# Application of Carbon Based Fertilizer To Increased Biomass For Biofuel Production.

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## Problem / Question

Conventional agriculture practices are responsible for the over application of chemical salt fertilizers, which have many adverse effects on our environment. Can crop biomass production be increased while minimizing these inputs?

## Hypothesis

- Amending soil with a carbon based fertilizer enhances biomass production of the intended crop.

## Materials and Methods

- Carbon fertilizer (Buhl) was applied at recommended label rate of 3 gal $ac^{-1}$ to separate field plots of high biomass sorghum at 35 days after sowing and energy beet at 71 days after sowing, and repeated again 2 weeks later. Plants were harvested 20 days after treatment and analyzed for nutrient concentration, dry weight (biomass), and percent Brix.
- Treated (T) Plots were compared to Untreated (UT) Plots for Results.

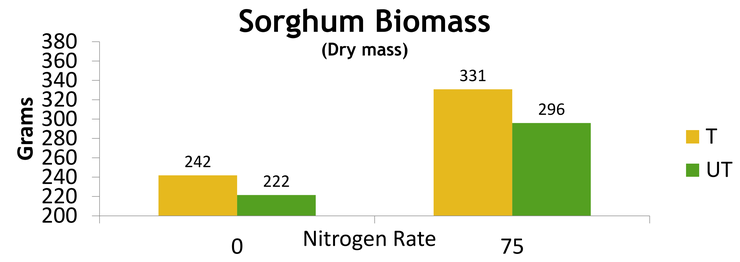
Applying Carbon Fertilizer

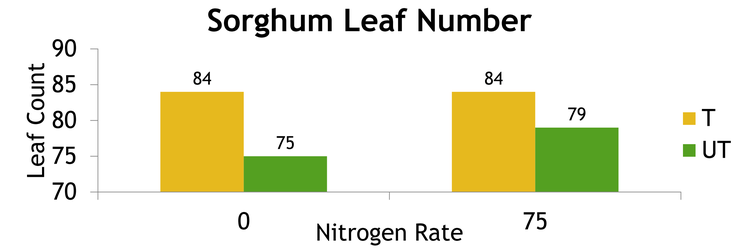
LI-3000 Leaf Area Meter

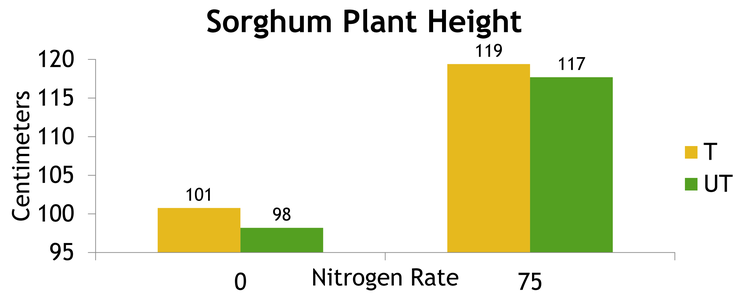
Refractometer

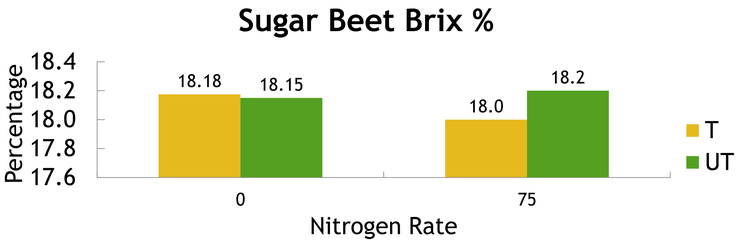
- Nitrogen (N) was applied to three replicated field plots of sorghum and energy beet at a rate of 0 and 75 lbs $ac^{-1}$. One half of each plot was applied with carbon fertilizer (Buhl) using a back pack sprayer.
- Fresh weigh of sorghum was measured immediately after harvest. The leaves were then removed for leaf number and leaf area was measured using LI 3000 (Licor Inc, Lincoln, NE).
- After being weighed, energy beets juice was extracted and measured for brix percentage using a handheld refractometer (Mettler Toledo, Columbus, OH) .

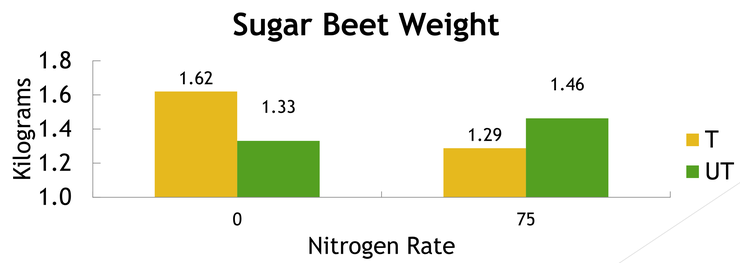
## Results




## In-Field Observations

- Treated sorghum plants showed less signs of midday wilt, possibly attributed to larger roots systems ability to forage for more water.
- Treated sorghum plants had thicker main stalks.
- Treated sorghum leaves were darker green in color, compared to control.
- Treated beets showed a significant increase (18%) in beet mass in 0 N treatment, but brix percentage was unaffected.
- Treated sorghum produced 10.6% more biomass with 75 lbs N and 8.3% more with 0 lbs N.
- Treated sorghum plants had an average 5 more leaves and were 2.5 cm taller.

## Conclusion

- Amending soil with a carbon fertilizer increased biomass, which would lead to a decrease in traditional chemical fertilizer inputs.
- Although there was significant biomass increase in both sorghum and energy beets, the energy beets showed no difference in brix percentage.
- Future research could evaluate application timings and rates for the addition of carbon fertilizers.

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## Acknowledgements

- I would like to thank Senior Agriculturist Jay Prater for the application of plot fertilizers and undergraduate student Real Kc for assisting in the preparation of samples for analysis.

# Conversion of Switchgrass to Butanol

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## Introduction

What is ABE (acetone-butanol-ethanol) fermentation)?

- Conversion of fermentable sugars to butanol, traditionally from food crops

Why Butanol?

Interest has grown in the properties of butanol over ethanol.

- The energy efficiency of butanol is more comparable to traditional gasoline than ethanol.
- Butanol does not absorb water as readily as ethanol and therefore presents advantages in its use with current gasoline-based infrastructure.
- Butanol is a 4 carbon compound and therefore has potential to be upgraded to long carbon chain fuels such as jet fuels

Why Switchgrass?

As the US strives for energy independence, switchgrass is considered to have high potential as a sustainable biofuel feedstock.

- Switchgrass can be grown on marginal land with low water and nutrient requirements, and it is also a high yielding plant with high cellulose content.
- However, there are toxic compounds such as phenolic compounds , furfural, hydroxylmethyl furfural in switchgrass-derived hydrolyzate that cause poor ABE fermentation requiring detoxification.

## Objective

To remove toxins from the switchgrass hydrolyzate in order to achieve butanol and ABE production that is comparable with a pure glucose medium.

## Materials and Methods

Pretreatment:

- Hydrothermal pretreatment.
- Water-solids slurry at 200ºC, 500 rpm, and high pressure for 10 min using a Parr reactor (shown right).
- Pretreated switchgrass was washed 4 times by total 2 L deionized water.

