

CB2E: CONVERTING CELLULOSIC BIOMASS TO ETHANOL



Overview: In this flexible lab sequence, students convert cellulosic biomass sources, such as sawdust, straw, or cardboard into sugars and then ethanol. As biomass samples are pretreated, enzymatically digested, and fermented, students use glucose meters and ethanol probes to measure the key products of this chemical conversion. In the process, students can test predictions about which biomass sources and treatment methods will be most effective for producing ethanol.

LEVELS

High School-Undergraduate

SUBJECTS

Chemistry, Environmental Science, Biology, Agriculture

OBJECTIVES

- Test predictions about how variables (biomass type and grinding method) affect conversion into sugars and ethanol
- Trace the transformation of cellulose into glucose and then ethanol
- Assess the action of cellulase enzymes on cellulose
- Measure the conversion of sugars to ethanol
- Use sugar and ethanol readings to evaluate initial predictions and draw conclusions about the effects of treatment variables

MATERIALS

CB2E Package

ACTIVITY TIME

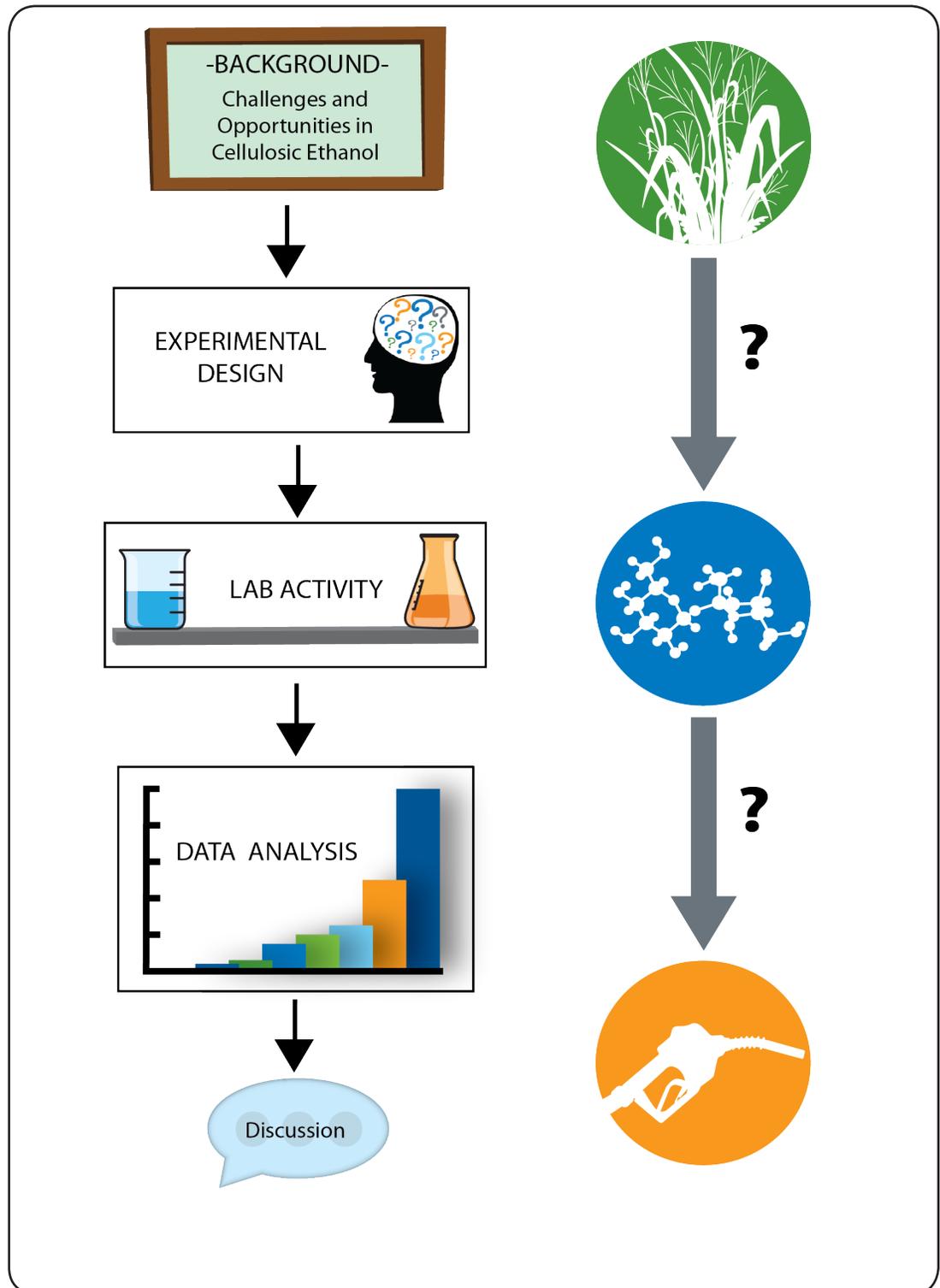
5-7 50-minute class periods

STANDARDS

Next Generation Science Standards (2013)

- Scientific and Engineering Practices: asking questions and defining problems; planning and carrying out investigations; analyzing and interpreting data; constructing explanations and designing solutions
- Disciplinary Core Ideas: matter and its interactions; ecosystems; earth and human activity; engineering design
- Crosscutting Concepts: Energy and matter
- Performance Expectations: See page 3 for details

NGSS Lead States. 2013. Next Generation Science Standards: For States by States. Washington DC: The National Academies Press



For Teachers - CB2E: Conversion of Cellulosic Biomass to Ethanol

Overview:

Students investigate the process of converting cellulosic biomass into sugars (glucose) and then ethanol. In this lab, biomass samples are moved through the conversion “pipeline” steps of pretreatment, hydrolysis (enzyme digestion) and fermentation. The chemical transformation of the biomass is measured at key stages using blood glucose meters and ethanol probes. Students can test and revise predictions about which biomass sources and cutting/grinding treatment methods will be most effective.

