CB2E: Converting Cellulosic Biomass to Ethanol

Introduction

What is **cellulosic biomass** and why are scientists and engineers working hard to discover efficient ways to convert it into **ethanol** transportation fuel? **Biomass** is any organic material that comes from organisms, such as plants. Plant biomass contains energy that can be used for food or fuel, depending on what part of the plant is used. You have most likely eaten some plant biomass today–perhaps in the form of fruit, cereal grains, nuts, sugar or plant oils. But you probably haven't eaten much cellulosic biomass today, since this is the part of plants that people cannot digest.

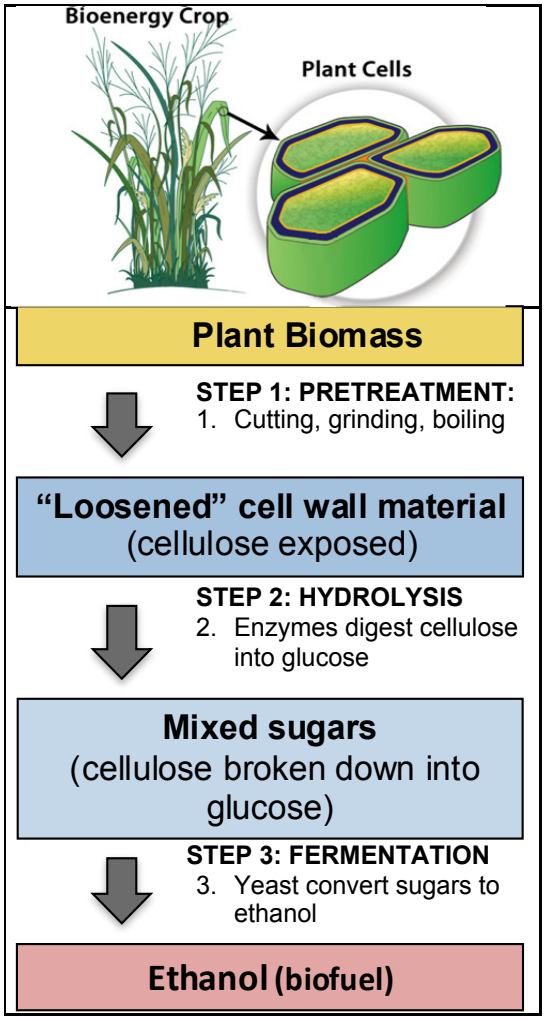
Cellulosic biomass refers to tough, fibrous, or woody plant parts, such as grass, leaves, stems, flowers, corn stalks, wood, or paper products. Although cellulosic biomass cannot be used for food, it contains a large amount of energy that can be used as fuel for transportation. If you have ever enjoyed the warmth of a wood fire, then you have observed the value of cellulosic biomass for fuel. Cellulosic biomass is mostly made up of a molecule called **cellulose**, which is the primary component of plant **cell walls**. Without cellulose, plants would not able to stand upright. This is one reason why cellulose is the most abundant molecule on earth and represents a huge potential pool of renewable energy if we could find a way to easily convert it into transportation fuel.

According to a recent study, a little over 1 billion tons of cellulosic biomass harvested every year could produce about 85 billion gallons of biofuels. This could supply approximately 30 percent of U.S. transportation fuel needs in the year 2030. In addition, this would represent a renewable fuel source that would greatly reduce U.S. greenhouse gas emissions compared to gasoline. For these and other reasons, the U.S. Department of Energy is investing heavily in research to discover methods to efficiently convert cellulosic biomass into **ethanol**.

In this lab, you will investigate the challenge of converting cellulosic biomass into ethanol. You will use some of the same strategies used by scientists and engineers at the Great Lakes Bioenergy Research Center (GLBRC). The process involves three key steps: 1) Pretreatment, 2) Hydrolysis (enzymatic digestion), and 3) Fermentation.

Biofuel Pipeline Overview

The diagram at the right provides an overview of the steps and describes the chemical changes occurring as cellulosic biomass is converted to sugar and then ethanol. We call this a "biofuel production pipeline" because the products generated from one step are used in the next step until ethanol is produced.



