# A comparison of pyrolysis and catalytic pyrolysis of switchgrass

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## Introduction

Pyrolysis is a thermochemical process that has demonstrated effectiveness in converting biomass into biofuels. In this process, biomass is subjected to rapid heating to temperatures of up to 600 ° C in the absence of oxygen.[1] Products of this reaction includes bio-oil condensed from the vapors, solid char and gaseous products as shown in Figure 1. Interest is given to the bio-oil as it shares properties of crude oil: a fluid that can be easily stored and transported having low sulfur and nitrogen contents.

Development of an efficient pyrolytic procedure for the production of high-quality liquid bio-oil from switchgrass is still underway.

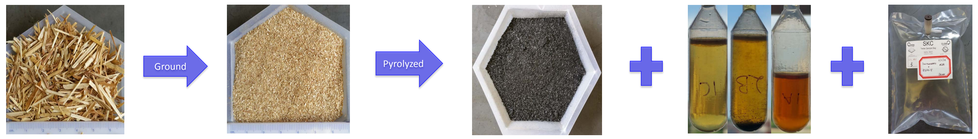



**Fig. 1 – Material Flow Diagram of pyrolyzed switchgrass**

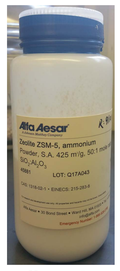
## Objective

To analyze the effects of the addition of a catalyst on mass conversion efficiencies of pyrolysis products at a batch scale.

## Materials

Kanlow switchgrass (*Panicum virgatum*) harvested by the Plant and Soil Sciences department at Oklahoma State University – Stillwater was selected as the biomass feedstock. The switchgrass was chopped using a Haybuster tub grinder to a size of 25 mm. To increase surface area further, the chopped switchgrass was ground using an industrial grade mill with a screen size of 1 mm.

The catalyst used was Zeolite ZSM-5 (Si/Al = 50). The ratio of catalyst to sample was 1:1 and thorough stirring was conducted before the material was added to the reactor for experimentation.




**Zeolite ZSM-5**

**ZSM-5 remains after bomb calorimetry**

**Parr reactor**

## Methods

Pyrolysis experiments were conducted in a fixed-bed batch-type Parr pressure reactor. Approximately 55 g of ground material was loaded into the reactor. Nitrogen gas was purged throughout the system to remove oxygen. Next, inlet and outlet valves were sealed and the reactor was heated to a temperature of 500 °C. At 400°C, collection of the gaseous product in a 1.0L Tedlar sampling bag with a combination valve occurred. After 30 minutes at 500 °C, the pyrolysis portion of the experiment was complete and the Parr reactor was allowed to cool down. The Higher Heating Value (HHV) of char and bio-oil samples were assessed using a Parr adiabatic bomb calorimeter. The char was bound with a binder and pressed into pellets. To ignite the liquid bio-oil, a benzoic acid tablet was used.

Due to time constraints, three trials of the control (switchgrass without catalyst) and two trials of the Catalyst/Switchgrass Mix (CSM) were completed.

## Results

Syngas production of the catalyst/switchgrass mix increased substantially compared to the control switchgrass samples. The mass of ZSM-5 was omitted to complete the mass balance of the Catalyst/Switchgrass Mix. Figure 2 shows the average mass composition of pyrolyzed switchgrass and Figure 3 shows the average mass composition of the Catalyst/Switchgrass Mix.

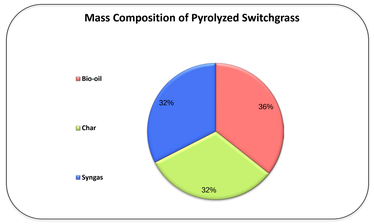



**Fig. 2**

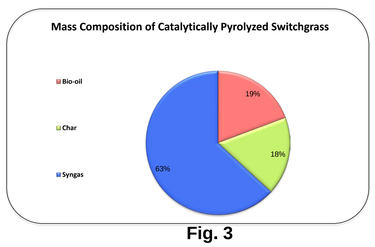



**Fig. 3**

Energy content was assessed using a bomb calorimeter. Pure cellulosic binder was combusted and produced 16.4 MJ/kg of gross heat. A standardization run calculated the HHV measurement of a benzoic acid tablet to be 26.712 MJ/kg. Average HHV measurements are reported in the table below.

| Product | Gross Heat [MJ/kg] |
|---|---|
| Char (control) | 28.606 |
| Char (CSM) | -0.486 |
| Bio-oil (control) | 2.490 |
| Bio-oil (CSM) | 1.386 |

## Observations and Conclusions

- Preliminary results show that the addition of the catalyst, ZSM-5, caused a decrease in bio-oil yield and heating value.
- Liquid condensed from the reaction was not separated into an aqueous faction and a bio-oil fraction. HHV of a pure bio-oil fraction is expected to be much higher.
- Process parameters such as heating rate and the maximum heating temperature are critically important to improving bio-oil yield.

**Acknowledgement:** This summer Research Experience for Undergraduates was funded by a grant from the National Science Foundation.

**References:**

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# Characterization of Biomass Produced by Five Different Algae Strains

**Batschelett, Garrett, Dunford, Nurhan Turgut and Ding, Qichen**
**Oklahoma State University, Biosystems and Agricultural Engineering Department and Robert M. Kerr Food and Agricultural Product Center**

## INTRODUCTION

This summer I was assigned to be mentored by Dr. Nurhan Dunford and paired with graduate student Qichen Ding to join the ongoing research on Oklahoma native algae strains. Microalgae are single cell organisms that can be grown in water and fix carbon dioxide by the photosynthesis process and produce biomass containing lipids, carbohydrates, and proteins. Algae were selected as feedstock to produce biofuel because of their advantages over terrestrial crops like corn. Algae can be grown on non-agricultural land and in waste water.

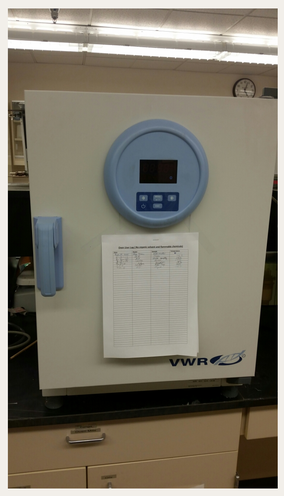
The objective of this project was to grow microalgae to produce biomass and determine the chemical composition of the biomass harvested.

We selected five Oklahoma native microalgae strains, them being SP 1, SP11, SP23, SP24, and SP25. With these strains we ran test to determine their chemical compositions including moisture, ash, and oil contents. Our goal is to find which Oklahoma native microalgae strain produces the most oil.

## METHODS & MATERIALS


**Biomass harvesting**

- Use centrifuge (8000 rpm for 10 minutes) to obtain wet biomass from algae growth medium




**Moisture content determination**

- Weigh wet biomass in a crucible then place the crucible into an oven at 105 degree Celsius for 5 hours and then determine the weight of the moisture evaporated


**Ash content determination**

- Put dry biomass in a crucible then place it inside of a furnace at a temperature of 525 degree Celsius for 5 hours and then determine the weight of the ash remaining in the crucible

**Oil content**

- Use glass beads to break the cell walls
- Add a mixture of chloroform/methanol (ratio of 2 to 1) to the biomass and extract oil
- Use rapidvap instrument to evaporate solvent and determine the residual oil weight

## CONCLUSIONS

- Oil content varies from strain to strain (Figure 1).
- Washing the algae biomass reduces the ash content of the sample (Figure 2).
- Moisture content of the harvested algal biomass was over 90% (Figure 3).
- Algae strain SP 1 had the highest oil content. (Figure 1)
- It appears that strain SP 1 has a better potential than the other strains examined in this study for biofuel production.