Hydrolysis:

- Enzymatic hydrolysis was preformed.
- Flasks contained pretreated switchgrass, the enzyme, water, and buffer.
- 50ºC and agitated for 48 hours.
- Hydrolyzate was detoxified using an adsorbent.

Fermentation:

- Strain: *Clostridium acetobutylicum* ATCC 824
- Treatments:
  (1)Glucose control,
  (2) glucose and xylose control as a simulation of hydrolyzate
  (3) non-detoxified hydrolyzate
  (4) detoxified hydrolyzate.
- Duration: 72 hours in an anaerobic chamber

## Results

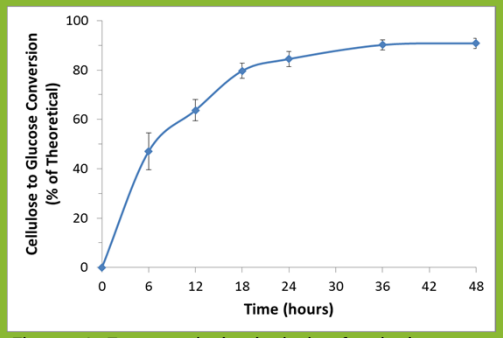


Figure 1: Enzymatic hydrolysis of switchgrass to glucose.

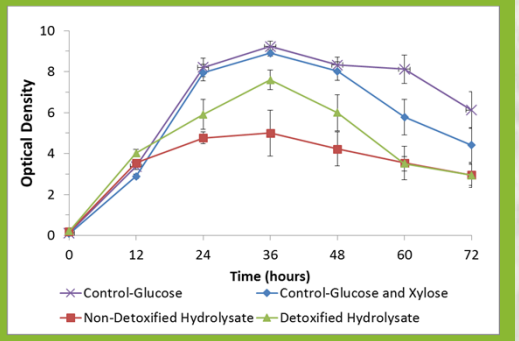


Figure 2: Cell growth profiles.

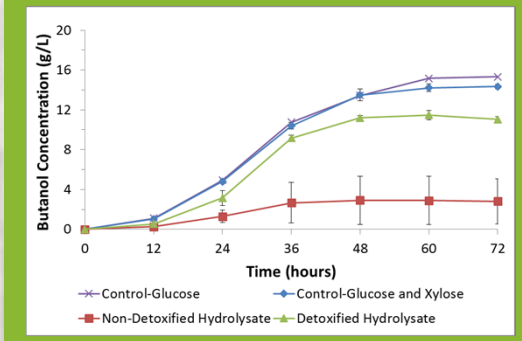


Figure 3: Butanol production profiles.

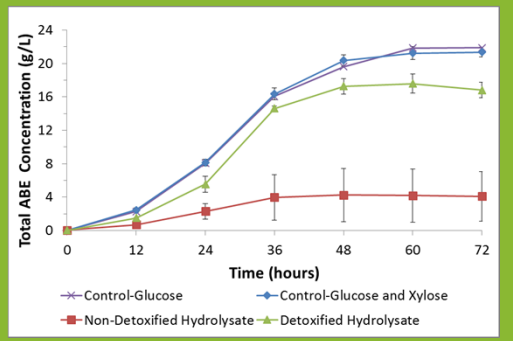


Figure 4: ABE production profiles.

## Conclusions

- Over 90% of cellulose in the pretreated switchgrass were converted to glucose in 48 h of enzymatic hydrolysis.
- Poor butanol (less than 2 g/L) was obtained in non detoxified hydrolyzate.
- Detoxification of the hydrolyzate successfully resulted in butanol production comparable to control treatments with pure sugars.
- Switchgrass has the potential use in butanol production using the process developed in this study.

## Acknowledgements

Special Thanks to Dr. Hasan Atiyeh, Dr. Kan Liu, and Oscar Pardo-Planas for their assistance.
Funding: NSF-Research Experiences for Undergraduates (REU), Sun Grant Initiative (U.S. Dept. of Transportation)and Oklahoma Agricultural Experiment Station.

# Canola Bioproduct Formation from Field to Factory

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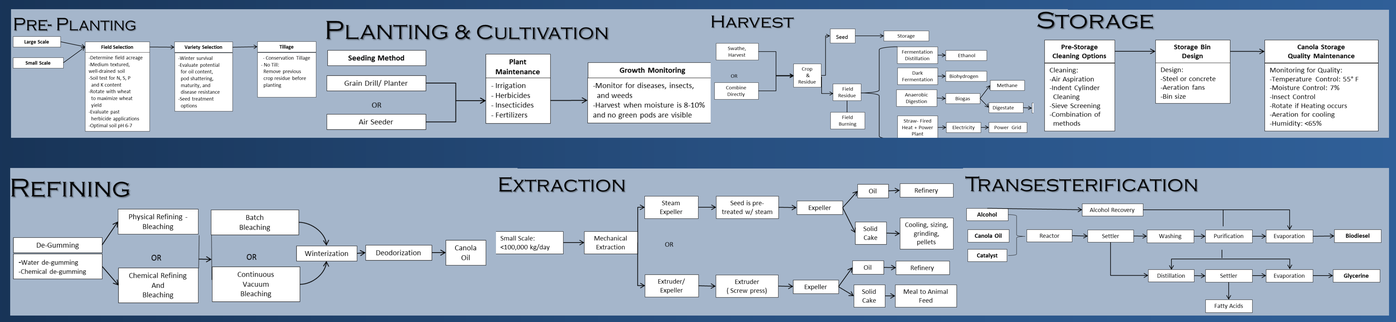
Project Mentor: Dr. Carol Jones, Ph.D., P.E.

## Background

In the state of Oklahoma, canola is a relatively new crop. In the 1970's, Canadian plant breeders naturally modified rapeseed into a non-toxic plant, canola (*Brassica napus*) with lower levels of erucic acid and glucosinolates. Subsequently, canola production has bloomed, making Canada the number one producer of canola in the world. In addition, canola production in the U.S. has grown substantially, and Oklahoma is the second leading producer of canola in the U.S. Local research initiatives have proved that winter wheat yields will increase 8-15% when grown in rotation after canola. Oklahoma farmers have been growing winter canola to manage weeds and maximize wheat yields. In order to maximize return on investments, would it be more cost-effective to develop the canola by-products (meal, oil, and biodiesel) on the farm or sell the seed to an off-site refinery?

## Canola Biomass Supply Chain




## Discussion

Research in the Great Plains growing region continues to prove that it is more lucrative to grow wheat in rotation with winter canola, instead of growing wheat continuously If Oklahoma farmers chose to establish farm-based refineries, an extruder-expeller extraction system should be used because it creates meal with higher protein levels. (Kenkel et al.)

Biodiesel production costs are directly related to the price of canola oil feedstock, and any slight change in the oil price causes a dramatic change in the cost of biodiesel production. Canola biodiesel production costs are comparable to soybean biodiesel production costs. On a per acre basis, a canola oil and meal refining systems would create the most revenue.

## Objectives

Develop the optimum supply chain structure for canola bioproduct formation from the canola fields to the factory, including field preparation, cultivation, harvest, storage, transportation, and by-product development logistics.