In the **pretreatment stage**, the goal is to loosen the cell wall structure so that the cellulose is exposed. Plant cell walls are made up of three primary components: **cellulose**, **hemicellulose**, and **lignin**. These molecules must be separated so that **enzymes** can reach the cellulose. Heating and grinding are effective pretreatment methods.

Cellulose is actually made up of long chains (polymers) of **glucose** molecules. In the **hydrolysis stage**, the goal is to break the long cellulose molecules down into individual glucose molecules. Special enzymes called **cellulases** are able to cut up the cellulose strands into **glucose**. Glucose is a simple sugar that can be used as food by many organisms.

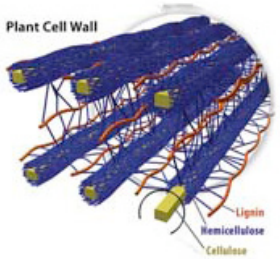
In the final step of the pipeline, **yeast** is added to the enzymatically digested biomass mixture. Without oxygen, yeast consumes the glucose and produces ethanol through a process called fermentation. The yeast used in this process is the same single-celled organisms used to bake bread or brew beer.

In this investigation, you and your research team will pick a cellulosic biomass sample to first convert into sugars, and then into ethanol through the process described above. You will track the conversion process by measuring sugar (glucose) and ethanol levels at key stages. This data you and your classmates collect will help you determine which biomass sources and pretreatment methods are most effective for producing sugars and ethanol and develop explanations for why some samples produce more sugar and ethanol than others.

Glossary



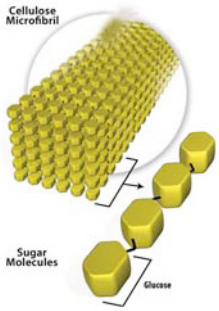
Biomass: An energy resource derived from organic matter. This includes wood, agricultural waste and other living-cell material that can be burned to produce heat energy.



Cell Wall: A diverse structure that primarily functions in shaping the plant cell to provide structural support and disease protection to plants.

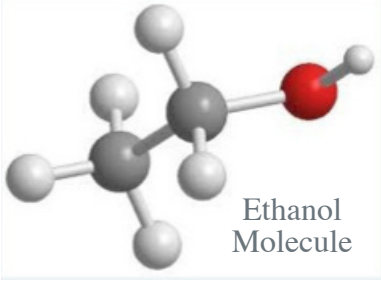
Cellulase: An enzyme that breaks cellulose into glucose.

Cellulose: The most abundant plant material on Earth. It is a type of carbohydrate made of a chain of beta glucose rings strung together that play a critical role in building the cell wall.



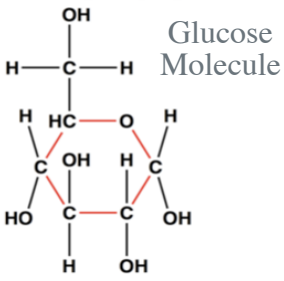
Enzyme: A protein molecule that functions as a catalyst; it helps break apart (or build) other molecules. Enzyme names usually end in "-ase." For example, cellulase (an enzyme) breaks apart cellulose (a carbohydrate).

Ethanol: An alcohol that can be produced by fermentation of plant material. Ethanol is the same alcohol found in beer, wine, and spirits. Denatured ethanol is used for transportation - it is drinkable ethanol that has additives to make it undrinkable.



Fermentation: A form of cellular respiration performed in an environment without oxygen. Yeast and bacteria are frequently used as fermenters; they consume sugars for energy and release byproducts such as ethanol and carbon dioxide.

Glucose: A simple sugar or carbohydrate. Cellulose and starch are both broken down into glucose before fermentation into ethanol.



Hemicellulose: A carbohydrate that is an important component of the cell wall with a branched structure.

Hydrolysis: A reaction breaking larger molecules into smaller ones, like breaking cellulose into glucose.

Lignin: A component of the cell wall, but not a carbohydrate. The intricate association of lignin and cellulose presents one of the major challenges in converting cellulose into ethanol.

Pretreatment: The opening of the structure of plant cell walls to allow access for enzymes to attack plant material. Often performed by boiling, grinding, or chemical usage.

Yeast: Various single-cell fungi capable of fermenting carbohydrates.

