CORNERSTONE
MINNESOTA STATE UNIVERSITY MANKATO

Journal of Undergraduate Research at Minnesota State University, Mankato

Volume 8 Article 2

2008

# Characterization of Fall Leaves as a Source of Cellulosic Ethanol

Justin Burum
*Minnesota State University, Mankato*

**Student Agreement:**
I am submitting my research article to be published in the JUR (The Journal of Undergraduate Research at Minnesota State University, Mankato), an electronic journal of the Minnesota State University Undergraduate Research Center.

I/We certify have followed the accepted standards of scientific, creative, and academic honesty and ethics.

I understand that my article submission will be blind-reviewed by faculty reviewers who will recommend acceptance for publication; acceptance with revisions; or reject for publication.

I understand that as author, I retain the right to present any part of the research in any form in other publications.

The JUR has the right to reproduce and reprint published submissions for instructional or promotional purposes.

For complete details, see *Journal of Undergraduate Research at Minnesota State University, Mankato policies page.*

**Mentor Agreement:**
I have reviewed the submission, and I support its inclusion in the JUR (The Journal of Undergraduate Research at Minnesota State University, Mankato). I understand that I will be acknowledged as the faculty mentor for the student author(s). To the best of my knowledge, the student has followed the accepted standards of scientific, creative, and academic honesty and ethics.

# Characterization of Fall Leaves for Ethanol Production

Justin Burum

(Department of Chemistry and Geology)

J. E. Rife

Faculty Mentor

(Department of Chemistry and Geology)

## Abstract

Ethanol is becoming increasingly popular as a fossil fuel additive or substitute. However, current production of ethanol from corn impacts food prices and appears to have an overall low net yield. New sources need to be identified and new processes developed for ethanol production. Cellulosic ethanol is one such new source. Plant material contains a large amount of cellulose and other polysaccharides which are potential feed stocks for ethanol production. The purpose of this experiment was to characterize the polysaccharide, lignin, and ash content of fall leaves to estimate their potential for ethanol production. A slight modification of the NREL procedure "Determination of Structural Carbohydrates and Lignin in Biomass" was used to characterize the leaves. Results of this analysis demonstrated that the leaves were composed of cellulose (23.8 +/- 1.6%), xylan (7.4+/- 0.7%), arabinan (7.9+/-0.8%), acid soluble lignin (22.3+/- 1.0%), acid insoluble lignin (27.6%), and acid insoluble ash (2.6%). It is estimated that approximately 60 gallons of ethanol could be produced per ton of leaf litter. In comparison to other cellulose sources, leaf litter has less sugars and will produce less ethanol. However, energy is already being expended to harvest leaf litter whereas addition energy would be consumed to harvest other cellulose sources.


## Introduction

Ethanol is an important fossil fuel additive or substitute. As fuel prices continue to rise and warnings of global warming attract public attention, more effort in the US is being made to find cleaner, renewable energy sources. Ethanol continues to be a

popular potential alternative to fossil fuels. While traditional fuels release new greenhouse gasses into the atmosphere, ethanol production actually recycles greenhouse gasses. Ethanol also enjoys growing popularity because it can be produced domestically. In the US, most ethanol is made from corn. However, there are problems with using corn as the sole source for ethanol production. First and foremost, it is food. By diverting corn to ethanol production, prices of food and livestock feed made from corn will rise. Another problem, is the amount of fuel it takes to plant, cultivate, harvest and transport corn. Considerable fuel is also consumed in the fermentation and distillation process before the corn becomes usable ethanol. The amount of fuel required for converting corn into ethanol is so great that the actual net yield for the process is low. Alternative sources and methods for producing ethanol need to be found if ethanol is to eventually replace fossil fuels. Cellulosic ethanol is one possible alternative.[1,2,3] Cellulosic ethanol is made by breaking down cellulose in plants and certain bacteria into glucose. The glucose can then be fermented into ethanol.

Cellulose is an important component of plant cell walls[4] and the main carbohydrate in plants.[5] Cellulose is composed of up to 15,000 glucose molecules connected by β-1,4 glycosidic bonds.[5] Cellulose forms a rigid crystalline structure due to hydrogen bonding within and outside the molecule.[5] Cellulose fibers are formed by intermolecular hydrogen bonds connecting strands of glycan. Glycan chains are then stacked, and further hydrogen bonding holds the whole structure together. Cellulose is responsible for the rigidity and strength of the plant.[5] Figure 1 shows the crystalline structure of cellulose.