1. Utilizing historical crop yield data and cost reports, predict canola biodiesel production costs.
2. Calculate expected revenue from production and sale of seed, meal, oil, and biodiesel.
3. Evaluate revenue estimates in order to determine which by-product will create the highest return on investment

## Results

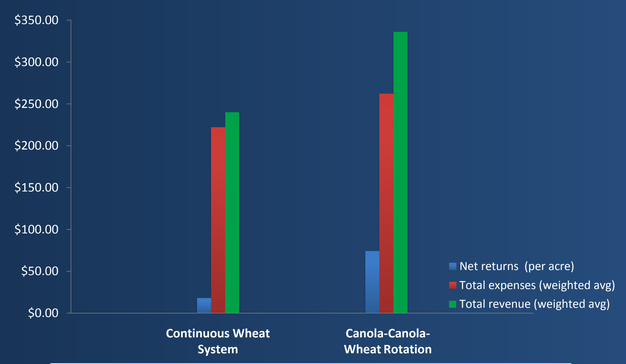



**Figure 1**: Profit comparison of continuous wheat systems vs. canola-canola-wheat rotation system


**Figure 2**: Biodiesel production cost comparison for canola and soybean oil feedstocks, respectively
Sources: Haas et al., USDA Economic Research Service

| Processing Cost ($/ton) | Extruder-Expeller (Kenkel et al. 2006) | Extruder-Expeller (2014) |
|---|---|---|
| Seed | $ 200.00 | $ 480.00 |
| Electricity | $ 8.19 | $ 8.19 |
| Total Utilities | $ 8.97 | $ 8.97 |
| Labor | $ 5.11 | $ 5.11 |
| Total Variable Costs | $ 27.54 | $ 27.54 |
| Overhead Costs | $ 5.83 | $ 5.83 |
| Total Cost | $ 233.38 | $ 535.64 |

**Figure 3:** Canola processing cost (per ton) using an extruder-expeller extraction system to produce canola oil and meal
(Sources: Kenkel et al. and OSU Canola Extension)

| Canola Product | Production (per acre) | Price (per unit) | Revenue ($/acre) |
|---|---|---|---|
| Seed (tons) | 1.23 | $ 355.00 | $ 436.65 |
| Oil (gallons) | 145 | $ 7.98 | $ 1,157.10 |
| Meal (lbs.) | 0.75 | $ 222.60 | $ 166.95 |
| Biodiesel (gallons) | 77 | $ 3.10 | $ 238.70 |

**Figure 4:** Predicted revenue from various canola bioproduct pathways, calculated on a per acre basis.

## Methods

In order to understand the entire supply chain structure, an in-depth study was carried out to gain an understanding of the structure's intricate details. Articles, reports, and studies were evaluated and sorted by topic relevant to the supply chain categories: pre-planting, cultivation, harvest, storage, extraction, refining, transesterification, and transportation. Accordingly, this data was used to develop an optimized supply chain flow chart. Next, a Canola Budget Generator from OSU's canola extension was updated and used to estimate current canola farming and harvest costs, expected revenue, and projected returns. In addition other budget spreadsheets were developed to predict production costs of canola biodiesel , oil, and meal. Last, the projected revenues were compared in order to determine which product could create the greatest profit per acre.

## Future Research

In the future, canola supply chains could be divided into individual steps and studied in more detail. Ideally, biomass supply chains are best modeled using specialized software. This modeling software is a better tool for predicting outcomes because it evaluates specific weather data, machine efficiency statistics, transportation logistics, and other figures to model the supply chain. More specifically, modeling software could be used to determine the feasibility of a large-scale canola biodiesel production facility. Similarly, modeling software could be used to estimate the number of facilities necessary to produce enough biodiesel to meet consumer demands.

## Acknowledgements

First of all, I would like to thank the National Science Foundation for funding this research opportunity for undergraduate students. In addition, I would like to extend a special thanks to my project mentor, Dr. Carol Jones, for assisting in the development of my project and for advising me along the way. Also, I would like to thank Dr. Kakani and all of the other Oklahoma State University faculty who made this program a success. Last, I am thankful for all of the great support staff and tour guides who graciously allowed us to visit the National Weather Center, the Noble Foundation, and the Abengoa Biorefinery.

# Red Cedar for Fuels and Chemicals: Thermal Devolatilization of Heartwood and Sapwood

## Introduction

The encroachment of red cedar (Juniperus virginiana) has created economic and ecological strains in many areas of Oklahoma. The National Research Conservation Service (NRCS) estimates that 760 acres are being infested per day in Oklahoma. This translates into 300,000 acres per year lost to red cedar and an estimated 447 million in loss in 2013. The invasiveness of red cedar has spurred landowners to develop strategies of control. Red cedar is viewed as a resource of no value and is usually burned. The energy lost in this process is never recovered and is never utilized. Red cedar can be changed from an underutilized biomass source into a valuable resource by thermal conversion technologies(incineration, pyrolysis, or gasification). This studies aim is to characterize red cedar by the devolatilization of its two major components sapwood and heartwood. Knowing the degradation behavior will allow for predictions of heating rates and expected products.

## Objective

To characterize the devolatilization of red cedar's components, sapwood and heartwood, in order to help optimize further processes such as pyrolysis or gasification

## Materials and Methods

Global devolatilization equations were used to determine reaction kinetics of red cedars heartwood and sapwood components. Regression in Excel was used to calculate activation energy, E(KJ mol-1 ), pre-exponential factor, A(s-1 ), and n the order of reaction . Feedstock used was locally obtained red cedar (Figure 3) that was ground to 0.5mm (Figure 2) in a Thomas-Whilley mill (Arthur H. Thomas Co., Philadelphia, PA). The proximate analysis was calculated using ASTM Standard E1756.08, ASTM Standard E1755.01 and ASTM Standard D3175.11. Table 1 displays the proximate analysis. The ultimate analysis was completed by Midwest Microlab Inc. and is displayed in Table 2. Thermogravimetric profile calculated using Thermo Scientific TGA (Figure 2). The reaction kinetics were modeled using a combined linear reaction rate and Arrhenius equation.