Lab Overview

The goal of this lab is to convert a cellulosic biomass sample into sugar and then ethanol. Your lab group should select a biomass sample and cutting/grinding treatment that you think will effectively produce ethanol. Your group will prepare both an experimental and a control treatment to evaluate the effects of the enzyme on the production of sugar and ethanol.

Experimental Sample:
- Biomass
- Cutting/Grinding

Treatment
- Enzyme

Control Sample:
- Biomass
- Cutting/Grinding

Treatment
- *No Enzyme*

Lab Research Stages	Activities
1. Experimental Design and Planning (1-2 days)	• Develop research plan • Choose biomass type and/or pretreatment options (cutting or grinding)
2. Sample Prep and Pretreatment (1 day)	• Set up experiement • Cut, grind and/or boil biomass • Measure initial sugar levels
3. Hydrolysis (Enzyme Digestion) (1 day)	• Add cellulase enzymes • Measure sugar levels (after 24 hours)
4. Fermentation (1 day)	• Measure initial ethanol levels • Add yeast • Measure final ethanol levels (after 24 hours)
5. Data Analysis, Conclusions, and Discussion (1-2 days)	• Graph final results • Summarize conclusions and communicate findings to class • Write up final results based on evidence from your other lab group results

Experimental Design and Planning

In your group, discuss and decide which biomass and grinding options will be best for producing ethanol. As instructed by your teacher, write down why you think it will produce the most ethanol. Be prepared to explain what you think will happen in this experiment and pinpoint what evidence you will use to determine whether your prediction was accurate.

Experimental Design and Planning Questions

The goal of this lab is to produce as much ethanol as possible from 1 gram of biomass mixed with 25 mL of water. Based upon the options provided by your teacher, work with your lab group to decide what biomass type and grinding option you would like to convert into ethanol. Answer the questions below and be prepared to share your answers with the class.

1. What biomass did you choose? Explain why.

2. If applicable, what grinding option did you choose? Explain why.

3. What evidence will you gather from this experiment to determine whether your biomass is effectively coverted into ethanol?

4. At what stage in the lab (pretreatment, enzyme digestion, fermentation) and in which treatment (control or experimental) do you expect to measure the highest glucose levels? Explain.

5. At what stage (pretreatment, enzyme digestion, fermentation) and in which treatment (control or experimental) do you expect to measure the highest ethanol levels? Explain.

Step 1: Sample Preparation and Pretreatment

Goal: Break down plant cell walls to release the cellulose fibers

Sample preparation:

1. Label two 50mL falcon tubes and caps with your team initials, date, and sample description (biomass source and any pre-treatment).

Control (No Enzyme)
Biomass Sample
Initials
Date

Biomass Sample
Enzyme
Initials
Date

Label for each of your 50mL falcon tubes

1.0 gram pretreated biomass

   a. The labels above are examples. Every group will have 2 tube setups with the same biomass.
   b. If any pre-treatment is required do so (cutting, grinding, drying, etc.)

2. Measure 1.0 gram of your biomass samples and put the 1.0 gram into the corresponding 50mL falcon tube.

3. Test the initial glucose concentration using the blood glucose test monitor and test strips. Record this data. Describe the biomass (appearance? odor?).

4. Test the initial ethanol concentration using the ethanol probes. Record this data. Describe the biomass (appearance? odor?).

Hot water pretreatment:

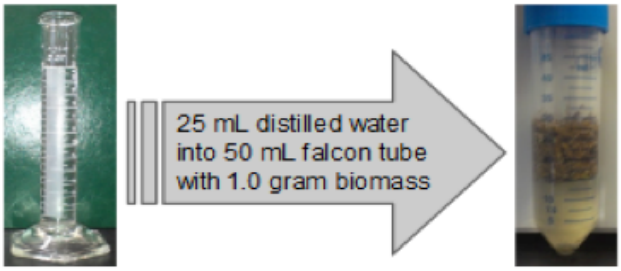
1. Start the hot plate to bring approximately 400mL water to a gentle boil in a 500 mL glass beaker. Use pre-heated water to fill your beaker.

2. Set up a falcon tube tube holder (i.e. chicken-wire screen or aluminum) foil for your 500 mL beaker as directed by your teacher. If you are partnering with another group, you can pack 4 tubes in a beaker without setting up a holder.