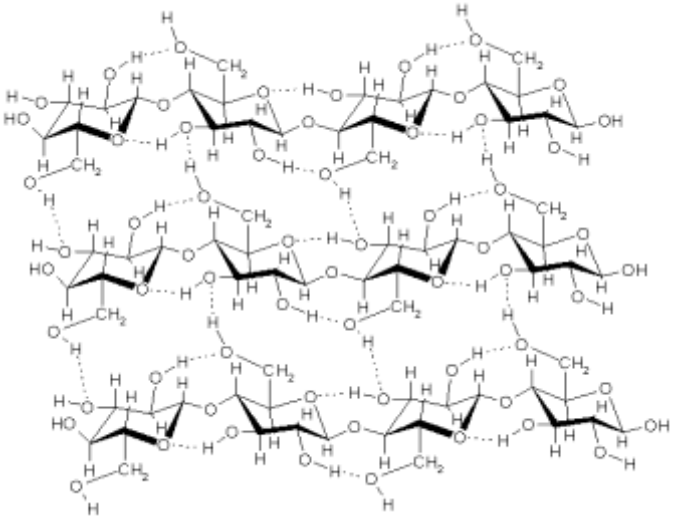

Figure 1: Structure of cellulose. This figure comes from http://www.doitpoms.ac.uk/tlplib/wood/figures/cellulose.png.

In nature, enzymes from microorganisms break down cellulose into glucose by cleaving the β-1,4 bonds.[1,5,6,7] Figure 2 shows the conversion of cellulose to individual glucose molecules. Once cellulose has been broken down, the glucose can be used in the production of ethanol. Work has been done manipulating microorganisms to produce decent yields of ethanol. In a study by Alterthum, et al. Escherichia coli were genetically altered with genes from Zymomonas mobilis.[8] Zymomonas mobilis produces enzymes that can convert sugar into ethanol.[8] The study involved turning multiple sugars such as glucose, xylose, and galactose, into ethanol.[8] A genetically altered strain of E. coli effectively converted all sugars into ethanol better than traditional bacteria strains.[8]

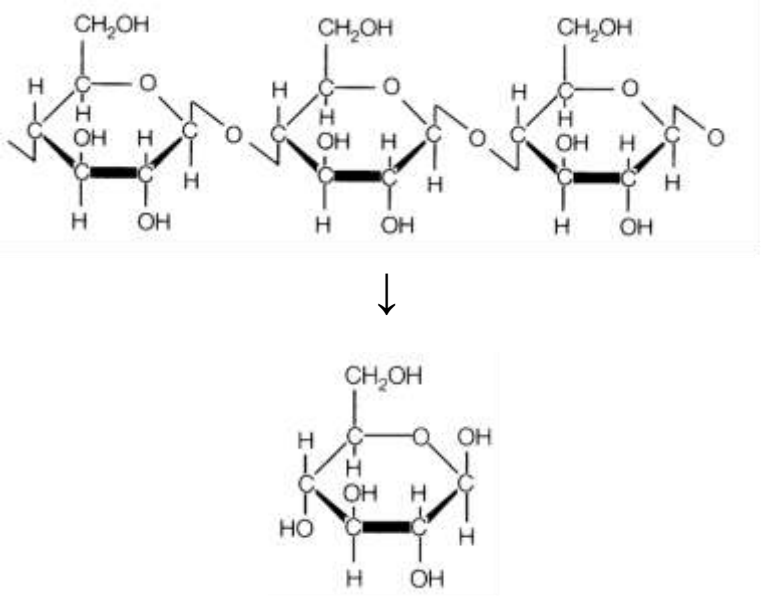

Figure 2: Cellulose to glucose. Structures come from http://www.greenspirit.org.uk/Resources/cellulose.gif.

Breaking cellulose into glucose is difficult as cellulose is usually associated with hemicellulose and lignins.[4,5] Lignins are a plastic-like, phenolic polymers that prevent enzymes from degrading cellulose and hemicellulose.[5]

The purpose of this experiment is to characterize leaf litter for the amount of sugars, lignins, and other components within the plant material. Leaf litter was chosen for two reasons. First, leaf litter requires little additional fuel to harvest for ethanol production as it is already has to be harvested in the fall when we rake our yards and transport the leaves to compost facilities. The second reason is that leaf litter is not currently used for anything productive other than production of mulch. For these two reasons, leaf litter is an attractive source for ethanol production.

## Materials and Methods

The method used was slightly modified from the protocol developed by the National Renewable Energy Laboratory (NREL).[9]

*Pre-treatment*

Leaves collected in Fall 2007 were ground into a powder. Samples were prepared in triplicate. Three hundred mg samples were weighed and 3 mL of 72% sulfuric acid were added to each sample. Samples were incubated at 30$^{o}$C in a shaking water bath for approximately one hour. Eighty-four grams of water were added to each sample before they were autoclaved at 121$^{o}$C for 1 hour. The residual solid was collected in a filtering crucible by vacuum filtration. Solid and liquid material were analyzed separately.