This lesson is designed to span five to seven 50-minute class periods over 5 to 14 calendar days. The actual conversion process can be completed in 4 days not including time for reflection and synthesis. This lab can be paused at any stage by refrigerating or freezing samples. This allows for flexibility to work around various class schedules.

Learning Outcomes: Students will...

- Test predictions about how variables such as biomass type or grinding affect conversion into sugars and ethanol
- Trace the transformation of cellulose into glucose and then ethanol
- Infer the action of cellulase enzymes on cellulose based upon sugar readings
- Measure the conversion of sugars to ethanol using ethanol sensors
- Use sugar and ethanol readings to evaluate initial predictions and draw conclusions about the effects of treatment variables

This lesson assumes prior knowledge of energy and matter transformations, fermentation, enzyme action, the relationship between monomers and polymers.

Standards

Next Generation Science Standards (2013)

Performance Expectations

High School:

- **HS-PS1-7.** Use mathematical representations to support the claim that atoms, and therefore mass, are conserved during a chemical reaction.
- **HS-LS2-3.** Construct and revise an explanation based on evidence for the cycling of matter and flow of energy in aerobic and anaerobic conditions.
- **HS-LS2-7.** Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.
- **HS-ESS3-4.** Evaluate or refine a technological solution that reduces impacts of human activities on natural systems.
- **HS-ETS1-2.** Design a solution to a complex real-world problem by breaking it down into smaller, more manageable problems that can be solved through engineering.

Scientific and Engineering Practices	Disciplinary Core Ideas	Crosscutting Concepts
Asking questions and defining problems	PS1: Matter and its interactions	Energy and matter: Flows, cycles and conservation
Planning and carrying out investigations	LS2: Ecosystems: Interactions, energy, and dynamics	
Analyzing and interpreting data	ESS3: Earth and human activity	
Constructing explanations and designing solutions	ETS1: Engineering design	

See Appendix for alignment with other standards.

Master Materials List:

For each item, the suggested quantities per group or class are listed. These quantities can be reduced if groups share equipment (i.e. scales, weigh boats, etc.) w/ combine samples in beakers. See footnotes for details. Columns on right indicate at what lab stage(s) items are needed.