Do this now! Caution: boiling water

Set-up will look like this later: two 50mL falcon tubes

3. Add 25 mL of distilled water to the to all three of your labeled 50mL falcon tubes.



4. Swirl to mix the biomass and the water. Let it sit for 1 minute.

5. Loosely screw the cap onto the falcon tube.

6. Wait for water in your beaker to come to a gentle boil on your hot plate.

7. Gently push your two falcon tube samples into the beaker though the aluminum foil or wire screen. If you are using the 4-tube method pack the 4 tubes into the 500mL beaker.
    a. Make sure that the biomass samples and the liquid are completely submerged below the surface of the boiling water in the beaker.

8. Leave tubes in the water for 10 to 25 minutes depending on how much time you have. The longer the time period, the higher the potential yield of ethanol will be.

9. Turn off the hot plate and remove your samples. Allow them to cool to room temperature. Use a cold water bath to make the tubes cool more quickly.

10. Test the glucose concentration using the blood glucose test monitor and test strips. Record this data. Describe any detectable changes in the biomass (appearance? odor?).

12. Test the ethanol concentration using the ethanol probes. Record this data. Describe any detectable changes in the biomass (appearance? odor?).

13. If samples will not be used in the next 2 days, refrigerate or freeze them immediately. This will suppress microbial growth.

Step 2: Enzymatic Digestion (Hydrolysis)

Goal: Digest the cellulose fibers into glucose (sugar)

1. Remove samples from refrigerator or freezer and bring to room temperature.
2. Make sure the common water bath or the incubator is at 50°C.
3. Add 1.0 mL of Celluclast™ or 0.05g Carolina Biological cellulase enzyme product to each test tube that is undergoing hydrolysis. The control will not have any enzyme added.
4. Screw caps on tightly. Mix gently.
5. Place both falcon tubes in a common water bath or incubator at 50°C.
6. Leave the tubes in the water bath for 24 hours.
7. After 24-hour hydrolysis data collection: Use the blood glucose test monitor and test strips to test post-enzyme glucose concentration of the sample. Record this data. Describe any detectable changes in the biomass (appearance, odor?).

8. Test the ethanol concentration using the ethanol probes. Record this data. Describe any detectable changes in the biomass (appearance, odor?).
   **Note: for more accurate ethanol readings, allow samples to reach room temperature before taking measurements.
9. If fermentation will not begin at this stage, freeze or refrigerate samples to prevent microbial contamination.

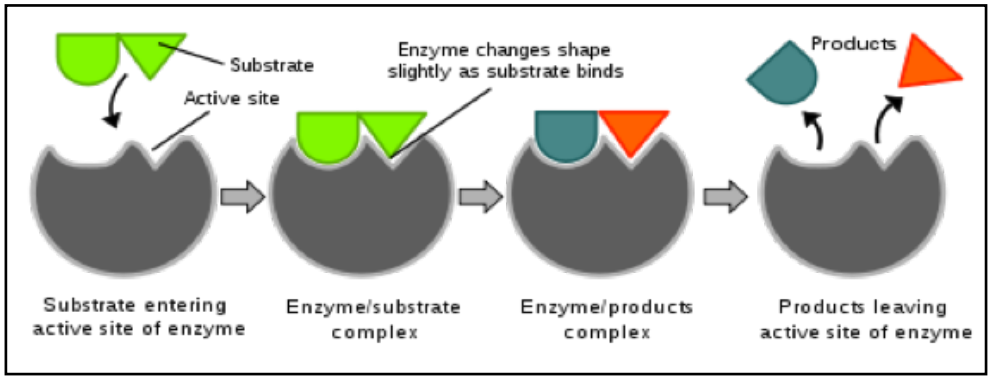


Illustration of enzymatic deconstruction. In this experiment the cellulase (enzyme) is binding to cellulose (substrate) in the biomass and breaking the cellulose up into individual glucose molecules (products).

Step 3: Fermentation

Goal: convert glucose (sugar) into ethanol (fuel).