## RESULTS

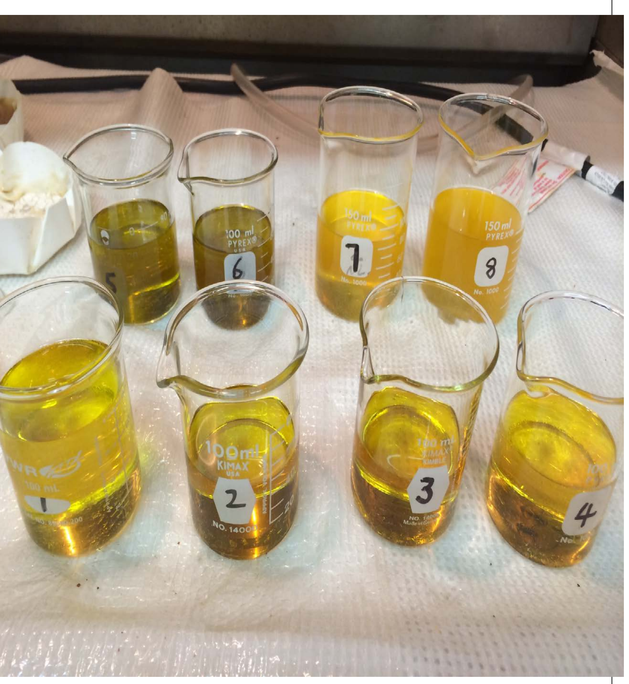


**Photo above**: oil extracted in solvent after running tests

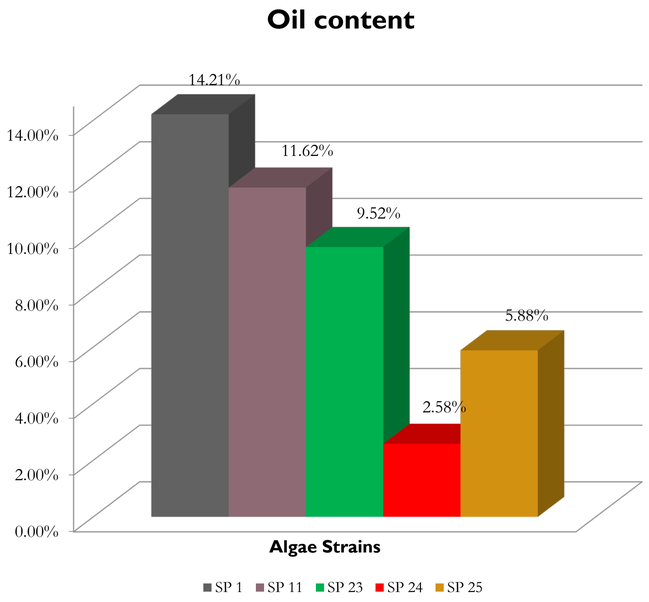



**Figure 1:** Comparison of oil content in algae strains.

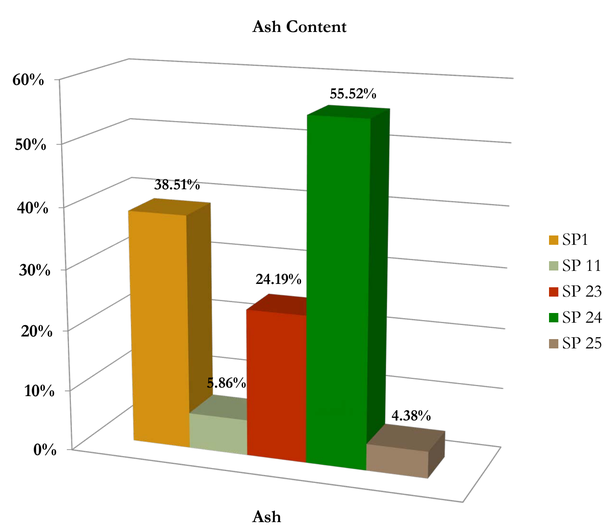



**Figure 2:** Comparison of ash content (Note SP 11 and SP 25 had biomass washed)

**Photo Above:** Algae Cultures that we harvest for biomass

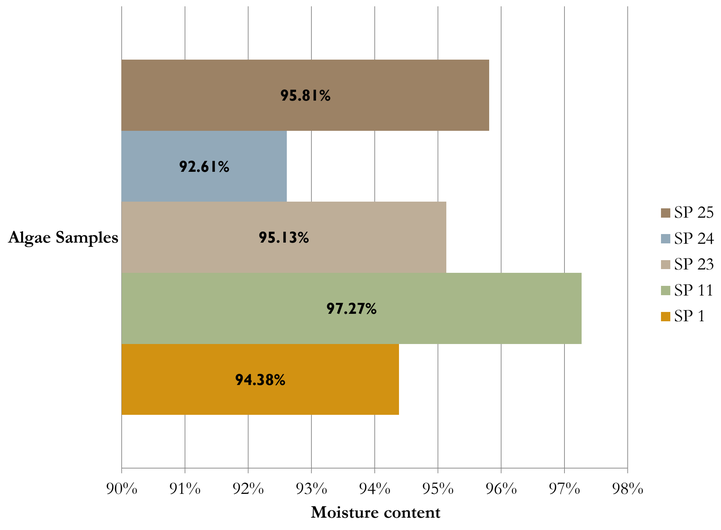



**Figure 3**: Comparison or moisture content of wet biomass harvested.

## OBJECTIVES

- The objective of this project was to grow microalgae to produce biomass.
- Determine the chemical composition of the biomass harvested.
- Find which strain would have the greatest potential for biofuel production.

## ACKNOWLEDGEMENTS

Thanks to the hard work of my assigned mentor Dr. Dunford and graduate student Qichen for helping and working with me over this past summer. I would like to thank the REU program for giving me this awesome opportunity for the learning and work.

# Production of Biohydrogen from Switchgrass Hydrolyzate by *Anaerobaculum hydrogeniformans*

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## OBJECTIVE

- To investigate the production of biohydrogen from switchgrass and pure sugar using *Anaerobaculum hydrogeniformans*

## INTRODUCTION

- Biohydrogen production from biomass has attracted the National Renewable Energy research sector due to it's lucrative and environmental preserving characteristics [1].
- Biohydrogen is deemed as one of the fuels that will be widely used in the future and a biobased hydrogen economy would be more sustainable.
- The combustion of hydrogen with oxygen produces energy and water vapor with no green house gases that are produced with conventional fossil fuels.

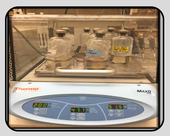

(Hydrogen car fueling, EERE)

- Switchgrass is one of the leading biomass crops in the USA and has an excellent potential as a bioenergy feedstock. Switchgrass was selected by the U.S. Department of Energy-Bioenergy Feedstock Development Program, as the herbaceous model species for biomass energy [2].
- *Anaerobaculum hydrogeniformans* sp. nov., an anaerobe that produces hydrogen from glucose. It is moderately thermophilic, halophilic (requires NaCl) and gram-negative [3].

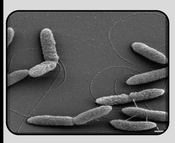
## MATERIALS AND METHODS

Mitchell et al. 2015 [5]

Pretreatment of ground switchgrass was performed by hydrothermolysis in a 1 L Parr reactor at 200 $^{o}$C for 10 minutes. Compositional analysis of pretreated and raw switchgrass was performed using National Renewable Energy Laboratory (NREL) protocols [4].



Enzymatic hydrolysis was carried out using an enzyme cocktail and pretreated switchgrass. Enzymatic hydrolysis was performed in four separate flasks at 50°C and 250 rpm for 48 hours.

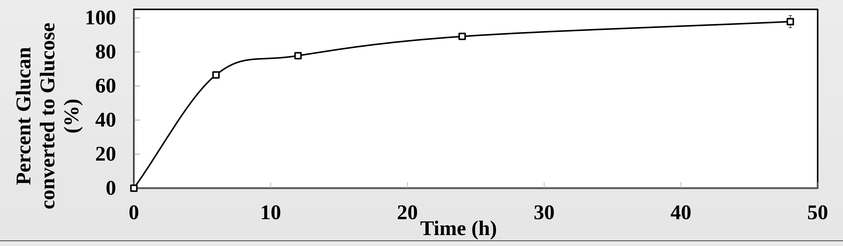


*Anaerobaculum mobile* [6]

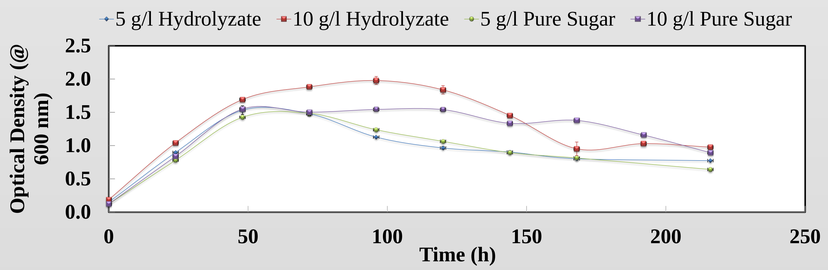
Fermentations were performed in triplicate in 250 ml bottles with 100 ml medium at *A. hydrogeniformans* optimum growth conditions of (55° C , pH of 7.3~7.8 and [NaCl ] = 10 g/l).