*Solid Analysis*

Solid material was dried in the crucible at 105$^{o}$C until a constant weight was measured. This measurement gave the combined mass of acid insoluble lignin and acid insoluble ash. The solid material was heated at 570$^{o}$C until constant weight. The remaining, solid material was acid insoluble ash.

*Liquid Analysis*

Acid soluble lignins were determined by measuring the UV absorbance at 198 and 240 nm. One mL of filtrate was diluted with 10 mL of water for absorbance measurements. Average absorbances and %ASL (Acid Soluble Lignin) were determined. The %ASL was determined with the following equation[9]:

$$\% \text{ ASL} = \{[(\text{Avg UV abs}) \times \text{filtrate volume} \times \text{dilution}] / [E \times \text{Sample Weight}]\}$$

The extinction coefficients, E, were based on those reported for bagasse[9].

Carbohydrates in the liquid portion were analyzed by High Performance Liquid Chromatography (HPLC). HPLC conditions were:

Column: Kromasil Amino Column (25 cm length x 4.6 mm diameter

Mobile Phase: 75% Acetonitrile 25% Water

Flow rate: 2 mL/minute

Refractive index detection

Sugar standards were used to determine the retention times of the sugars and to prepare standard curves for quantification of sugars found in the filtrates. Sugar standards were made for arabinose, xylose, glucose, galactose, mannose, and cellobiose. Standard curves were made for each sugar by plotting HPLC peak areas versus sugar concentrations in the standards.

## Results

*Solid Material*

Analysis of the solid material in the filtering crucible determined that an average of 27.6% of the initial 300 mg leaf powder sample was acid insoluble lignin while 2.6% was acid insoluble ash.

*Liquid Material*

Table 1 shows the results for measuring the acid soluble lignins in the liquid material. Amount of acid soluble lignins in 300 mg of leaf litter was determined to be 23.4+/-1.1% using the absorbance 240 nm and 21.2+/-0.8% using the absorbance at 198 nm. An average of these two values is 22.3+/-1.0%

| Sample | 198 (1+10) | 240 (1+10) |
|---|---|---|
| A1 | 2.9348 | 1.1623 |
| A2 | 2.8622 | 1.1941 |
| A3 | 2.897 | 1.1903 |
| Avg | 2.898 | 1.18223 |
| Dilution | 11 | 11 |
| %ASL | 0.2264 | 0.2463 |
| B1 | 2.6974 | 1.098 |
| B2 | 2.6751 | 1.1045 |
| B3 | 2.6974 | 1.1122 |
| Avg | 2.68996 | 1.1049 |
| Dilution | 11 | 11 |
| %ASL | 0.2121 | 0.2323 |
| C1 | 2.6539 | 1.0577 |
| C2 | 2.6974 | 1.0616 |
| C3 | 2.6751 | 1.0666 |
| Avg | 2.67546 | 1.06196 |
| Dilution | 11 | 11 |
| %ASL | 0.2117 | 0.2241 |

Table 1: Acid soluble lignin data from absorbance measurements at 198 and 240 nm

Standard sugar and filtrate samples were analyzed by HPLC. Standard curves for xylose, arabinose, mannose, galactose, glucose, and cellobiose were created. Sugar peaks in the filtrate were identified by comparing their retention times with the sugar standards. The amount of each sugar in the filtrates was determined by inserting

the peak area from the HPLC chromatogram into the standard curve line equation of that particular sugar. The measured amount of each monosaccharide was corrected to its anhydro mass to reflect the amount of the corresponding polysaccharide in the original plant material. Cellulose (23.8+/- 1.6%), xylan (7.4+/- 0.7%), and arabinan (7.9+/- 0.8%) were found in significant quantities. Other sugars found were negligible. The observation that cellobiose was negligible indicated that the acid hydrolysis was essentially complete.

Table 2 summarizes the overall amount of lignins, ash, and polysaccharides found in the leaf litter samples. A column for cattails was included for comparison (Lama, Rife and Marg, unpublished results).

| Substance | Leaf Litter Percent | Cattail Comparison |
|---|---|---|
| Acid Soluble Lignin | 22.3 +/- 1.0% | 3.7 +/- 0.1% |
| Acid Insoluble Lignin | 27.6% | 22.1 +/- 0.3%* |
| Acid Insoluble Ash | 2.6% | Not Available |
| Cellulose | 23.8 +/- 1.6% | 37.4 +/- 4.4% |
| Xylan | 7.4 +/- 0.7% | 24.5 +/- 7.0% |
| Arabinan | 7.9 +/- 0.8% | 11.5 +/- 5.3% |

Table 2: Percentage results for leaf litter and cattails for acid insoluble lignins, acid insoluble lignins, acid insoluble ash, cellulose, xylan, and arabinan.