Item	Suggested Quantities	Set-Up & Pretreatment	Hydrolysis	Fermentation
Vernier or Pasco data-collection interface	4/class	✓	✓	✓
Vernier or Pasco Ethanol Probe	4/class	✓	✓	✓
50mL Falcon Centrifuge Tubes ¹	2/group	✓		
TRUEbalance Blood Glucose meter ²	1/group	✓	✓	✓
Blood glucose test strips ³	~10/group	✓	✓	✓
Wax paper or parafilm	1 sheet/group	✓	✓	✓
Cellulosic biomass: sawdust, straw, corn stover, switchgrass, cardboard, etc.	~50 grams of each	✓		
Weight boats	1/group	✓		
Electronic balance	1/group	✓		
Grinder for biomass samples ⁴	4/class	✓		
Scissors, saws, shears, etc. for cutting biomass ⁵	~ 1/group	✓		
25 or 50mL graduated cylinder	1/group	✓		
600mL beakers	1/group	✓		
Hot plates	1/group	✓		
Pens or tape to mark Falcon tubes	1/group	✓		
Pipettes ⁶	1/group	✓	✓	✓
Thermometer	1/group		✓	✓
Water bath or incubator with racks to hold tubes	1/class		✓	✓
Cellulase enzyme (Celluclast: available from Sigma or Cellulase: available from Carolina Biological) ⁷	~10mL or 0.5g/class		✓	✓
Yeast (standard dry active baker's)	1/2 tsp/group			✓
1/4 teaspoon measurer ⁸	1/group			✓

1. These reusable tubes are ideal because of their size and screw on cap. Available through Amazon.com for ~\$20 for 25 tubes. Standard glass test tubes will also work fine.

2. TRUEbalance meters are available from many online medical retailers for ~\$7 to \$15 each.

3. Available from Amazon.com for ~\$14 for 100. Buy extra to accommodate student sampling errors.

4. Standard coffee or spice grinders work well.

5. This is optional depending on whether the instructor wants to offer a range of cutting and grinding options to compare.

6. A standard dropper will work fine as well, but is less accurate for measuring out enzymes and taking samples.

7. Celluclast aqueous solution (Item # C2730) costs \$92 for 50 mL from [Sigma](#). This should be plenty of enzyme for 3-5 sections of ~30 students. Or Cellulase powder (Item # 853630) costs \$35 for 25g from [Carolina Biological](#) which provides enough enzyme for 50 sections.

8. This is the easiest method, but since 1/4 tsp is approximately 1 gram, an electronic balance will work as well.

-BACKGROUND-
Challenges and
Opportunities in
Cellulosic Ethanol

Sequence:

Part 1. Discussion & Background Information: Framing the Problem
(1-2 50-minute periods)

The supplemental discussion questions can be used as a formative assessment to uncover students' current understanding about ethanol production for fuel. Questions can be answered individually or in small groups and then discussed as a class. The questions are designed to uncover students' fundamental knowledge about how ethanol is produced and to engage them in some of the larger social and environmental issues surrounding ethanol production. It is likely that students will not be familiar with cellulosic ethanol production, but the discussion can serve as a launching off point for that topic.

The GLBRC *Fermentation in a Bag* activity can serve as a nice introduction to the challenge of producing ethanol from cellulosic biomass and serve as an engagement point for this investigation.

A combination of the introduction pages to the lab (pages 1-3), the supplementary reading "*Why is it so Difficult to Make Cellulosic Ethanol,*" and the included presentation slides and videos can be used to provide additional background information to students related to basic cell wall structure, the steps in the conversion process, and energy and matter transformations.

Students can also construct concept maps to organize ideas, key terms and to assess their initial understanding of the process of converting biomass to ethanol. A sample concept map template is included with the "Supplemental Materials" for this activity. Creating, reviewing, and revising concept maps through the course of this investigation can serve as a useful formative assessment tool.

EXPERIMENTAL
DESIGN



Part 2. Experimental Design and Planning (50 minutes) (pages 4-5)

Organize students into groups of 3-4 students (3 is preferable). Review the steps for converting biomass to ethanol (pretreatment, enzyme digestion, fermentation), the options for the experiment (biomass type, grinding) and the data to be collected (sugar and ethanol readings). In the basic version of this lab, students have the option to choose the biomass type and how they “pretreat” it through grinding or cutting and measure changes in biomass glucose and ethanol levels after the conversion steps.

Provide students with several biomass options such as wood/sawdust, straw, cardboard, corn cobs, corn stover, etc. If applicable, identify the pretreatment options such as grinding and/or cutting. Students choose biomass-treatment combination and set up two test tubes, one to which cellulase enzyme is added and the other to which no enzyme is added. The tube without enzyme serves as a control.