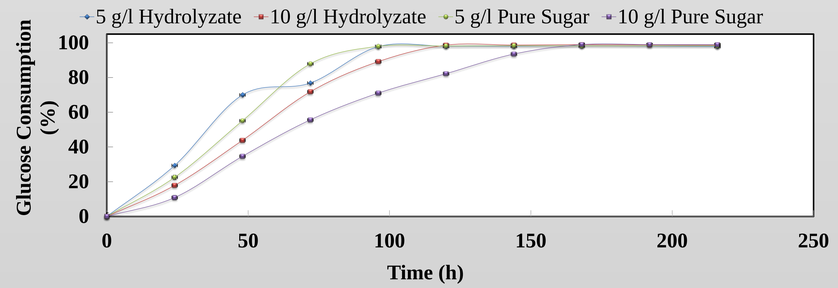
## RESULTS

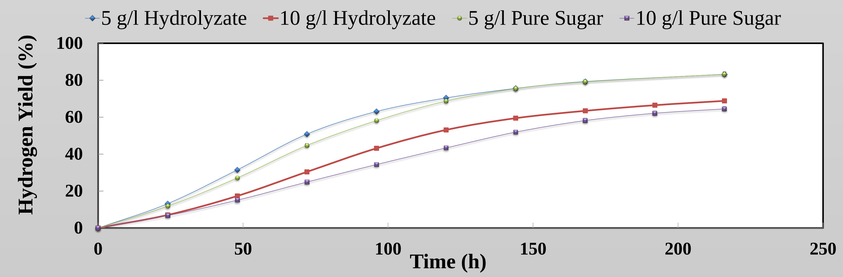
### Hydrolysis




### Fermentation

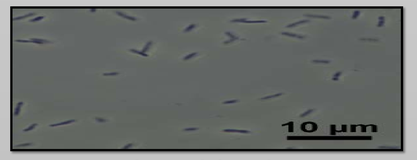


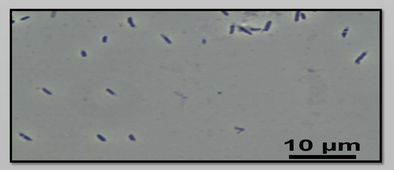




### *Anaerobaculum hydrogeniformans* Growth

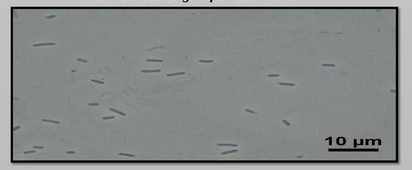

*Hydrolyzate 5 g/l after 120 hours*




*Pure Glucose 5 g/l after 120 hours*




*Hydrolyzate 10 g/l after 144 hours*




*Pure Glucose 5 g/l after 144 hours*

### SAS-ANOVA ANALYSIS

% Hydrogen Yield — Tukey Grouping

| Treatment | Mean± S.E. | ❖Same letter are not significantly different |
|---|---|---|
| Hydrolyzate 5g/l | 83.27 ± 0.29 | A |
| Pure Sugar 5 g/l | 83.23 ± 0.79 | A |
| Hydrolyzate 10 g/l | 68.82 ± 1.10 | B |
| Pure Sugar 10 g/l | 64.51 ± 1.16 | B |

## CONCLUSIONS

- Glucose yield from pretreated switchgrass was 98%.
- Treatments with 5 g $L^{-1}$ glucose exhibited a higher conversion efficiency than with 10 g $L^{-1}$ glucose.
- Treatments with 10 g $L^{-1}$ grew to a higher optical density; however, they exhibited increased acetic acid production and reduced hydrogen efficiency.
- Hydrogen production was statistically similar in treatments with switchgrass hydrolyzate and pure glucose at the same initial sugar concentration.
- Future research should focus on an in-depth analysis of growth parameters of *A. hydrogeniformans.*

## ACKNOWLEDGEMENTS

- This research was supported by the National Science Foundation – Research Experience for Undergraduates (NSF-REU) 2015, USDA-NIFA Project No. OKL03005 and Oklahoma Agricultural Experiment Station.
- We also would like to thank Dr. Ralph Tanner for providing the microorganism used in this research.

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# Microbial Degradation of Lignin-Model Compounds for Lignocellulosic Biomass Pretreatment

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## Abstract

Lignocellulosic biomass is readily available for the conversion of biofuels yet, lignin, a recalcitrant, complex, aromatic polymer, prevents efficient saccharification of cellulose and hemicellulose into sugars; thus making current biofuel manufacturing processes inefficient. Lignin's heterogeneous structure consists of around 70% ether-type bonds and the remaining 30% ester-type bonds or carbon-carbon bonds. Four consortia of bacteria, previously enriched on alkali lignin (Sigma-Aldrich), were inoculated onto media containing two synthetic lignin model compounds, benzylvanillin and anisoin, representing ether and carbon-carbon bonds of lignin respectively. Pure cultures that degrade benzylvanillin and anisoin as the sole carbon source were isolated. Experiments were set up to test the isolate's ability to degrade alkali lignin, switchgrass or alfalfa. Periodically, samples were withdrawn to determine viable cell counts and assayed for lignin peroxidase, DyP peroxidase and laccase activity. Preliminary results demonstrate that the isolated organisms are capable of degrading lignin and plant biomass.

## Introduction

Lignocellulosic biomass is one of the most abundant renewable resources for the conversion of biofuels. However, lignin is a complex heteropolymer that encases cellulose and hemicellulose. The complex structure of lignin acts as a barrier to enzymatic and microbial degradation, thus preventing further saccharification. Currently, the production of second-generation ethanol utilizes expensive pretreatment methods, such as mechanical, thermomechanical and thermochemical pretreatment processes, that can also generate toxic intermediates. Biological pretreatments exploit microorganisms to modify lignin to access fermentable plant sugars. This offers a natural, low-input means for lignocellulosic biofuel production. Though the majority of studies to date focus on lignin degradation by fungi, fungal performance is poor under low oxygen conditions and in nitrogen rich substrates. Fungi also present the unique challenge of protein expression and gene manipulation. On the contrary, bacteria have a high potential for their ligninolytic capabilities because of their immense environmental adaptability and biochemical versatility. This experiment looked to isolate bacteria on model-lignin compounds, benzylvanillin and anisoin, and then test their ability to degrade lignin (alkali lignin, switchgrass and alfalfa).

## Methods

***Previous Laboratory Work***

Lignin degrading enrichment cultures were obtained from various biomass decaying sources including decaying wood from Cow Creek (CC) in Stillwater, OK, Green Stream (GS) and mesothermal ponds (YS) at Yellowstone National Park and termite gut and rumen fluid (RF). The four consortia and have been maintained for over five years on mineral salt medium (MSM) amended with 0.02% organosolv lignin (insoluble lignin, Sigma-Aldrich) as the source of carbon.

***Current Laboratory Work***

*Isolation of Benzylvanillin and Anisoin Degrading Bacteria*

Samples from each consortium were used as inocula to isolate pure cultures that degrade benzylvanillin and anisoin as the sole carbon sources. Four flasks with 100 mL of MSM amended with 0.02% benzylvanillin or anisoin and 0.05% yeast extract were inoculated with 1 mL of culture from each consortia. A fifth flask was inoculated with autoclaved nanopure water to serve as a control. After three weeks of incubation, 0.01 mL of culture from each flask was plated onto MSM plates containing 0.02% anisoin or benzylvanillin as the sole carbon source. Plates were incubated for four days and colonies were then streaked for purity onto respective MSM plates containing 0.02% anisoin or benzylvanillin. A week later, distinct colonies, one from benzylvanillin plate (BV-1) and two from anisoin plates (ANI-1 and ANI-2) were selected and transferred to 100 mL of 10% LB broth.

*Degradation of Benzylvanillin and Anisoin by the Isolated Pure Cultures*

Flasks containing 100 mL MSM and 0.02% alkali lignin, switchgrass, or alfalfa were setup and inoculated with BV-1, ANI-1, or ANI-2 and incubated at 30C. Samples were (1.0 mL) withdrawn at time points and assayed for various lignin degrading extracellular enzymes including lignin peroxidase, DyP-peroxidase and laccase. In addition, samples were also withdrawn initially and at the conclusion of experiment and growth of the organisms on each lignin substrate was determined by plating the samples on LB plates.

## Results

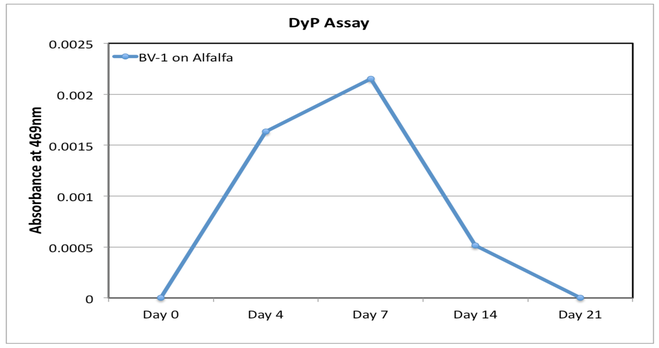
All isolates demonstrated growth on alkali lignin as well as both plant biomass. All isolates also showed enzyme activity (DyP-peroxidase, lignin peroxidase and laccase) in alfalfa grown cell supernatant. No such activity was observed in cultures grown on lignin or switchgrass. Among the organisms, BV-1 demonstrated the most DyP-peroxidase and lignin peroxidase activity.