## Discussion

A large portion of leaf litter is made up of lignins. Acid insoluble lignins and acid soluble lignins make up as much as 50% of leaf material. Cellulose, arabinan, and xylan were found in significant quantities within leaf litter. Cellulose (23.8+/-1.6%) makes up nearly a quarter of the leaf followed by arabinan and xylan at significantly lower amounts. Galactose, mannose, and cellobiose amounts in leaf litter samples were negligible. Based on overall calculations, fall leaf litter has the potential to yield approximately 60 gallons of ethanol per ton of leaves. Table 2 shows that cattails have significantly higher amounts of cellulose, arabinan, and xylan. Cattail material can be made into nearly twice as much ethanol/ton. It is important to note that although cattails can be made into a higher amount of ethanol per ton, cattails have to be harvested. This will require energy that will decrease the overall net yield. Leaf litter is still a good source of cellulosic ethanol because much of the energy needed to harvest and transport the leaves is already being expended. Future work for leaf litter will involve maximizing the amount of sugars recovered from leaf litter as well as optimizing the fermentation.

## Acknowledgments

I would like to thank J. E. Rife for being by mentor, introducing this project, and his valuable guidance through the procedure. I would also like to thank Tenzing Lama for his data on cattails, the Department of Chemistry and Geology and the Department of Biology for allowing use of their equipment, the Department of Energy for funding, and the URC for allowing me to present my results.

## References

1. Mandels, M.; Hontz, L.; Nystrom, J. Enzymatic hydrolysis of waste cellulose. *Biotech. Bioengrg*., 2004, 16 (11), 1471-1493.

2. Pimentel, D.; Patzek, T. Ethanol production using corn, switchgrass, and wood; Biodiesel production using soybean and sunflower. *Natural Resources Research, 2005*, 14(1), 64-76.

3. Sun, Y.; Cheng, J. Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresource Tech*., 2002, 83, 1-11.

4. Lynd, L.R.; Weimer, P.J.; van Zyl, W.H.; Pretorius, I.S. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol. Molec. Biology Reviews*, 2002, 506-577.

5. Beguin, P.; Aubert, J. The biological degradation of cellulose. *FEMS Microbiol. Reviews*, 1994, 13 (1), 25-58.

6. Jeoh, T.; Ishizawah, C.; Davis, M.; Himmel, M.; Adney, W.; Johnson, D. Cellulase digestibility of pretreated biomass is limited by cellulose accessibility. *Biotech. Bioengrg*. 2007, 98 (1), 112-122.

7. Wood, T.; McCrae, S. Enzymes involved in the generation of glucose from cellulose. *Developments in Food Science*,1979, 2, 257-263.

8. Alterthum, F.; Ingram, L. Efficient ethanol production from glucose, lactose, and xylose by recombinant Escherichia coli. *Appl. Environ. Microbiol*, 1989, 55(8), 1943-1948.

9. Sluiter, A.; Hames, B.; Raymond, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of structural carbohydrates and lignin in biomass. *NREL Biomass Program*, 2006.

**Biographical Sketches**

Justin Burum just graduated with a BS in biochemistry from Minnesota State University, Mankato and also holds a BA in biology from Gustavus Adolphus College. Justin has presented at the Sigma Xi Conference at Gustavus on “Thin-Section Evidence of a Sulphophilic Stage on Ancient Whale Falls”. At the 2008 URC, he gave an oral presentation titled “Characterization of Fall Leaves for Ethanol Production”. He has begun a master's degree at the University of North Dakota with Dr. Steven Ralph. His master's thesis will likely involve identifying genes that respond and defend the plant against insect foraging.

James E. Rife (faculty mentor) attended Blackburn College in Carlinville, Illinois for two years before transferring to the University of Illinois at Champaign-Urbana where he obtained a B. S. in Chemistry in 1972. After serving two years in the military, he entered graduate school at the University of Wisconsin. There he worked in the laboratory of Dr. W. W. Cleland studying the kinetics of Bovine Liver Glutamate Dehydrogenase. He earned a Ph.D. in Biochemistry in 1978. He worked as a post-doctoral student for two years in the laboratory of Dr. Marion O’Leary in the Department of Chemistry at the University of Wisconsin studying the carbon isotope effects of Phosphoenol Pyruvate Carboxylase from maize. He taught chemistry at Mundelein College in Chicago from 1980 until 1986. In the Fall of 1986, he joined the faculty in the Department of Chemistry and Geology at Minnesota State University, Mankato where his teaching duties have focused on biochemistry courses and chemistry for health science majors.