Controls: Some cellulase enzyme mixtures, such as Celluclast, contain glucose. In addition, yeast and water will produce small quantities of ethanol. For these reasons, we recommend that the instructor set up several controls (i.e. enzyme and water, water only) to provide baseline data on how much glucose is coming from the enzyme and how much ethanol can be produced by the yeast alone.

The overall goal of the lab is to convert biomass into glucose and then into ethanol. Students should discuss in groups which biomass and treatment they think will be most effective. The pre-lab planning questions can be used to guide their discussion and develop an explanation about why they think their choice will be most effective. Groups can share plans with other groups so as to coordinate experiments and collect data that can answer larger questions. Allow groups to briefly share their research plans so that everyone is aware of the scope of questions investigated and samples to be compared.

Part 2 (continued): Experimental Design and Planning

By challenging groups with the task of producing the most ethanol we have framed the investigation as an engineering problem. Instructors should feel free to adapt the lab to the inquiry method with which they feel most comfortable. For example, students can plan investigations to test predictions, hypotheses or evaluate scientific claims. Included with the “Supplemental Materials” for this activity are worksheets and a sample rubric for students to develop claims and scientific arguments using the “Claim, Evidence, Reasoning” framework.

This lab protocol can be simplified or expanded to include more or less complexity depending upon the goals, classroom environment and audience.

Ideas to simplify:

- Give students only one variable to work with, i.e. either biomass type or grinding/cutting treatment.
- Proceed only through hydrolysis (enzyme digestion) phase if the goal is to compare pretreatment methods and evaluate enzyme activity.
- Measure only ethanol or CO₂ production (eg. Vernier gas pressor sensor - see *Fermentation Challenge* activity). This option compromises the educational value of the lab as students observe the transformation of cellulose into glucose then ethanol.



Part 3: Run the experiments (Three 50-minute class periods) (Handout pages 6-9)

The process of converting biomass samples to ethanol can be completed over the course of four class periods (Day 1: sample preparation, Day 2: pretreatment/start hydrolysis, Day 3: finish hydrolysis/start fermentation, Day 4: end fermentation). If it is necessary to pause the conversion process (ie. over the weekend), samples can be frozen or refrigerated to slow chemical reactions and inhibit microbial growth.

The lab sequence also presents excellent opportunities to intersperse student activities such as concept mapping or model building to reinforce understanding of chemical changes to cell wall structure, enzyme interactions and matter and energy flows through the conversion process. One option is to have students use a large sheet of paper or whiteboard to build a large concept map and model of the conversion process for their biomass sample that they revise and update through the lab sequence. See sample concept map template in activity “Supplemental Materials.”

Notes on lab steps:

- Sample Preparation and Pretreatment (1-2 50-minute periods) (page 6)

Initial measurements: Students can take initial glucose and ethanol measurements of the biomass and water samples in order to develop a baseline comparison for the rest of the experiment. This will help students observe what is occurring as the glucose and ethanol measurements change at each stage. Have students predict and explain how glucose and ethanol levels will change before each stage and then explain differences and/or agreement between their predictions and the data collected.

Sample preparation: To save time, you can prepare “pre-ground” or “pre-cut” biomass samples. However, it is valuable for the students to experience the process first hand. To make results comparable across groups, provide guidelines for cutting or grinding.

Using buffer (optional): Cellulase enzymes perform best at a pH of approximately 5.5, while the normal pH of distilled water is 7 and different biomass types can affect the pH differently. To increase enzyme activity and reduce variation due to biomass type add a citrate buffer to the tubes. See “Supplementary Materials” for more information and a guide to making and using the appropriate buffer.

Heat pretreatment (boiling samples): To save time, preheat water for groups. Also, the teacher can boil all samples together in a larger vessel. However, it is educationally valuable for students to carry out, observe and think about the boiling treatment in individual groups.

****Safety precaution:** Students should wear gloves and goggles, especially during boil pretreatment!

Post-pretreatment measurements and discussion: Take glucose and (if time permits) ethanol readings before and after pretreatment, recording observations in the table provided in student pages. Students will likely be surprised that glucose levels do not change significantly after boiling and grinding. This provides the opportunity to review the function and effects of pretreatment on biomass (opening plant cell walls and making cellulose accessible) and to discuss how strong the bonds are in the cellulose molecules, such that boiling/grinding will not break them.