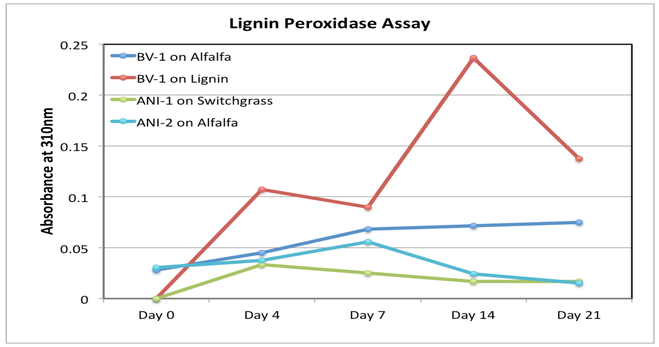
## Results

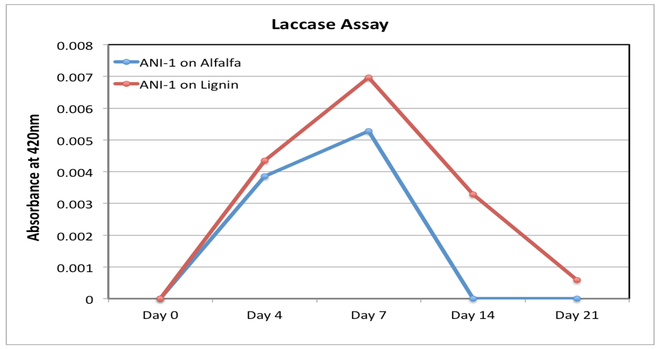
*Cell Growth*

| Isolate | Substrate | Number of CFUs | |
|---|---|---|---|
| | | Day 0 | Day 28 |
| BV-1 | Switchgrass | 4.50E+04 | 5.75E+04 |
| | Alfalfa | 4.75E+04 | 7.75E+04 |
| | Lignin | 4.25E+04 | 6.25E+04 |
| ANI-1 | Switchgrass | 1.40E+09 | 1.78E+09 |
| | Alfalfa | 1.48E+09 | 1.88E+09 |
| | Lignin | 1.48E+09 | 1.63E+09 |
| ANI-2 | Switchgrass | 1.73E+09 | 1.83E+09 |
| | Alfalfa | 1.70E+09 | 1.95E+09 |
| | Lignin | 1.65E+09 | 1.93E+09 |

*Enzyme Activity*




## Conclusion

Our preliminary data suggest that the isolated bacteria are capable of degrading lignin in plant biomass. However, direct studies are needed to determine the degradation of lignin in plant biomass.

# Optimization of Polymerase Chain Reaction of Switchgrass Laccase Genes

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## Introduction

Combustible fuels synthesized from plant biomass (biofuels) are being researched all across the world as an alternative to fossil fuels such as coal and gasoline. The most common plant used in this process is corn, but with food demand rising the agricultural industry has raised many concerns. Using corn (starch) to create carbon fuels such as ethanol can deduct from the amount of corn that is used for food, so an alternative plant source is necessary to continue bioenergy development without cutting into the food supply. Plants such as *Panicum virgatum* (Switchgrass) and Corn stover can be processed to utilize cellulose sugars instead of starch. The end product is known as "Cellulosic ethanol", which is ethanol produced from the extracted cellulose in these types of plants. Lignin, which keeps plants rigid and provides some defense against disease, surrounds the sugars in plant cells. Without an enzyme to break down lignin, the extraction of cellulose is very difficult. Laccases are enzymes that degrade lignin, alleviating the difficulty of removing cellulose. Switchgrass, along with other plants, naturally produces laccase enzymes which have been defined and sequenced. My project involves the optimization of amplification for future cloning of genes encoding laccases.

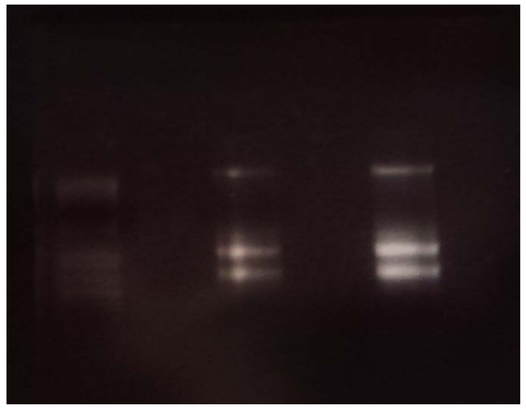
## Objective

Three GC-rich Switchgrass laccase genes were selected to be cloned in this project, labeled 681, 374, and 841. Each gene contained 909 bp, 1340 bp, and 2135 bp, respectively. Amplifying long genes with a large amount of GC bonds can be difficult due to the higher number of hydrogen bonds between Guanine and Cytosine (3 compared to the 2 of AT(U) bonds). By adjusting the annealing temperature and time, the type of Taq Polymerase enzyme, and other parameters, the amplification of these genes is possible. Using cDNA synthesized from whole Switchgrass RNA, the Polymerase Chain Reaction (PCR) must be optimized to successfully amplify each specific gene.

## Methods

*The process of amplifying and cloning can be separated into five broad steps:*

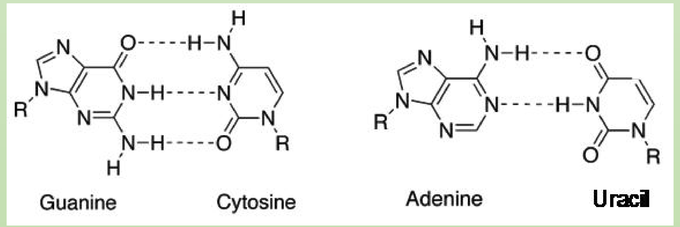
1. **cDNA synthesis from whole Switchgrass RNA.** This is achieved by using the SuperScript II Reverse Transcriptase enzyme/system.
2. **PCR amplification of Laccase genes.** Along with specific primers and Switchgrass cDNA, the OneTaq Master Mix (with GC-enhancer) was used to attempt to amplify the genes.
3. **Ligation of gene fragment into a vector.** T4 DNA ligase was used to ligate genes of interest into a P-GEM T-Easy vector (non-expression). This vector included a gene encoding Ampicillin resistance.
4. **Transformation using JW107 *E.coli* cells.** Competency was achieved via calcium chloride treatment. When plating on agar, ampicillin was added to select for colonies that successfully expressed the vector.
5. **Verification and confirmation of gene insert.** Simple colony PCR was performed to confirm that the fragment of interest was integrated into the vector. Full plasmid sequencing was then used to confirm the identity of the inserted gene.



Left to right: 1kb plus DNA Ladder, SG RNA (X2)
*28s rRNA is the top band, 18s rRNA is the bottom band*

## Future Directions

Longer genes require special Taq Polymerase mixtures that can "proof-read" the DNA being replicated. One such mixture is the Platinum Taq system. This procedure is specifically engineered to amplify long, GC-rich genes better than native Taq enzymes. Using Platinum Taq, future amplification is possible. The P-GEM T-Easy vector is a non-expression vector, meaning that over-expression of the inserted gene is not possible. Using a vector such as pET28 during ligation and transformation will ensure that the over-expression of the laccase gene is possible. After optimization and cloning, simple enzyme assays using lignin as a substrate will measure the lignin-breakdown activity of each of the three laccases.

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# Effect of Enzyme Loading on Extrusion & Alkali Extrusion Pretreatments of Sweet Sorghum Bagasse

Matthew Maxwell[1] ,Naveen Pessani[2]; Danielle Bellmer[1,2]
[1]Oklahoma State University, [2] Food and Agricultural Products Center

## ABSTRACT

Sweet sorghum is a promising bioenergy crop due to its high productivity, low input requirements and versatility. It has tremendous carbohydrate potential if the available starch, cellulose, and hemicellulose can be converted to fermentable sugar in addition to the existing soluble sugars. Extrusion pretreatment using twin screw extrusion technology has been used on various feedstocks such as switchgrass, corn stover, etc. In addition, alkali soaking in combination with extrusion has been used to help break down the lignin in order to better access the cellulose and hemicellulose in lignocellulosic feedstocks.

The objective of this experiment was to evaluate the effect of extrusion and alkali extrusion as a pretreatment method for enzymatic hydrolysis of sweet sorghum.