Table 1: Proximate analysis

| | Heartwood | Sapwood |
|---|---|---|
| Moisture Content(d.b.) | 20.2% | 19.9% |
| Heating Value | 19.2561MJ/kg | 18.244MJ/kg |
| Ash content | 1.5% | 1.2% |
| Volatile content | 63.5% | 65% |
| Fixed Carbon | 35% | 33.8% |

Figure 1: Ground samples heartwood on left and sapwood to the right

Table 2: Ultimate Analysis

| Element | Heartwood | Sapwood |
|---|---|---|
| Carbon | 48.23% | 46.45% |
| Hydrogen | 5.44% | 5.33% |
| Nitrogen | 0.21% | 0.19% |
| Oxygen | 45.96% | 47.64% |
| Sulfur | 0.15% | 0.16% |

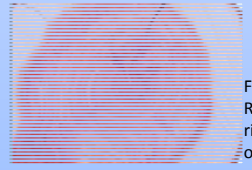


Figure 3: Cross section of Red Cedar, composed of inner ring of heartwood and an outer ring of sapwood

Figure 2: Thermo Scientific Versa Thermogravimetric Analyzer

## Results and Discussion

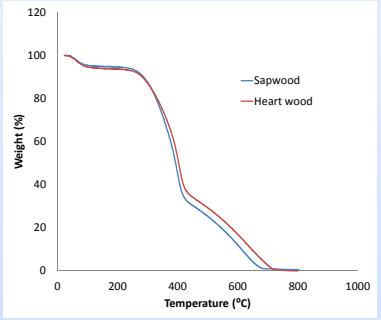



Figure 3: Weight loss in an inert atmosphere

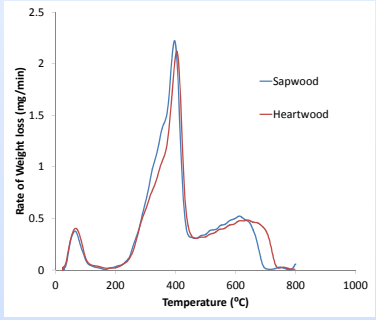



Figure 4: Rate of weight loss

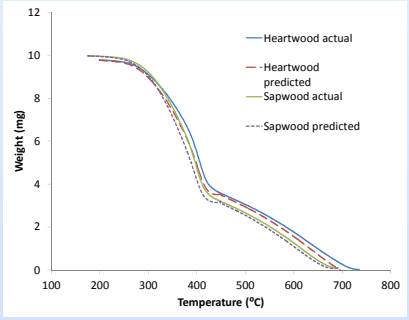



Figure 5: Plot of Predicted weight loss and actual weight loss

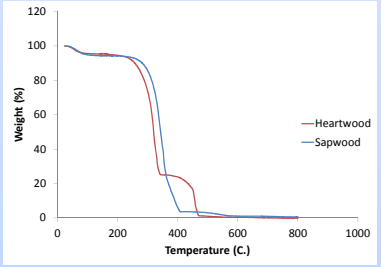



Figure 6: Weight loss in an oxidative atmosphere

Table 3. Reaction Kinetics in $N_2$(inert) environment

| Sample | Pre-exponential factor, A (s-1) | activation energy, E(KJ mol-1 ) | Reaction order, n | R Squared |
|---|---|---|---|---|
| Heartwood Peak1 | 122607.2 | 67.83 | 0.67 | 0.97 |
| Heartwood Peak 2 | 17.6 | 33.16 | 0.42 | 0.94 |
| Sapwood Peak 1 | 128299.9 | 67.43 | 0.71 | 0.97 |
| Sapwood Peak 2 | 46.6 | 38.03 | 0.42 | 0.97 |

$$\ln\left(\frac{-\frac{dw}{dt}}{W_f - W_i}\right) = lnA - \frac{E}{RT} + nln\left(\frac{W - W_f}{W_i - W_f}\right)$$

Figure 7: Combined linear Arrhenius and Reaction rate Equation

- Both sapwood and heartwood showed three separate devolatilization stages.
- Figure 3 displays weight loss vs. temperature. Both devolatilized before the standard gasifier temperature of 800°C
- Figure 4 displays the rate of weight loss vs. temperature. Both showed similar curves with the heartwood fully devolatilizing at 750°C as compared to sapwood at 700°C
- Figure 5 was calculated using the results displayed in table 3. The two peaks were analyzed separately using Excel's regression function.
- Figure 6 displays the oxidative atmosphere weight loss profile
- The predicted weight loss profile was calculated using the equation in Figure 7

## Conclusion

- In the inert and oxidative atmosphere both sapwood and heartwood displayed three devolatilization stages
- The most weight loss was in the second stage which contributed to about 60% of weight loss in an inert atmosphere
- In an oxidative atmosphere heartwood deviated from sapwood in the second and third stages
- Both samples converted before the standard gasifier temperature of 800°C
- Both samples had approximately 20% of weight left by 600°C, the standard pyrolysis upper bound

**Acknowledgments**

1. The National Science Foundation Research Experience for Undergraduate program for providing funding
2. Dr. Ajay Kumar for mentoring me throughout the project
3. Cody Collins, Zixu Yang, Kezhen Qian, Pratik Chaphekar, and Mark Gilstrap for helping with experimental design and implementation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# Cloning of Laccase Gene from Switchgrass

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## Introduction

Lignin is a polymer that is found in plant cell walls and helps in keeping the plant rigid and resistant to pathogens (Ruben Vanholme et al. 2010). However, lignin makes it difficult to extract the cellulose from the cell walls, and therefore to produce bioenergy from these plants. We hypothesize that laccases involved in lignin breakdown in switchgrass (*Panicum virgatum)*, can be manipulated and in turn facilitate cellulose extraction. In this project, the cloning of GC rich laccase genes from switchgrass was optimized. In total three laccase genes from switchgrass sharing thirty-seven percent homology were cloned.

## Objectives

The goal of this research project was to clone laccase genes from switchgrass that were identified using LigPred, a machine based learning technique (Weirick et al., 2014). One of the major challenges in this project was to clone this GC-rich genes of switchgrass genome. It is difficult to amplify genes that are GC rich because unlike adenine and thymine bases which are held together by two bonds, cytosine and guanine bases are held together by three; it is challenging to break apart three bonds in order to amplify the gene (GenScript).

## Methods

Three putative laccase genes that were less than 2 kilobases each (coding sequence) were selected for this project. *Setaria italica laccase-12/13-like (LOC101763003), mRNA, Setaria italica putative laccase-5-like (LOC101764906), mRNA* and *Setaria italica laccase-7-like (LOC101781236), mRNA.* To produce recombinant laccase proteins, the corresponding full-length coding sequences have to be cloned. As a first step, the genes were amplified by PCR from cDNA (obtained from mRNA) and ligated into a cloning vector (pGEM-T vector system, Promega). Subsequently, the recombinant plasmids were transformed into an *E. coli*. Successful cloning was tested with colony PCR and verified by DNA sequencing.

**1. PCR Amplification:** Switchgrass mRNA isolated from bud tissue was converted to cDNA by reverse transcriptase reaction. Primers (20-25 nts) were designed based on EST sequences of the putative laccase genes. The PCR reaction mixture contained 10x High Fidelity PCR Buffer, 10 mM dNTP mixture, 50 mM MgSO4, forward and reverse primers, cDNA, Platinum Taq High Fidelity, GC- Rich Enhancer, and water. The primer annealing temperatures were standardized (Fig. 1).

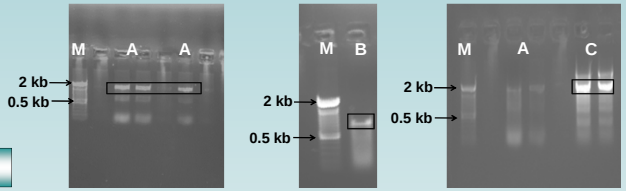


**Fig. 1. Agarose gel electrophoresis of PCR Amplified laccase genes**

**M**: Marker
**A**: *S. italica* laccase-12/13-like gene Gene size: 1.34 kb
**B**: *S. italica* putative laccase-5-like Gene size: 0.9 kb
**C**: *S. italica* laccase-7-like Gene size: 2.135 kb

**2. Ligation:** Using the Promega pGEM-T® Easy vector system, the amplified gene was ligated into pGEM-T vector and incubated overnight at 4˚C.