Before adding enzyme (next step), ask groups to reflect on the sugar and ethanol readings after the boil pretreatment. Review the chemical structure of the cell wall and discuss the effects of the pretreatment on the cell wall. The supplementary slides and videos provide helpful graphics (see appendix). If time permits, have students revise their initial prediction and explanation based upon new evidence.

- Hydrolysis (Enzyme digestion):

If time permits, enzyme can be added after the boiling heat pretreatment and initial glucose and ethanol readings have been taken.

****Be sure to allow samples to cool to 50°C before adding enzymes.** At higher temperatures, the enzyme can denature and lose function.

Before students add enzyme to the samples, demonstrate the proper use of a pipetter or dropper if using the aqueous cellulase (Celluclast). Have students practice with water if they are not experienced.

****Safety precaution:** Cellulase enzymes can cause skin and eye irritation. Students should wear gloves and goggles when handling the enzyme.

Discuss why no enzyme will be added to the control sample. What information will this provide in this experiment?

After enzyme is added to samples, the remaining class time can be used to review enzyme function and the relationship between cellulose and glucose. The supplementary videos and slides can help students visualize the interactions between cellulase enzymes and cellulose fibers.

****Note:** The Celluclast enzyme product contains a detectable amount of glucose, which is included as a stabilizer for the enzymes. The powdered cellulase from Carolina does not. If using Celluclast or a similar aqueous product, some of the increase in glucose over time can be attributed to this glucose, but the majority is produced through the hydrolysis of the biomass. To tease out the effect of this glucose on readings, prepare a control sample with only water and enzyme. Track glucose and ethanol levels over time. It is easiest for the teacher to prepare 1-2 of these control samples and share the data with the class.

- Hydrolysis: Continued

With available time, have groups discuss their final post-hydrolysis glucose readings, share observations and discuss whether the results are in line with what was predicted. Identify groups that had the highest and lowest glucose readings. Discuss why.

- Fermentation:

Yeast can be added to samples after 24-hr hydrolysis glucose and ethanol readings are recorded.

**Cool samples to less than 37°C (roughly room temperature) before adding yeast. Higher temperatures can kill the yeast.

Review the process of fermentation and have groups discuss expected changes in glucose and ethanol levels during this stage.

Notes on taking glucose measurements:

- From our experience the TRUEbalance blood glucose meter is the most accurate measure for this experiment, as it is more specific to glucose than other monitors such as True2Go or TRUEtest.
- Only a small droplet of sample is needed to take a reading.
- When extracting a droplet for a reading, avoid including particles of biomass.
- When taking a reading, touch only the very edge of the test strip to the droplet.
- Each test strip can be used only once.
- It important to use test strips with the same batch number, which can be found on the side of the container and is labeled “LOT.” Test strips from the same batch receive the same calibration and will allow results to be more accurate. Comparing results from strips with different batch numbers might not be valid.
- A piece of wax paper, aluminum foil or parafilm is a good surface for arranging and labeling droplets to be measured for glucose.
- A “Lo” reading means that the sample has <20 mg/dL of glucose.
- A “Hi” or “E5” reading indicates that the samples has >600 mg/dL glucose. If this happens, take a second measurement to confirm. If second reading is also “Hi” use a 2-fold dilution combining sample droplet with droplet of distilled water and take another reading. Actual glucose concentration is 2X the diluted reading.
- From our experience, blood glucose meters do not provide accurate absolute measurements of glucose concentrations in biomass samples but are effective at detecting relative changes over time through this lab procedure. Therefore, use caution when drawing broader conclusions from results.
- An accurate method to measure changes in sugar concentrations is a digital refractometer. However, refractometer readings are only accurate in the absence of ethanol.

Notes on taking ethanol readings:

- Use a Vernier or Pasco ethanol sensor.
- For best results, follow instructions for calibrating sensors.
- If possible, replace the permeable membrane between each class use.
- The sensor height above the liquid affects readings. For best results, calibrate sensors and take measurements at the same height above liquid as directed in the vendor’s sensor instructions.
- Temperature affects ethanol readings. Higher temperatures can produce higher readings. For best results, take measurements at room temperature.
- It may take several minutes for the sensor to approach a final reading. Encourage students to be patient, since hasty measurements can be inaccurate.
- For standardizing measurements, one option is to use a stop watch and have students wait at least 2 minutes before recording measurement.