A twin screw extruder was used to pretreat sorghum at 110 °C with and without alkali pretreatment (4% sodium hydroxide). Sorghum samples were diffused to extract soluble sugars, and the bagasse was used as a starting material for extrusion pretreatment. Samples from each pretreatment were subjected to enzymatic hydrolysis of cellulose and starch at a solid loading of 6%. Samples were loaded with three different levels of cellulase and xylanase enzymes. The temperature was maintained at 55°C and the flasks were agitated at 150 rpm. Samples were analyzed for carbohydrates using an HPLC. Control samples containing no enzyme were used to adjust for the overestimation of sugar yields due to the diffusion of soluble sugars from plant material.

Sugar yields were compared to enzymatic hydrolysis of non-pretreated sorghum samples. Results show that extrusion pretreatment with and without alkali results in increased carbohydrate conversion to sugars compared to untreated samples. As expected, increased enzyme levels resulted in increased sugar yields.

## INTRODUCTION

Sweet sorghum is a known high-energy, drought tolerant crop with the ability to adopt to various climates and soil conditions. Different parts of this plant can be hydrolyzed to obtain fermentable sugars that can then be used to produce biofuels.

Sweet sorghum's stem juice can be readily extracted to collect its fermentable sugars, but in order to utilize all of sweet sorghum's available sugars, including starch, cellulose, and hemiceullose, the bagasse must be pretreated.

This research project focuses on the use of extrusion with and without alkali as a pretreatment method for sweet sorghum. Enzymatic hydrolysis was conducted using three different enzyme levels to determine the most efficient use of enzymes to achieve maximum sugar yields.

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## METHODS AND MATERIALS

The two pretreatments tested in this experiment were extrusion and alkali extrusion. Enzymatic hydrolysis was carried out using a solid loading of 6%, three different levels of cellulose and xylanase enzymes, and a 96 hour reaction time in a rotating incubator at 55 $^{0}$C.

Sodium Azide was added to prevent the growth of microorganisms within the 250 mL sample flasks. Cellulase was added at .23(X), .34, and .45 mL/gram, while xylanase was added at .14, .22, and .31 mL/gram. Controls were obtained and contained no added enzymes.

Samples were centrifuged for 8 minutes at 10,000 RPM to separate the supernatant which was then filtered using a .2 µm nylon filter. The filtered samples were then analyzed for specific carbohydrates using an HPLC equipped with HPX-Aminex 87-P column (Bio-RAD, USA)

**Glucose Yields from Alkali Extrusion Pretreatment**

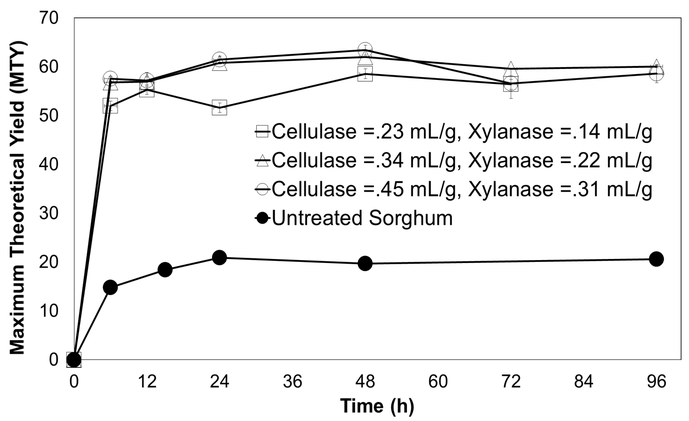


**Figure 1: Glucose yields obtained by enzymatic hydrolysis at three different enzyme loadings of alkali extrusion pretreated sorghum and untreated sorghum.**

**Glucose Yields from Extrusion Pretreatment**

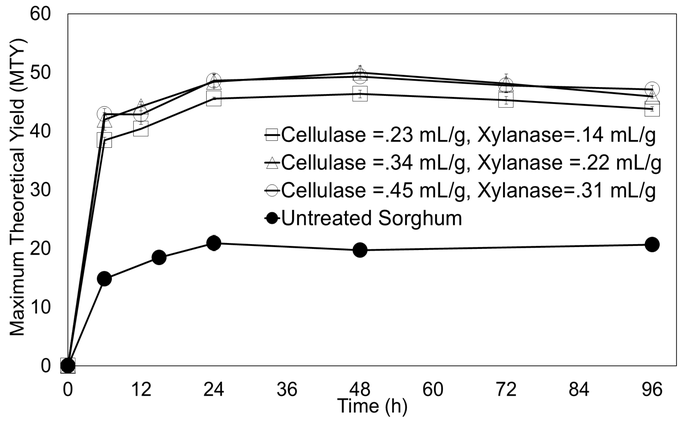


**Figure 2: Glucose yields obtained by enzymatic hydrolysis at three different enzyme loadings of extrusion pretreated sorghum and untreated sorghum.**

**Xylose Yields from Alkali Extrusion Pretreatment**

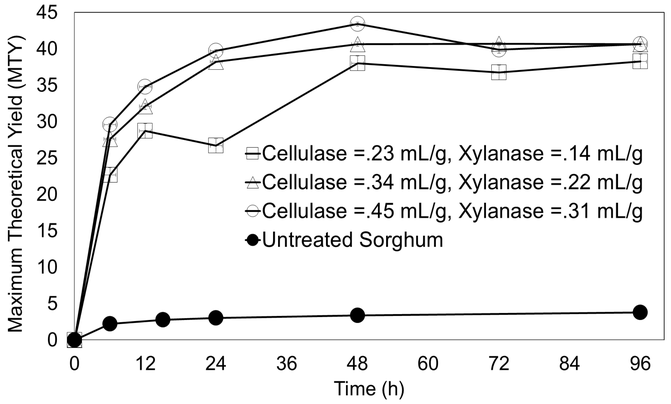


**Figure 3: Xylose yields obtained by enzymatic hydrolysis at three different enzyme loadings of alkali extrusion pretreated sorghum and untreated sorghum.**

**Xylose Yields from Extrusion Pretreatment**

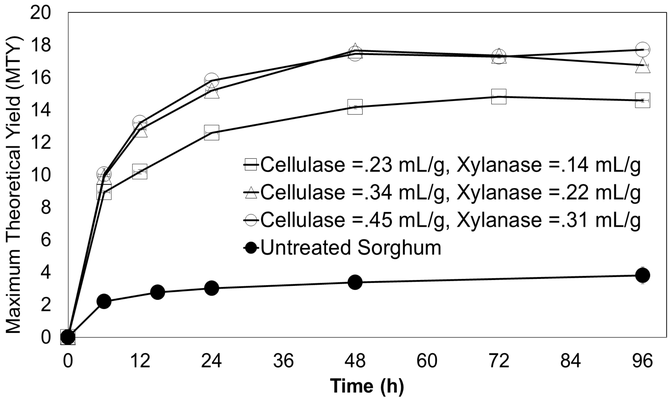


**Figure 4: Xylose yields obtained by enzymatic hydrolysis at three different enzyme loadings of extrusion pretreated sorghum and untreated sorghum.**

## RESULTS & DISCUSSION

- As expected, with increasing enzyme loadings we see an increase in sugar yields. The highest two enzyme loadings appear to be very close together in terms of sugar yields. Based on this assumption one could save enzymes by using the lower loading of the two highest enzyme loadings and achieve near the same sugar yields.
- The alkali extrusion pretreatment repeatedly outperforms the extrusion pretreatment in max glucose and xylose yields. With the highest glucose yields for alkali extrusion being close to 66% as opposed to extrusion with a 53% max yield. The highest xylose yields from alkali extrusion were around 43% as opposed to a 18% yield with extrusion.
- With alkali extrusion we see around a 40% increase in max yields from untreated sorghum for both glucose & xylose. With extrusion we see around a 30% increase from untreated sorghum for max glucose yields & only a 14% increase in max xylose yields.

## CONCLUSIONS

The use of alkali extrusion and extrusion pretreatments improved sugar yields of sorghum bagasse compared to untreated sorghum. The addition of alkali to the extrusion process greatly improved sugar yields compared to extrusion alone, and required no detoxification step though it contained an added chemical step.

## AKNOWLEDGEMENTS

This project has been supported by the NSF-REU Bioenergy program at Oklahoma State University, the USDA Forests Products Lab, and the Robert M. Kerr Food and Agricultural Products Center.

