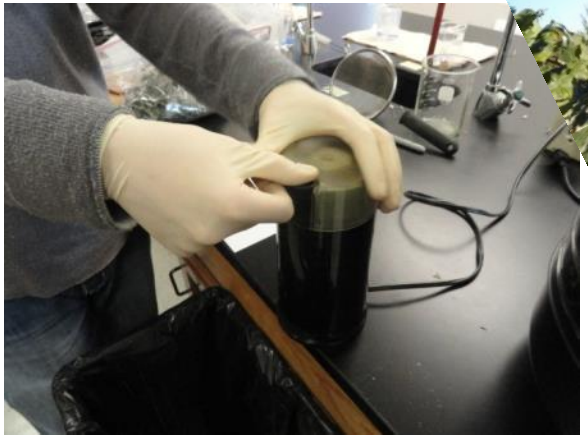
Riley, Mark R. "Efficient Extraction Method to Collect Sugar from Sweet Sorghum." *Journal of Biological Engineering*. Journal of Biological Engineering, 10 Jan. 2013. Web. 23 Oct. 2013. <http://www.jbioleng.org/content/7/1/1>.

"The Science of Color in Autumn Leaves." *The Science of Color in Autumn Leaves*. United States National Arboretum, 6 Oct. 2011. Web. 22 Oct. 2013. <http://www.usna.usda.gov/PhotoGallery/FallFoliage/ScienceFallColor.html>.

Wang, Nam S. "Glucose Assay." *Glucose Assay*. University of Maryland, n.d. Web. 22 Oct. 2013. <http://www.eng.umd.edu/~nsw/ench485/lab4a.htm>.



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