Part 4: Data analysis, Discussion and Conclusions (pages 10-13)

Have students organize their data into graphs and/or tables (see sample data below). In groups, students should review their initial predictions and explanations and make revisions based upon new evidence. The interpretation and discussion questions can guide the process and help gauge student understanding.



Have students complete the interpretation questions (page 11) in their lab groups. Then give groups an opportunity to share their findings and explanations. Encourage questions, discussion and comments between groups. The goal is for students to share results and ideas so that they can collectively come to a deeper understanding of the biomass conversion process and generate broad conclusions about which biomass/treatment options work better and why.

Part 4: Data analysis, Discussion and Conclusions (pages 11-13): Continued

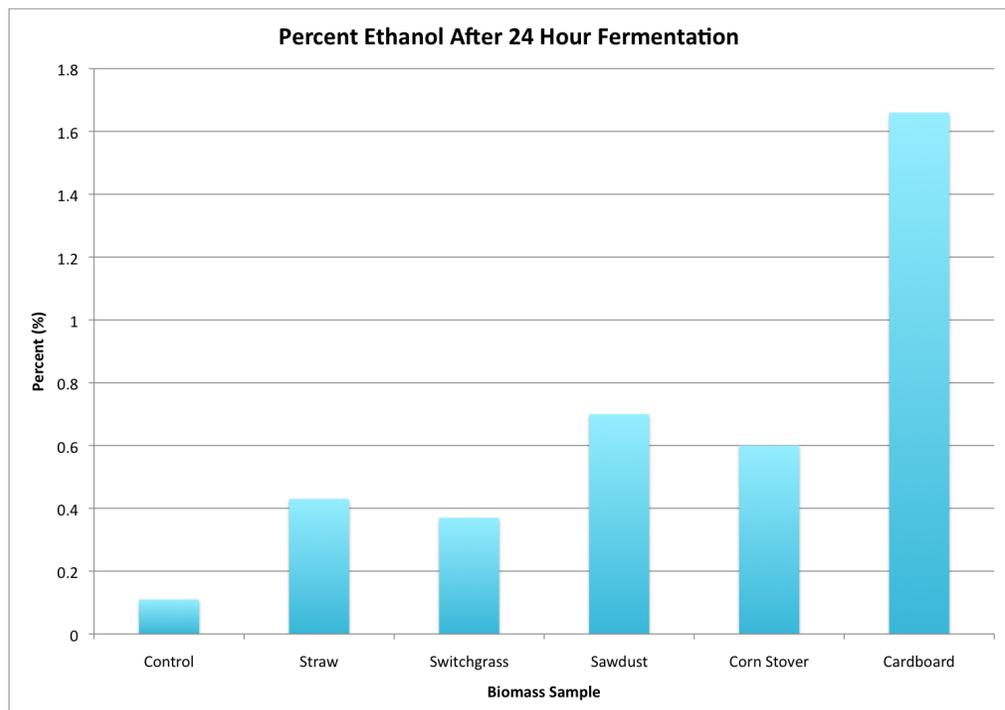
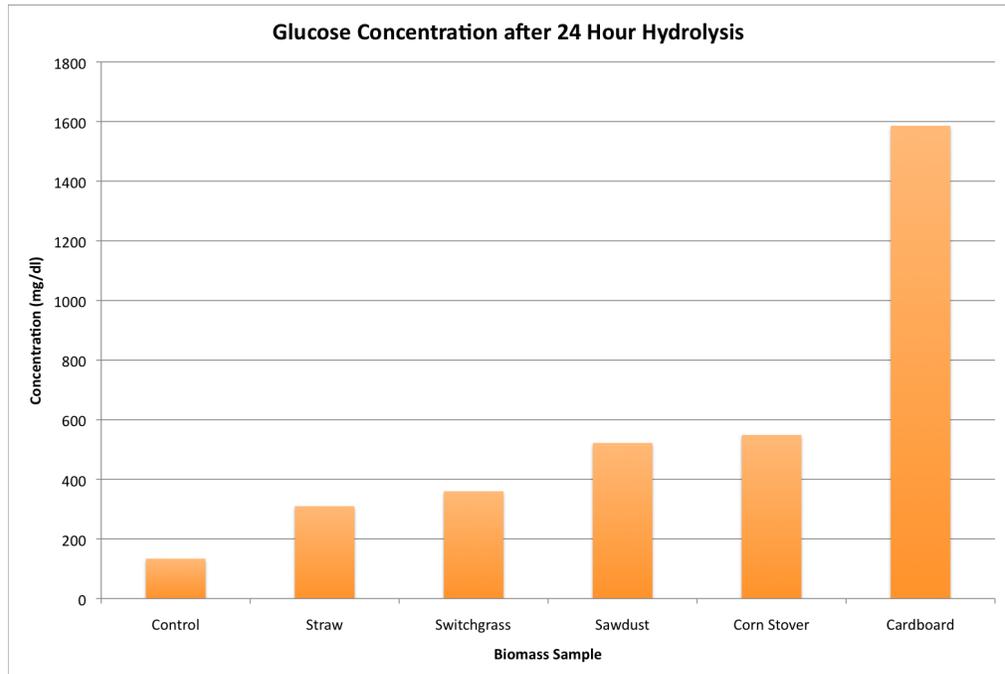
Discuss which groups had the highest and lowest glucose and ethanol yields and explore why. What would students do differently next time to generate higher glucose and ethanol yields? Many variables can be adjusted in the process such as biomass type, grinding method, length of boiling time, length time in hydrolysis stage, amount of enzyme added, amount of yeast, temperature, pH, etc. It is important that students base their ideas for improvements on evidence gathered during the lab.