# Life Cycle Inventory Data Acquisition Methods for Pyrolysis Biofuels

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Langston University, Langston, OK

## Abstract

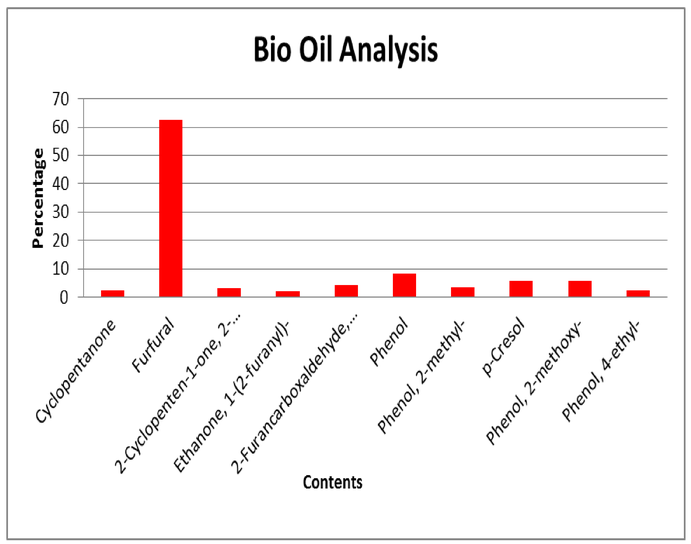
Life Cycle Assessment (LCA) examines the inputs and outputs of a product and the effects they have on the environment. Using the Argonne National Lab GREET® LCA software (Greenhouse gases, Regulated Emissions, and Energy use in Transportation), outputs for E-10 reformulated gasoline and pyrolysis oil gasoline were calculated and compared. The inputs to the LCA are known as the Life Cycle Inventory (LCI). This research details the main steps and analysis involved in producing the LCI used by programs such as GREET to give the final overall LCA of the two gasoline types. Switchgrass was pyrolysized using the HP/HT Pressure Reactor provided by the Parr® Instrument Company. The products were then analyzed for use as LCI inputs.

## Introduction

Switchgrass has been used in numerous bioenergy conversions processes. Switchgrass is capable of undergoing pyrolysis, which is thermal decomposition sans oxygen. From this procedure, syngas, bio-oil, and biochar are generated and analyzed. These analysis are the origins for the information in the LCA model GREET. The data received from the analysis's provide models such as GREET with information needed to conduct an LCA. For this research endeavor, switchgrass was pyrolysized and its syngas and bio-oil were analyzed using Gas Chromatography and Mass Spectrometry respectively.

## Mass Spectrometry

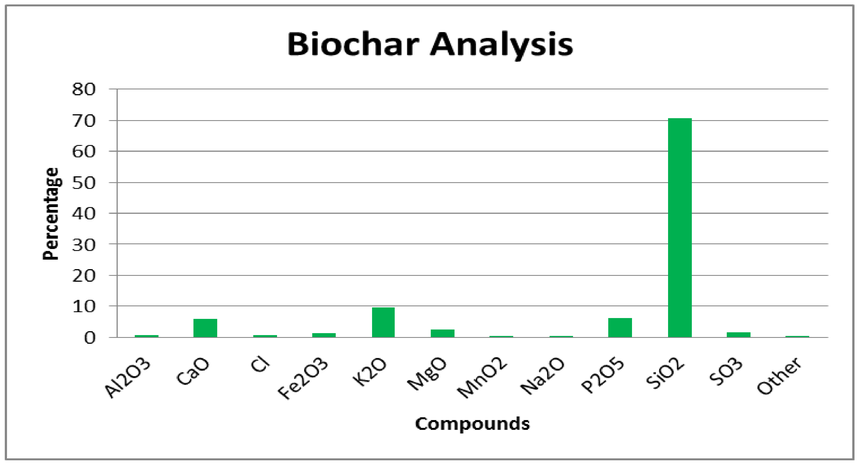
The liquid product of pyrolysis provides the basis for what will eventually become biofuel used in transportation and other energy activities in lieu of fossil fuels. The bio oil obtained is analyzed using a mass spectrometer. This machine ultimately identifies all of the components of the liquid product. Below is a graph showing the contents of bio oil that was pyrolysized switchgrass.




The bar graph shows that Furfural is the most present in the bio oil. Furfural is an organic compound derived from a variety of agriculture byproducts. Its chemical formula is $OC_4H_3CHO$. Furfural is a skin irritant.

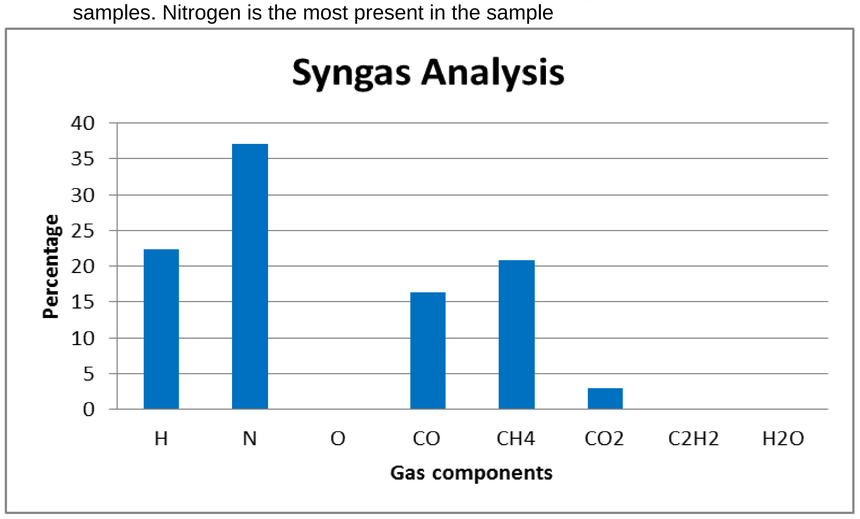
## Biochar

After pyrolysis of biomass has occurred, a solid known as biochar is generated. The content of what would be produced from switchgrass via pyrolysis is 20% biochar and 80% ash. The ash accounts for the elements listed in the graph below (Brewer et al.)




## Gas Chromatography

The syngas obtained from the reaction underwent gas chromatography. It is a common type of chromatography used for identifying compounds in gaseous samples. Nitrogen is the most present in the sample

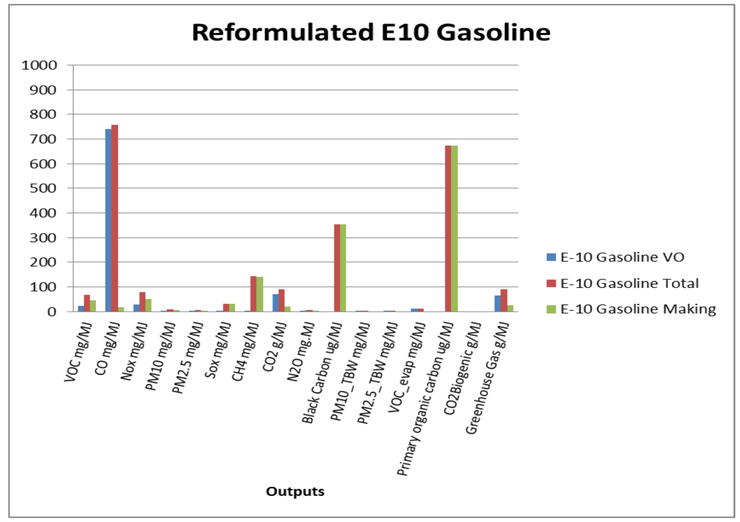


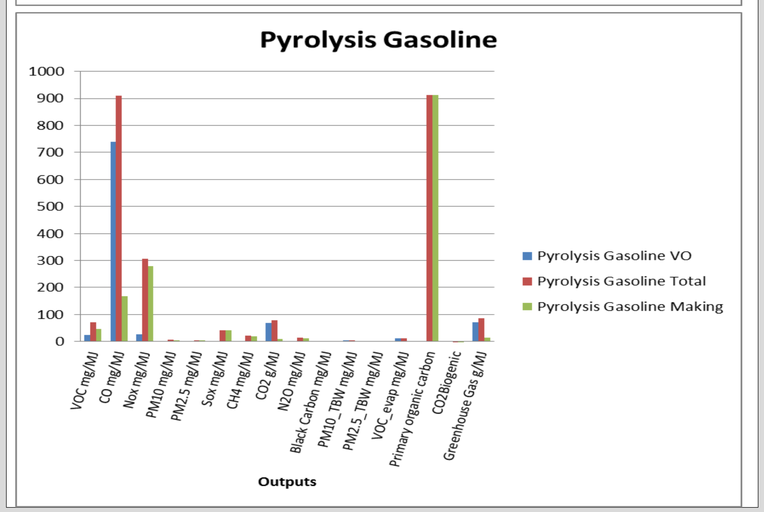

## Acknowledgments

- National Science Foundation's Research Experience for Undergradutes 2015
- Department of Biosystems and Agricultural Engineering at Oklahoma State University
- Lab support from Dr. A. Kumar, Brian Burris, Zixu Yang, OSU BAE

## Fossil Fuel Versus Biofuel

The results generated LCI data only accounts for the pyrolysis reaction. To further complete the LCA, GREET determines the Well to Wheel (WTW) portion. Below are graphs comparing reformulated E10 gasoline to pyrolysis gasoline(switchgrass).