**3. Transformation:** After ligation, the DNA was transformed into DH5α *E. coli* strain and incubated at 37˚C overnight.

**4. Colony PCR:** Growing colonies were tested by colony PCR using gene specific primers (Fig. 2).

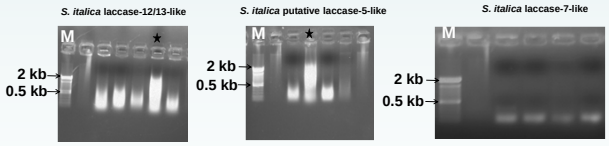


**Fig. 2. Agarose gel electrophoresis of colony PCR**

**M**: Marker ★: Positive colony

**5. Plasmid Isolation:** Colonies giving positive amplification of expected size were grown overnight and plasmid was isolated, using Thermo Scientific Gene JET Plasmid Miniprep Kit Protocol

**6. Sequencing:** The plasmid was sent for sequencing at the OSU core facility and the obtained sequence was blasted against NCBI and Noble Foundation switchgrass databases.

Results of the BLAST Analysis of the sequence data is shown below (Fig. 3).

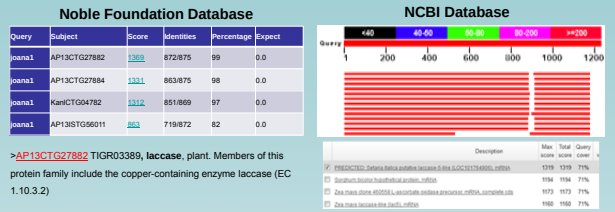


**Fig. 3. Noble Foundation and NCBI BLAST results .** The first hit in both databases was a laccase gene. In the Noble Foundation database the subject was verified in unitranscript ID in Noble Foundation Switchgrass Functional Genomics Server.

Additionally, restriction enzyme reaction was also performed to confirm the presence of the insert (Fig. 4).

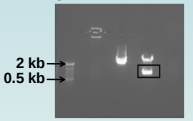


**Fig. 4. Restriction Enzyme Analysis**
Recombinant laccase-5 plasmid was restricted with EcoRI restriction enzyme to release and confirm the presence of insert gene.

## Conclusion and Outlook

One out of the three genes being studied was successfully cloned. For future study, the gene will be re-cloned into a suitable expressing vector and the recombinant laccase proteins in *E. coli* will be purified. Finally, commercial lignin will be used to identify if the recombinant laccase has any lignin degrading activity.

## References

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2. Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W (2010) Lignin Biosynthesis and Structure. Plant Physiology 153:895-905.
3. Tyler Weirick, Sitanshu S. Sahu, Rakesh Kaundal and Ramamurthy Mahalingam (2014) Machine learning based computational analysis for a novel laccase classification scheme and subtype prediction system (Accepted in BMC Bioinformatics).
4. http://blast.ncbi.nlm.nih.gov/Blast.cgi
5. http://switchgrassgenomics.noble.org/

## Acknowledgements

I would like to thank OSU Recombinant DNA core facility for providing the PCR machines and sequencing services. This study was funded by the Science Foundation, Research Experiences for Undergraduates Program

# Constructing a Biofuel Processes Life Cycle Inventory Database

Hannah Blankenship
Mentor: Dr. Scott Frazier

## Life Cycle Assessment Overview:

- An LCA gives values to the effects of a project/ process on the environment from "cradle-to-grave".
- It evaluates all the inputs and outputs of that process to determine potential environmental impacts, to compare multiple products for the least resource dependent, or which step of a process releases significant pollution.
- The Life Cycle Inventory step consists of quantifying energy and material inputs for all steps, as well as all outputs of wastes and emissions, into a database.
- An LCA evaluates environmental aspects of a process and does not include economic or social aspects.

## Project Objective:

- Begin creating the LCI database portion of a switchgrass to butanol conversion LCA.
- Analyze energy requirements:
  1. from agricultural processes to produce and harvest 1 acre of switchgrass.
  2. from commercial scale biochemical process to convert biomass to.
- Determine net energy production of entire process.
- Compare $CO_2$ equivalents in production and combustion for butanol/ethanol and gasoline.

## Methods:

- The conversion process was observed in the lab and detailed notes were taken to log all components of inputs and outputs. Figure 2 depicts a single step of the detailed flow diagram that was created.
- Once the diagram was complete for all steps, quantity data was researched and compiled to begin meeting the requirements of the LCI database.
- Energy yield and $CO_2$ emissions are based on estimates from other studies until the LCI is complete.

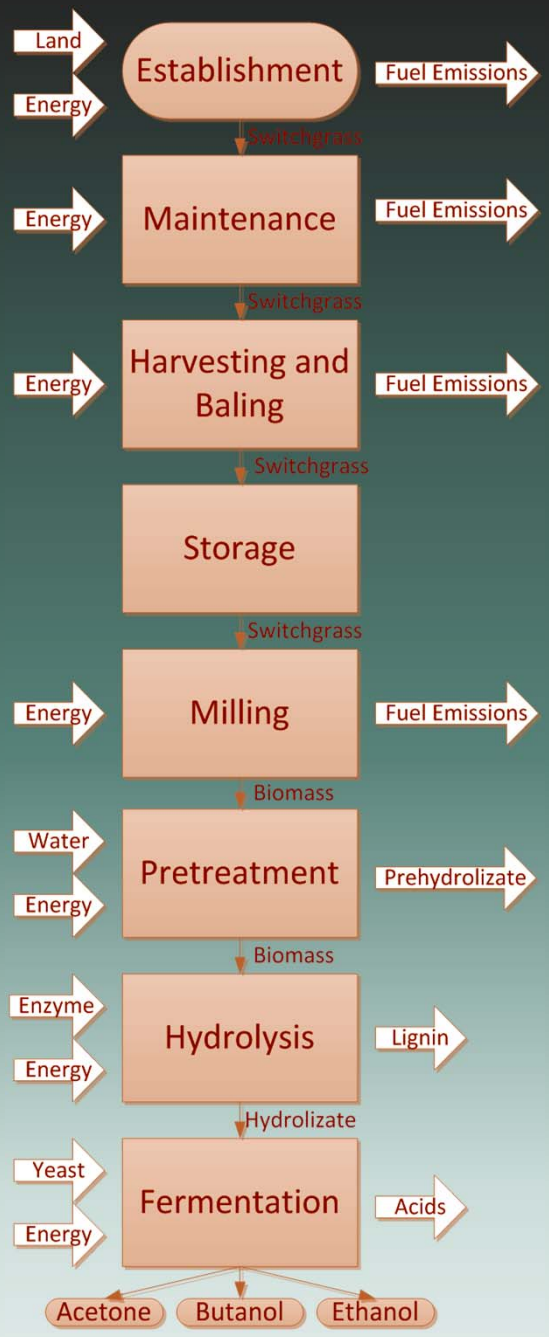



Figure 1: Simple Flow Diagram

| Table 1: Net Energy Return of Biofuel Production | |
|---|---|
| **Process Step** | **Energy Yield (MJ/ acre)** |
| Agriculture | 8,244.27 |
| Transportation | 733.48 |
| ABE Conversion | 47,279.90 |
| Total | 56,256.80 |
| Fuel Energy Density | 60,643.80 |
| Net Energy Return | 4386.97 |
| Energy Input to Output Ratio | 1.1 |