If students are disappointed by the low ethanol readings in this lab, point out that this process is not easy to make work. Plant cell walls and their cellulose fibers are difficult to break down and convert to ethanol. GLBRC scientists and engineers are working hard to figure out efficient methods to do this using advanced technology, better enzymes and special engineered yeast. This process is not easy!

Sample results: Below are glucose and ethanol yields for sample biomass sources that might be used in this lab sequence. These results show a realistic range of expected glucose and ethanol yields. Actual results can vary quite a bit due to differences in chemical composition of biomass, grinding and boiling intensity, and experimental error. Sources of variation are discussed in more detail below, but the % cellulose of biomass is a major factor. Note the inclusion of the control (water only) which indicates how much background glucose and ethanol comes from the yeast and enzyme product alone. Note: the Celluclast enzyme product was used to generate these data. From our experience using the powered enzyme from Carolina Biological Supply produces lower glucose and ethanol yields but similar overall trends.

Biomass Type	Treatment	Before Pretreatment		Post Pretreatment		Post Hydrolysis		Post Fermentation	
		Glucose (mg/dL)	Ethanol (%)	Glucose (mg/dL)	Ethanol (%)	Glucose (mg/dL)	Ethanol (%)	Glucose (mg/dL)	Ethanol (%)
Sawdust (ground)	Enzyme	<20 ("Lo")	0.01	<20 ("Lo")	0.01	522	0.01	<20 ("Lo")	0.70
	No Enzyme	<20 ("Lo")	0.01	<20 ("Lo")	0.01	<20 ("Lo")	0.01	<20 ("Lo")	0.11
Corn Stover (ground)	Enzyme	<20 ("Lo")	0.01	128	0.01	549	0.01	<20 ("Lo")	0.60
	No Enzyme	<20 ("Lo")	0.01	122	0.01	30	0.01	<20 ("Lo")	0.18
Straw (ground)	Enzyme	<20 ("Lo")	0.01	<20 ("Lo")	0.01	310	0.01	<20 ("Lo")	0.43
	No Enzyme	<20 ("Lo")	0.01	<20 ("Lo")	0.01	<20 ("Lo")	0.01	<20 ("Lo")	0.12
Cardboard (ground)	Enzyme	<20 ("Lo")	0.01	<20 ("Lo")	0.01	1586	0.01	<20 ("Lo")	1.67
	No Enzyme	<20 ("Lo")	0.01	<20 ("Lo")	0.01	<20 ("Lo")	0.01	<20 ("Lo")	0.16
Switchgrass (ground)	Enzyme	<20 ("Lo")	0.01	<20 ("Lo")	0.01	360	0.01	<20 ("Lo")	0.37
	No Enzyme	<20 ("Lo")	0.01	<20 ("Lo")	0.01	<20 ("Lo")	0.01	<20 ("Lo")	0.18