1. Make sure the common water bath or incubator is at 37°C.

2. Add ¼ teaspoon or 1.0 gram of active yeast to each tube. These measurements are roughly equivalent.

3. Gently mix in the yeast. The yeast will grow more quickly if evenly mixed.

4. Loosely screw on the cap to the tubes. It is important that the tubes not be air-tight for the fermentation. Yeast will produce CO_2 and will build up pressure in the tube uness the gas is allowed to escape.

5. Place falcon tubes upright in the 37°C water bath or incubator. Use a test tube rack or similar aparatus (chicken wire) to keep falcon tubes upright.

6. **OPTIONAL: After 30 minutes measure ethanol and glucose concentration, record data and other observations about changes occuring in the tubes.

7. Return your falcon tubes to the 37°C common water bath or incubator for 24 hours of fermentation.

8. After 24 hours, remove your falcon tubes from the 37°C water bath.

   **Note: If 24-hour measurement does not fit with class schedule, instructor can remove samples from water bath and refrigerate or freeze until final measurements can be taken.

9. Take final glucose readings: Use the blood glucose test monitor and test strips to test post-enzyme glucose concentration of the sample. Record this data. Describe any detectable changes in the biomass (appearance, odor?).

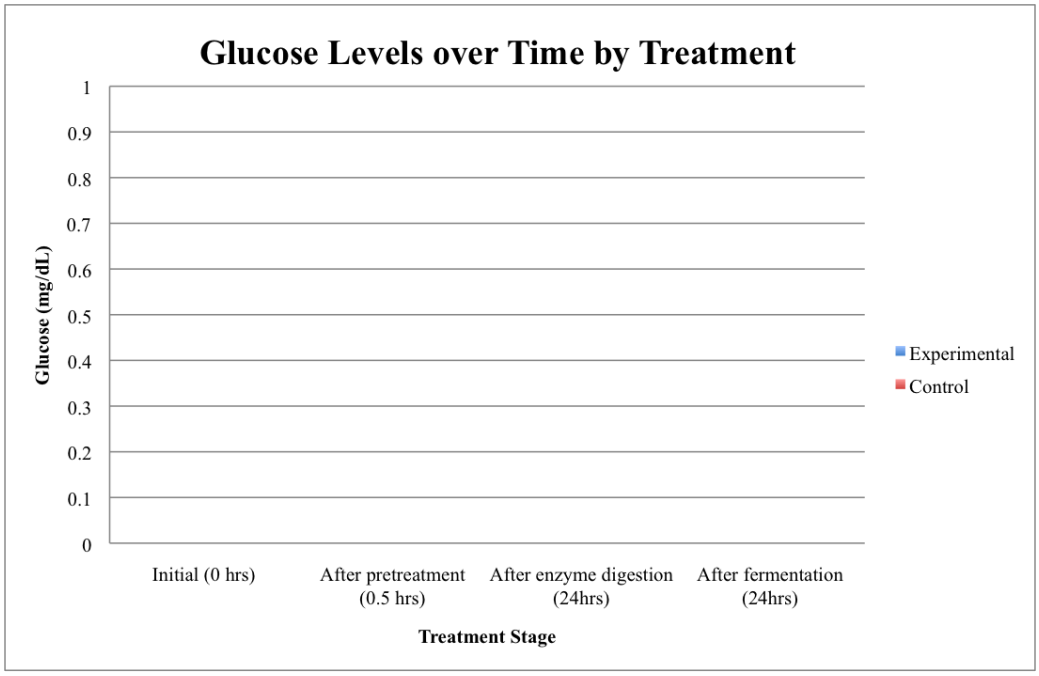
10. Take final ethanol readings: Test the ethanol concentration using the ethanol probes. Record this data. Describe any detectable changes in the biomass (appearance, odor?). **Note: For more accurate ethanol readings, allow samples to reach room temperature before taking measurements.