Data source: (Swana, et al., 2010)

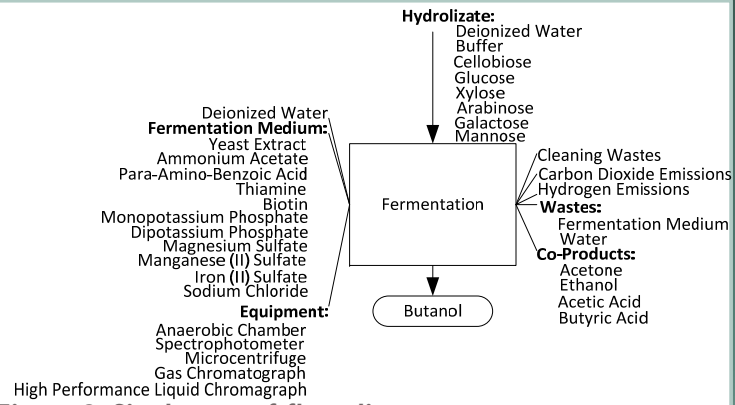



Figure 2: Single step of flow diagram

| Table 2: Kg of $CO_2$ Equivalent Based on 388 gal/ acre | | | |
|---|---|---|---|
| **Butanol/ Ethanol** | | **Gasoline** | |
| Production | 589 | Production | 814 |
| Combustion | 0 | Combustion | 2791 |
| Total | 598 | Total | 3605 |

Data source: (Marano and Ciferno, 2001) ; (Mclaughlin and Walsh, 1998)

## Future Research:

- Complete the LCI beginning with switchgrass establishment and ending with commercial-scale biochemical butanol production.
- Use SimaPro and GREET modeling software to compare the life cycle of butanol with gasoline on the basis of environmental impacts and other petroleum fuels.

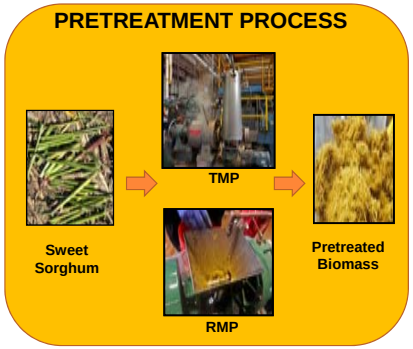
# Effect of enzyme level During Hydrolysis of sweet sorghum pretreated With Refiner Mechanical pulping (RMP) & Thermo Mechanical pulping (TMP) .

**H.Lemma, N. Pessani, D. Bellmer**

Robert M. Kerr Food and Agricultural Products Center, Biosystems and Agricultural Engineering,
Oklahoma State University, Stillwater, OK

## INTRODUCTION

Sweet sorghum (*sorghum bicolor* ) is an annual plant that can be cultivated in widespread areas from tropical to temperate climates with the potential to produce ethanol. It has a high yield of biomass and different parts of this plant can be hydrolyzed to fermentable sugar before further bioconversion to ethanol. The pretreatment is an important process before hydrolysis in order to remove or alter lignin and increase the accessibility of enzyme to cellulose. Mechanical pulping is the process by which fibers are produced through different mechanical methods. Refiner Mechanical pulping and Thermo mechanical pulping are the methods that used in the pulp and paper industry. The objective of this study was to compare the effect of enzyme loading on hydrolysis of TMP &RMP pretreated sweet sorghum**.**




## METHODS

Sweet sorghum was harvested at the OSU agronomy station . The harvested stalks were chopped using a seydelman bowl chopper. The chopped sweet sorghum was pretreated using Refiner Mechanical Pulping and Thermo Mechanical Pulping equipment at the USDA Forest products lab in Madison, Wisconsin. Xylanase, Cellulase, α-amylase and Amyloglucosidase are the four enzymes used for enzymatic hydrolysis of the pretreated sweet sorghum. The enzymatic hydrolysis was carried out using a solids loading of 12%, three different levels of enzyme loading, and 96 hours reaction time at 55°c .Sodium azide was loaded to prevent the growth of microorganisms. Cellulase and Xylanase were loaded at 0.5 , 0.7 µL and 1.0 µL. Controls were maintained that did not have any added enzymes. Samples were centrifuged for 10 minutes at 15000rpm to separate the supernatant and filtered using a 0.2µm nylone filter. Samples were taken at 0, 9, 18, 24, 48 and 96 hours to analyze for glucose level. The filtered samples were then analyzed using HPLC that was equipped with HPX-Aminex 87-P (Bio-RAD, USA) column.

## RESULTS

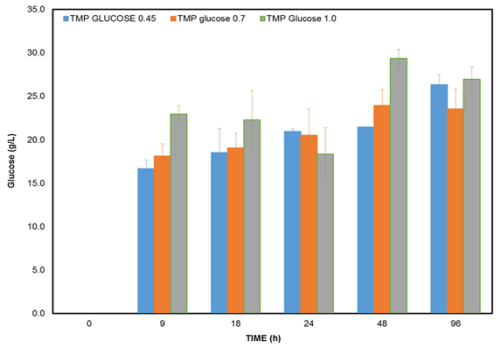



**Figure 1. Effect of enzyme level on Glucose obtained from TMP pretreated sweet sorghum.**

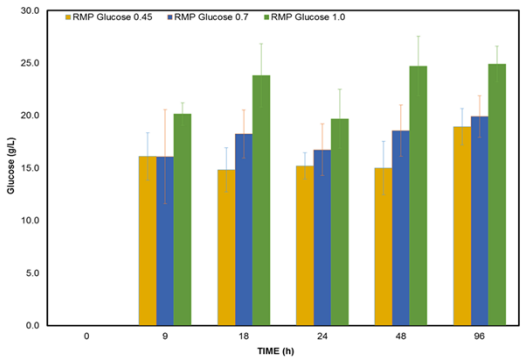



**Figure 2.Effect of enzyme level on Glucose obtained from RMP pretreated sweet sorghum.**

## SUMMARY OF RESULTS

- The TMP 1.0 mL enzyme resulted in about 40 % maximum theoretical yield of glucose , the 0.7 mL enzyme resulted in about 39% and 0.5 mL enzyme resulted 35%.
- The RMP 1.0 mL enzyme resulted in about 37% maximum theoretical yield of glucose, the 0.7 mL enzyme resulted 30 % and 0.5 mL enzyme resulted 28%.
- Most of the glucose level was produced during the first 9 hours.
- TMP giving a higher amount of glucose compared to RMP.
- Both RMP and TMP pretreated sweet sorghum resulted in higher glucose with increasing amount of enzymes.

## Acknowledgements

This work has been supported by a grant from the bioenergy NSF-REU program at Oklahoma state university, the USDA Forest Products Lab, and the Robert M. Kerr Food and Agricultural Products Center.