## Conclusion

- pyrolysis gasoline reduces Greenhous Gas emissions by 5% compared to reformulated E10 gasoline.
- E10 gasoline produces significantly more Carbon Black than pyrolysis gasoline.
- However pyrolysis gasoline produces more CO and N2O than its competitor

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# Varietal Differences in Oven Drying of Canola Seed

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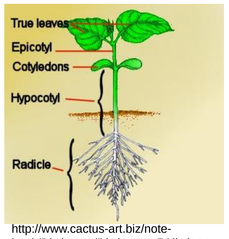
## Background

Before canola seed is processed, it is often stored for a certain period of time. The seed must sometimes be dried in order to prevent quality deterioration during storage. If seed is too wet, fungal growth is likely to occur. This leads to an increase in FFA and undesirable odors, which will take additional processing to remove during refining of the oil that has been harvested from the stored seed. A reduction in moisture content ensures that seed quality is maintained during storage. However, if the seed is dried at a temperature that is too high, the seed viability will be reduced and its ability to germinate will be affected. The Canola Council of Canada recommends that canola be dried under 45ºC in order to preserve germination. The goal of this study was to determine if there is a difference in germination between varieties of canola seed after they are dried, and to find the drying temperature at which germination begins to be affected.

http://www.oilmillequipment.com/canola_oil_processing_unit_281.html

## Germination Testing

The germination test procedure used in this experiment involved placing a piece of filter paper in a petri dish and wetting it with 5 mL of distilled water. After drying, 50 random seeds with no visible damage were selected from each sample and placed in a dish. The dish was then covered and the seeds were allowed to sit at room temperature for 5 days. Because some of the seeds germinated so quickly, after 3 days the seeds that had already germinated were counted and removed from the dish. This prevented the roots from tangling together and making it difficult to get a proper count. After 5 days a final count was made of seeds that had germinated, and the total was taken as a percent out of 50. These percentages were recorded and analyzed.




http://www.cactus-art.biz/note-book/Dictionary/Dictionary_R/dictionary_radicle.htm

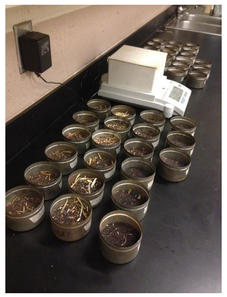
A difficulty that emerged during testing was determining which seeds to consider as germinated. Many had full roots and distinguishable cotyledons, which were counted as germinations. Conversely, seeds that had not changed at all and had remained in the seed coat were counted as non-germinations. However, there were many seeds that fell in between these two parameters. In this case, seeds that possessed a hypocotyl yet no radicle were not counted, nor were the seeds without stable cotyledons.

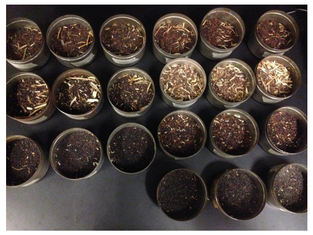
## Methods

Seven varieties of canola seed were used to conduct this experiment: Mercedes, 44-10, Riley, Star 915W, 1336, 46W94, 115W. The theoretical volume of water needed to bring the moisture content (dry basis) to 12.0% was calculated and added to each variety of seed. Three samples of each variety, each weighing around 80 grams, were placed in an oven set to six different temperatures: 30ºC, 40ºC, 70ºC, 80ºC, 90ºC, and 100ºC. The samples were left in the oven for 48 hours. When the seed was removed from the oven, a 5 day germination test was conducted to evaluate viability.

| NAME | TYPE | TRAIT |
|---|---|---|
| DKW 44-10 | OP | RR |
| 46W94 | Hybrid | RR |
| HyCLASS 115W | OP | RR/SURT |
| Star 915W | OP | RR/SURT |
| Riley | OP | non-GMO |
| Mercedes | Hybrid | non-GMO |
| 1336 | Unknown | Unknown |

**Key**
OP = Open Pollinated
RR = Roundup Ready
SURT = Sulfonylurea Carryover Tolerant





## Conclusion

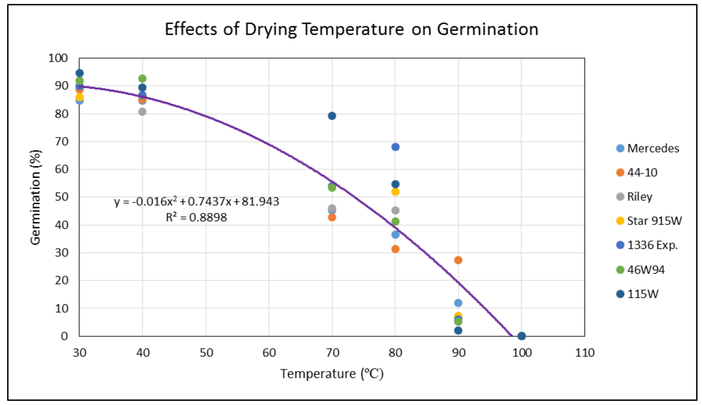
Although the results of this experiment did not show any significant differences in germination between the varieties of canola, further testing is required to determine the ideal drying temperature of the seed. Since germination remained above 80% at a drying temperature of 40ºC but dropped below 55% at 70ºC, drying the seed at 50ºC and 60ºC would help in more precisely determining the temperature at which germination begins to fall below 80%. Also, doing a larger number of replications at 70ºC and 80ºC would be useful in identifying the source of variability in germination results.

## Acknowledgments

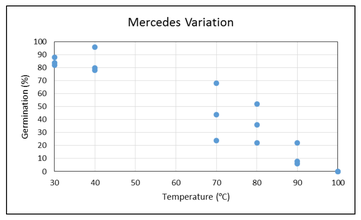
Special thanks to the Bioenergy REU Program of the National Science Foundation for sponsoring this project and to Dr. Angela Post for providing the necessary canola seed.

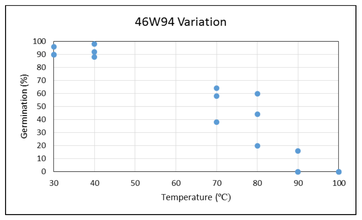
## Results and Discussion

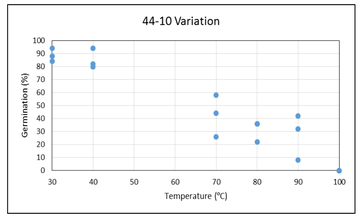
There were no significant difference in germination between these varieties of canola seed. The results showed a general trend of decreased germination as temperature was increased. As expected, germination began to be affected after 40ºC. When the temperature was increased past 80ºC, most germinations went below 15%.

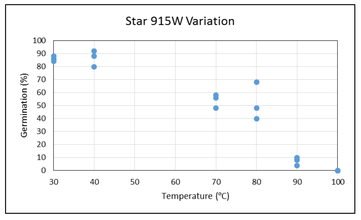



During the germination testing, there seemed to be a lot of variation in the results between replications of the same variety. It is possible that some of this error comes from the placement of the samples in the oven. However, when the data was analyzed a pattern started to emerge throughout many of the varieties. It seemed that as the drying temperature reached 70ºC and 80ºC, the variation increased noticeably. This could mean that at this temperature a lot of damage begins to occur to the embryo inside the seed. Additional testing is required to determine the source of the variation.

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# Establishing & Managing A Switchgrass Hybrid And Inbred Performance Trial


## Dillon Fields

REU Bioenergy Program – Oklahoma State Universty


## Introduction & Overview

Switchgrass is a warm season, perennial, deep rooted crop that occurs naturally in most parts of the United States. Its adaptability to varying climate characteristics as well as tolerance to a wide variety of soils allows it to be so widespread. Its applicability ranges from erosion control and prairie restoration to wildlife habitat but what is most intriguing about switchgrass is its potential to be a valuable raw biomass for biofuel synthesis. The U.S. Department of Energy considers switchgrass one of its principal raw biomass sources due to its high yielding cultivars, broad geographical adaptation, ease in planting, compatibility with conventional farm equipment and positive environmental impacts (Wright, 2007). While there are many switchgrass cultivars, this study will be discussing the following established cultivars amongst other experimental entries: Alamo, Blackwell, Kanlow and Cimarron.

Establishing and managing a switchgrass (*Panicum virgatum* L.) hybrid and inbred performance trial for the purpose of determining applicability for biofuel synthesis is a delicate procedure involving many details and specifications. If the proper field trial is to be executed in determining the feasibility of experimental pedigrees then a level of consistency must be maintained while also ensuring the plants achieve their maximum growing potential. When comparing control pedigrees with experimental pedigrees certain traits must be considered in determining their viability for biofuel production. Future research on the established switchgrass plots will detail specific pedigree traits and parameters necessary for considering biofuel production. The trial consists of preparing 11 different switchgrass pedigrees plots with 4 replications. All parameters in the preparation, planting and plot management are kept constant in order to determine the pedigrees with optimal traits for biofuel synthesis.