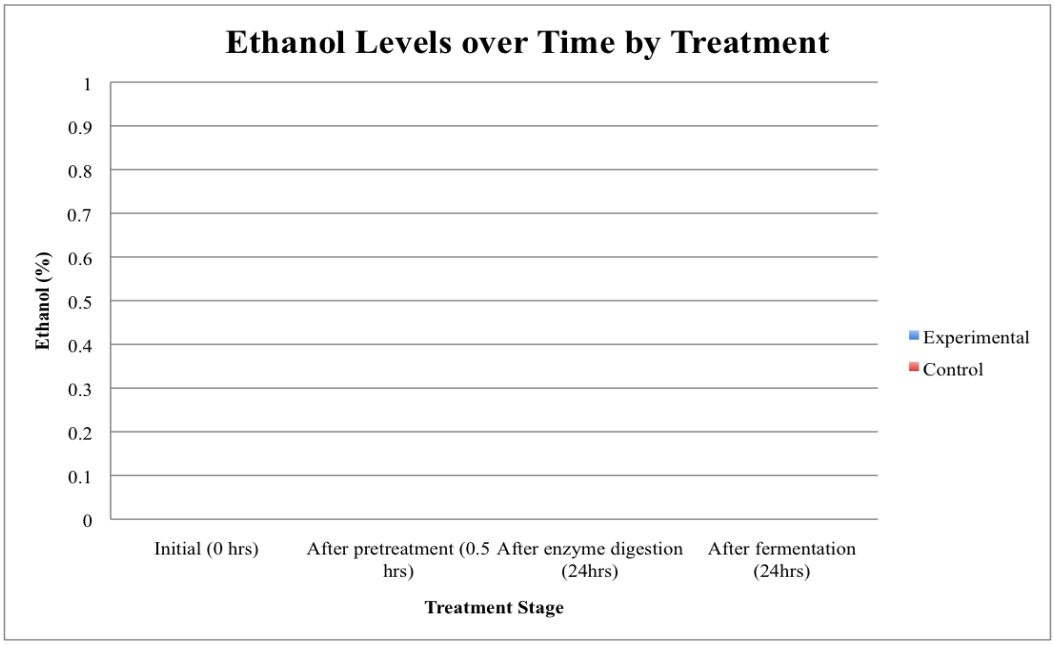
11. Clean tubes and lab area as instructed by your teacher.

Data Chart: Record glucose readings, ethanol readings and other notes and observations for your samples.			**Control (No Enzyme)** Biomass sample description:		**Experimental (Enzyme added)** Biomass sample description:	
Lab Sequence (Date)	Lab Stage	Total Hours	Glucose (mg/dL)	Ethanol (%)	Glucose (mg/dL)	Ethanol (%)
Date, Time:_____	**Initial Measurement**	0				
Notes and Observations						
Date, Time:_____	**Pretreatment** Post liquid hot water treatment	.5				
Notes and Observations						
Date, Time:_____	**Hydrolysis** Post-24-hour Cellulase treatment	24				
Notes and Observations						
Date, Time:_____	**Fermentation** Post-24-hour Yeast treatment	48				
Notes and Observations						

Analysis, Discussion, and Conclusions

To organize and draw conclusions form your data, it is helpful to compare changes in glucose and ethanol levels over time using bar graphs. Using a computer program such as Microsoft Excel (or by hand), create two bar graphs to summarize your results. The empty graphs below can serve as a guide. Discuss the graphs with your lab group. Do these results match your initial prediction? Why or why not? How do you explain your results? Explain, summarize and communicate your results as instructed by your teacher.





Interpretting Results, Discussion and Conclusions

Interpreting results: Use your graphs and lab notebook data to answer these questions about the results of this experiment. Be prepared to share your answers with the class.

1. Did you observe any changes in glucose and ethanol levels after the enzyme digestion stage (hydrolysis)? Explain why or why not.

2. Where does the glucose come from in this experiment?

3. Did you observe any changes in glucose and ethanol levels after fermentation? Explain.

4. Why do you think that glucose levels went up then then went down in over the course of this experiment?

5. Did your observed results match what you expected would happen? Explain why or why not.

Discussion and conclusions: Share your results and initial conclusions with the class. Learn from your classmates' results and observations so you can determine what might be the most effective ways to convert biomass into ethanol.

1. Of all of the samples tested in your class, what biomass treatment produced the most glucose and ethanol? Explain why you think this treatment was most effective.

2. Of all of the samples tested in your class, what biomass treatment produced the least glucose and ethanol? Explain why you think this treatment was least effective.

3. If you were to try this experiment again to produce more ethanol what would you do differently? Explain why.

4. Explain how you would design an experiment to determine whether the boiling pretreatment had an effect on how much glucose and ethanol is produced.