# Bacterial Degradation of Lignin and Lignin- like Compounds for Biomass Pretreatment

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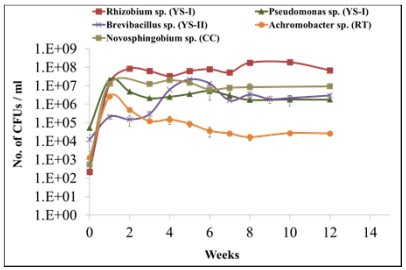
Department of Microbiology and Molecular Genetics, Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK

**Introduction:** Years before Bio-fuel production, plants were discovered to contain ligno-cellulosic polymers, such as lignin, cellulose and hemicellulose. Cellulose and hemicellulose are held together by lignin. Although, cellulose and hemicellulose are easily fermentable sugar compounds that can produce ethanol as a bio-fuel, lignin is much more stable and difficult to breakdown due to its heterogeneous aromatic polymer properties. Therefore, microbial degradation is necessary for cost-effective bioconversion of plant biomass into biofuels. To date, much work has been done using fungi to delignify plant biomass, but not much is known about the ability of bacteria to degrade lignin despite their metabolic versatility. Major emphasis of this project is to assess the role of bacteria in delignification of plant biomass.

**Background:** This project was started 4 years ago by Dr. Babu Fathepure of Oklahoma State University. The project initially started by isolating different potentially lignin degrading bacteria from various sources including rumen fluid, termite gut and decaying wood from thermal ponds in Yellowstone, WY, as well as Cow creek in Stillwater, OK. Next, the bacteria were enriched on lignin-based media for a period of 12 months. Pure cultures were isolated using agar plates containing 0.01 % lignin (Sigma-Aldrich). Individual colonies were picked and tested for their ability to grow on lignin as the sole carbon source. The isolates were identified using 16S rRNA-gene sequences and the NCBI database. Overall, five lignin degrading pure cultures were isolated (Figure 1). Among these, 2 bacteria showed the best growth. These were *Rhizobium* sp. and *Pseudomonas* sp. and were both from Yellowstone. Mother inocula of the two organisms were made for future work.

**Figure 1**




**Objectives:**

•Assess the ability of *Rhizobium* sp. and *Pseudomonas* sp. to degrade lignin monomers and dimers.

**Current Work:** To determine the ability of *Rhizobium* sp. and *Pseudomonas* sp. to degrade lignin and lignin- like compounds, organisms were grown in the presence of polymeric lignin, monomers (Veratric acid, Vanillic acid), and dimers (Benzylvanillin, Anisoin) as the only carbon substrates. Growth of the organisms on the above compounds was monitored by withdrawing the culture on a weekly basis and plating appropriately diluted cultures on to Luria-Bartani (LB) agar plates. Plates were incubated at 30°C and colonies were counted.

**Methods:**

Making the Medium:

The study started by making Mineral Salts Medium (MSM) and pouring the medium in a total of 30 flasks, 100ml per flask. Replicate flasks were used for each compound. The flasks containing 0.1% lignin, 0.02% Veratric acid, 0.02% Vanillic acid, 0.02% Benzylvanillin, or 0.02% Anisoin were inoculated with *Rhizobium* sp. or *Pseudomonas* sp. All flasks were incubated at 30°C.

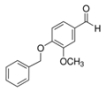
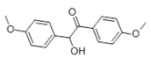
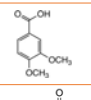
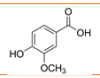
Inoculating the medium:

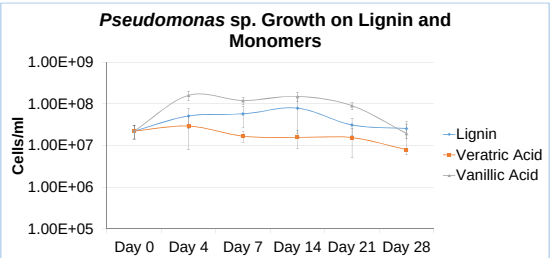
A starter culture of each bacteria was created and grown to high turbidity. The cells of the starter culture were then pelleted, washed and resuspended in MSM. 2ml of the washed starter culture was then inoculated into their respective flask.

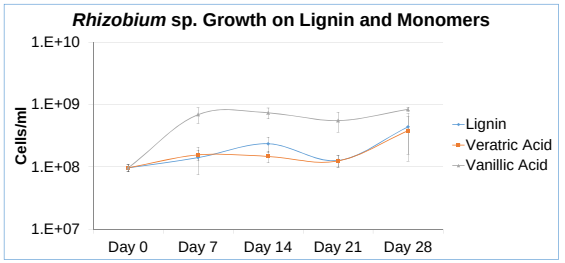
Measuring the growth:

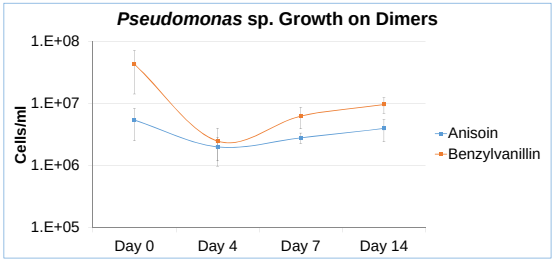
A 1mL sample was taken from each active flask for growth analysis on a weekly basis. The samples were then plated at an appropriate dilution and colony forming units were counted.

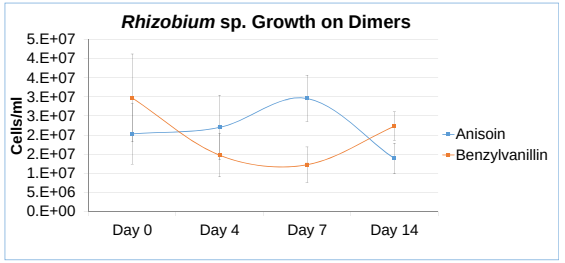
# Results:

| Compound Name | Compound Structure | Number of Cells/mL of Medium | | | | | |
|---|---|---|---|---|---|---|---|
| | | Days | 0 | 7 | 14 | 21 | 28 |
| Lignin |  | *Pseudomonas* sp. | 2.23E+07 | 5.75E+07 | 7.93E+07 | 3.13E+07 | 2.56E+07 |
| | | *Rhizobium* sp. | 9.63E+07 | 1.41E+08 | 2.35E+08 | 1.27E+08 | 4.39E+08 |
| Benzylvanillin |  | *Pseudomonas* sp. | 4.26E+07 | 6.26E+06 | 9.94E+06 | In progress | In progress |
| | | *Rhizobium* sp. | 2.97E+07 | 1.22E+07 | 2.23E+07 | In progress | In progress |
| Anisoin |  | *Pseudomonas* sp. | 5.40E+06 | 2.80E+06 | 3.96E+06 | In progress | In progress |
| | | *Rhizobium* sp. | 2.03E+07 | 2.96E+07 | 1.39E+07 | In progress | In progress |
| Veratric Acid |  | *Pseudomonas* sp. | 2.23E+07 | 1.68E+07 | 1.57E+07 | 1.56E+07 | 8.00E+06 |
| | | *Rhizobium* sp. | 9.63E+07 | 1.56E+08 | 1.47E+08 | 1.24E+08 | 3.81E+08 |
| Vanillic Acid |  | *Pseudomonas* sp. | 2.23E+07 | 1.20E+08 | 1.51E+08 | 9.05E+07 | 1.96E+07 |
| | | *Rhizobium* sp. | 9.63E+07 | 6.93E+08 | 7.37E+08 | 5.53E+08 | 8.40E+08 |




**Conclusion:**

•Several pure cultures of bacteria capable of degrading lignin and lignin-like compounds have been isolated.