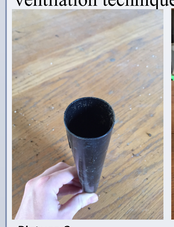
Picture 1

Picture 2

## Materials and Methods

Greenhouse Methods

Initially seeds were placed in germinating mix in a cylindrical tray (Picture 4) with dimensions of 24 cm in diameter and 6 cm long. After 1 to 2 weeks the seedlings were removed from the cylindrical tray and planted individually in cone shaped container (Picture 3) filled with more germinating mix. The cone shaped container is 24 centimeters tall and 4 centimeters in diameter at the opening. These containers were held in holding trays (Picture 5) that had a 98-container capacity. Plants were watered throughout the 6 to 8 weeks from the seed planting time to field planting time. Plants were trimmed and maintained so that the plants didn't exceed 10 to 12 inches. Plants were primarily kept in the 6 to 8 inch range. Plants were kept in a greenhouse that was aerated using a cooling ventilation technique.



Picture 3

Picture 4

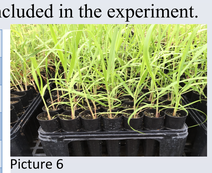
Picture 5

Seedling Count Methods

There were 14 switchgrass pedigrees initially involved in the study. 4 established switchgrass pedigrees, Alamo, Cimarron, Kanlow, and Blackwell, were used as the control entries. There were then 11 experimental entries. Experimental entries include SL93 sib-mating plots 1, 2, and 3. Entries from NL94 X SL93 crossing plots 4, 5, 6, and 7, and entries from NL94 sib-mating plots 8, 9, and 10. The entries were labeled given a number 1 through 14 and are described below (Table 1). For inclusion in the study each plant was to have a minimum 180 seedlings in order to establish a trial. Due to insufficient quantities of data for entries number 3, 10 and 14 they have not been included in the experiment.

| No. | Pedigree | Seedling No. | Table No. |
|---|---|---|---|
| 1 | SL93 S3 | 392 | 1 |
| 2 | SL93 S1 | 392 | 1 |
| 3 | SL93 S2 | 7 | 1 |
| 4 | SL93 S3 x NL94 S3 | 490 | 1 |
| 5 | SL93 S3 x NL94 S3 | 392 | 1 |
| 6 | SL93 S3 x NL94 S3 | 196 | 1 |
| 7 | SL93 S1 x NL94 S1 | 392 | 2 |
| 8 | NL 94 S1 | 686 | 2 |
| 9 | NL 94 S2 | 376 | 2 |
| 10 | NL 94 S3 | 45 | 2 |
| 11 | Alamo | 541 | 2 |
| 12 | Cimarron | 854 | 3 |
| 13 | Kanlow | 363 | 3 |

Table 1

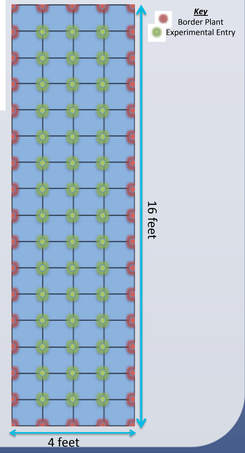


Picture 6

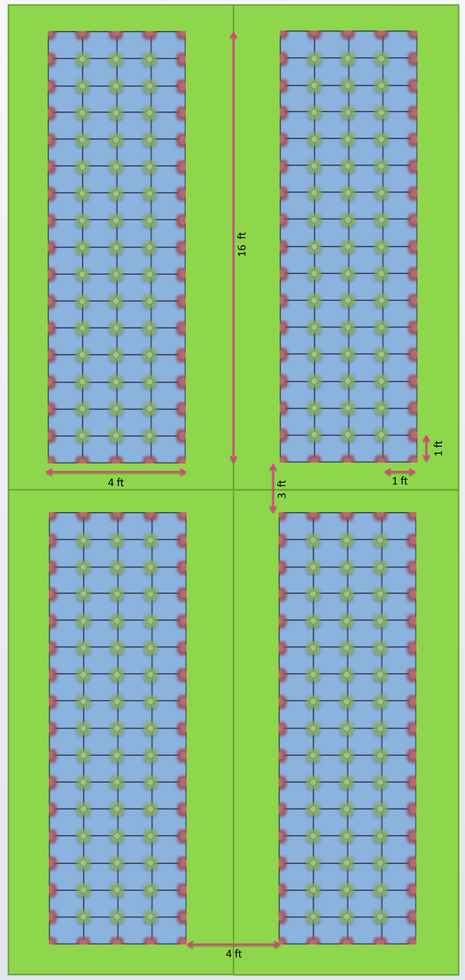
Picture 11

Plot Design Methods

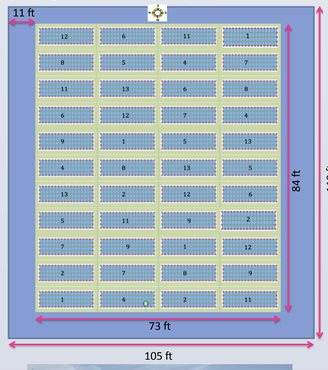
Plants were transplanted with 1 ft spacing within a row. Row length is 16 ft. The spacing between rows is 1 ft with 5 rows per plot. Plots will have 3-foot alleys on length sides and 4-foot alleys on width sides. Border plants will be used, and not tested on, for the edge plants in each plot. The total area of the planting operation is 73 ft in width and 84 ft in length hence a total area of 6,132 sqr ft.




Uniform Plot Design With Spacing Dimensions




Complete Planting Layout




The complete planting layout consists of the 4 columns with each of the 11 entries in each column. This makes 4 x 11 plots in the field with the 11 entries randomized within each column. The blue shaded area consists of the available space that had been tilled for the purpose of this planting.

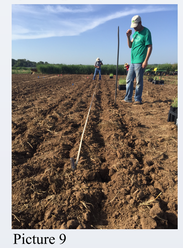
Picture 7

Picture 12

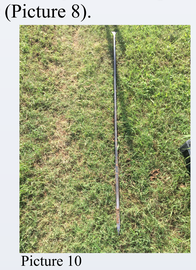
Planting Methods

The dimensions of the available land was measured and flagged before the planting took place. 11 sets of 5 grooves were made in the soil across the planting site with the proper 1-foot spacing in the North-South direction. A tape measure was used to help determine where the holes were needed (Picture 9). Using a long pointed metal pole (Picture 10) holes were created in each of the grooves every foot within the specified flags (Picture 12). Following the Complete Planting Layout blueprint, specific plant entries were planted accordingly. For switchgrass entry number 6 which did not have sufficient plants in order to contain border plants of entry number 6 variety, Cimarron plants were supplemented for border plants. Once the planting was completed a sprinkler irrigation system was placed in the spacing between plots (Picture 8).

Picture 8



Picture 9



Picture 10

## Methodologies Discussion

Greenhouse Planting

The initial step of planting the switchgrass seeds in a germination mix was done in order to maximize seed survival rates. Planting in an exclusive environment ensures no competition of weeds or risk of disease that would be found in outside soil. Drainage was also improved by using a germination mix over planting seeds in outdoor soil. The positive draining from lesser-compacted soil allows for air pockets to form in moist soil so that root formation can extend around the container (Smucker et al., 1982). After the seedlings individual root systems were sufficient enough for transplant they were placed in a tall and thin cone shaped containers so that the roots grew deep into the soil. A deeper root system is vital for plant survival especially in climates where water supply is often scarce. Zhu et al. (2002) state that deeper-rooted systems allow for the access to water that is inaccessible to shallower rooted plants. This can balance high rates of transpiration by the plants during times of minimum rainfall (Zhu et al., 2002). This also posed as an advantage for the switchgrass plants against weeds when they were planted in the soil hence minimizing sources of error in experimental results.

Field Planting

In designing a suitable planting layout the main consideration to be made was spacing. In a study performed by Muir et al., (2001), findings show that the ideal row spacing for switchgrass plants is approximately 25 centimeters as opposed to more distant spacing. This is caused by the quicker canopy enclosure and weed control caused by plants in close proximity (Muir et al., 2001). To reduce sources of error when planting, spacing was increased approximately 5 centimeters so that the spacing between plants was 1 foot. Each plot was initially designed to hold 45 experimental plants with the dimensions 15 plants by 3 plants. Border plants were planted around the edge in order to avoid the boarder effect on experimental plants.

## Conclusion & Future Results

In order to attain proper and useful information on switchgrass cultivar performance, the methodology must be precise as well as uniform for experimental entries. Considering the extended period of time it takes to run a field trial, there is ample amount of time for sources of error to build up and throw off results if the methods of the trial aren't performed carefully. Future analysis on the established field trial will detail results for which definitive conclusions can be made.