•Both *Rhizobium* sp. and *Pseudomonas* sp. have been shown to grow on lignin and its monomeric and dimeric substructures; these preliminary observations indicate that bacteria are capable of degrading lignin in plant biomass.

Acknowledgement: National Science Foundation -REU

# Switchgrass Selfing Confirmation Using Duplex SSR Markers and Field Planting of Bermudagrass

Ethan Ozment, Shiva Makaju, and Yanqi Wu
Department of Plant and Soil Sciences, Oklahoma State University, Stillwater, OK 74078

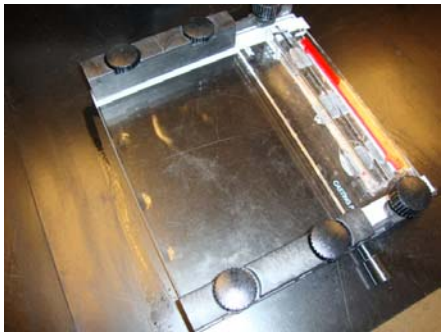
**My work this summer focused on the genotyping of switchgrass. Genotyping was used to determine whether or not individuals were the product of self-pollination, and was done using Simple Sequence Repeats (SSRs) molecular markers. Eight loci were analyzed for each individual. The overall objective of the project was to produce several generations of inbreds, and therefore largely homozygous plants, then perform a cross. Based on the principle of hybrid vigor, this should yield offspring superior to both its parents. My work specifically involved the fourth generation of putative inbred plants (S4).**

**The greenhouse, where plants weremaintained while we determined which ones were inbreds.**

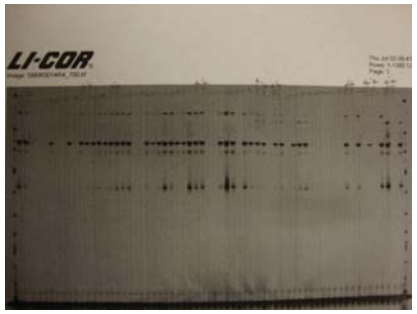
**We worked on the 4th generation of plants, a few of which are shown here. Those that turned out to be inbreds will be moved to the field.**

**Some of the chemicals used to extract DNA from leaf tissue. Among them are EtOH, NaOAc, and a mix of phenol, chloroform, and isopentanol in a chemical hood.**


**Tubes of DNA collected from plants, and ready to be analyzed.**



**An acrylamide gel in the process of polymerization.**




**A picture of a gel showing DNA bands from which we can tell which individuals are inbreds.**

**A field of bermudagrass plants I helped plant. Dr. Wu is genetically improving this species for use as turf.**

## Summary

**I learned a lot about switchgrass research, and other aspects of this experience included everything from advice on graduate school applications to a tour of the Noble Foundation. It has been quite possibly the shortest yet most enriching 9 weeks of my life. I feel very fortunate to have been selected to participate in this program.**

## Acknowledgements

National Science Foundation-Research Experience for Undergraduates Summer 2014 Project. OSU Dr. Gopal Kakani, Dr. Tillin Fang, Pu Feng, Dan Chang, Yuanwen Guo, and Gary Williams.

# The Process of Identifying Micro RNA and the Gene it Targets

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Jedaiahlovevann@gmail.com

## Introduction

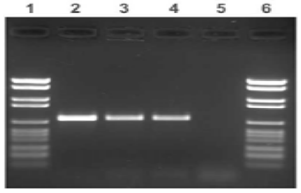
MicroRNAs are a fairly new discovered group of non-coding RNA that play important roles in the regulation of gene expression. They typically bind to the un-translated region of their target mRNA and repress protein production by destabilizing and translational silencing. An abundance or over expression of microRNA in plants can lead to substantial abnormalities in development. Plant microRNA are key adjusters in biotic and abiotic responses to stress responses and in nutrient homeostasis.

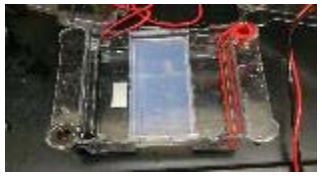
## Objective

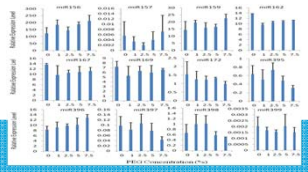
Identify the micro RNA and the gene it targets involved in drought resistance and heat resistance in switch grass. By identifying these micro RNAs the gene can be enhanced thus making the switch grass highly tolerant to harsh conditions which can in turn increase crop production and biomass.

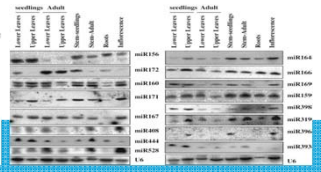
## Methods

The RNA from biological samples were purified using a process called RNA isolation where plant material is crushed into a fine powder using liquid nitrogen and sent through a series of various steps that eventually is composed into a simple solution. The proteins in the solution is the measured on Nano-drop and sent through a process called agarose gel electrophoresis. Agarose gel electrophoresis is a method used in biochemistry and molecular biology to separate a mixed population of nucleic acids in a matrix of agarose. The gel cast is then placed on an ultraviolet Trans illuminator to observe the migration of the nucleic acid fragments. A process called PCR; the polymerase chain reaction is a biochemical technology in molecular biology used to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence; was also used in the identification of micro RNA. Once the Micro RNA is identified MFOLD was used for detection of novel Micro RNA and to get a better understanding of the structure .







## Conclusion

All the processes listed are used to identify Micro RNAs and the Genes they target. Being that miRNA is a fairly new discovery, there are still numerous advancements being made on an every day basis. These advancements can assist with the overall production of biofuel. When the biomass and crop production is increased so is the production of biofuel which has been the ultimate goal in these processes and studies.

## Further information

Understanding the expression of micro RNA in switch grass can result in the enhancement of fuel production and maximum sustained yield

## Literature cited

Xie, Fuliang, Taylor P. Frazier, and Baohong Zhang. "Identification and characterization of microRNAs and their targets in the bioenergy plant switchgrass (Panicum virgatum)." *Planta* 232.2 (2010): 417-434.

Matts, J., Jagadeeswaran, G., Roe, B. A., & Sunkar, R. (2010). Identification of microRNAs and their targets in switchgrass, a model biofuel plant species. *Journal of plant physiology*, *167*(11), 896-904.

http://mfold.rna.albany.edu/?q=mfold/rna-folding-form

## Acknowledgments

The REU-NSF summer 2014 internship has given undergraduates the opportunity to expand their knowledge and gain experience in a field that is soon to be a need for the advancement of the world.