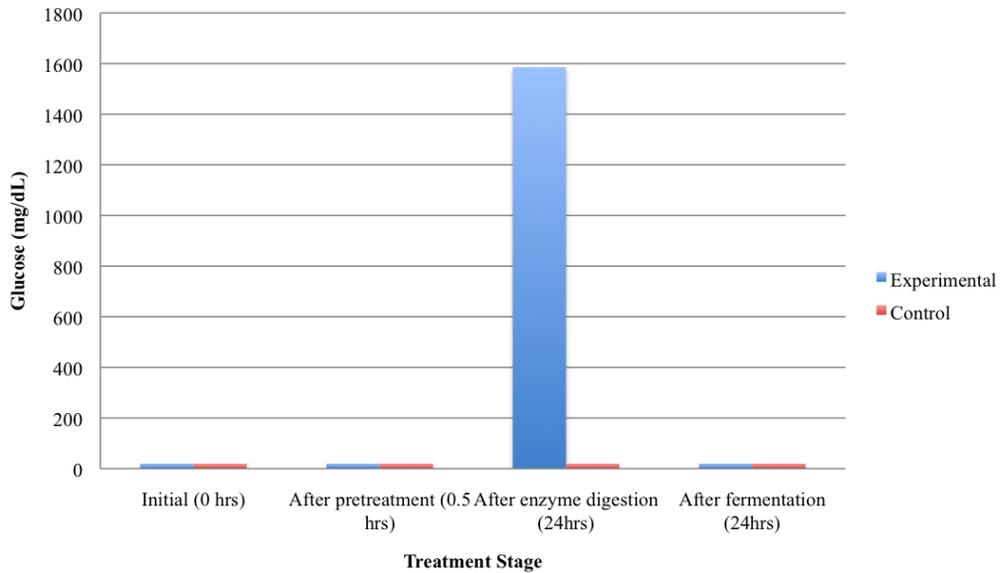
Sample results: Peak glucose and ethanol yields for each biomass type. It is useful for lab groups to share and graph results. The graphs below illustrate the close correlation between final glucose yields after enzyme digestion and final ethanol yields after fermentation.



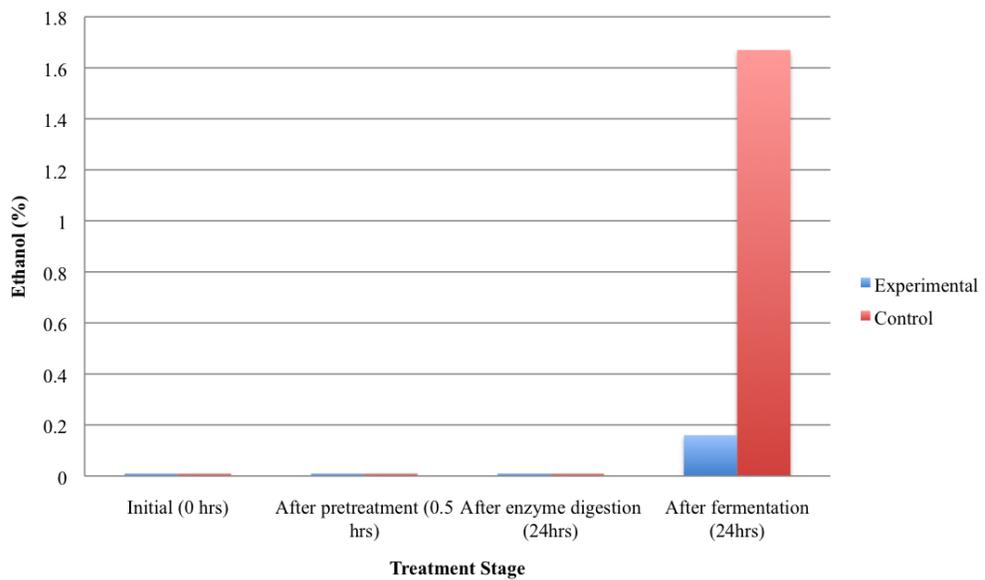
Sample data and expected results:

Below are sample graphs that were generated for cardboard’s glucose and ethanol levels using the protocol in this lab:

Cardboard: Glucose Levels over Time by Treatment



Cardboard: Ethanol Levels over Time by Treatment



Why was cardboard the “winner?” In the process of making cardboard, the wood undergoes a harsh chemical and heat “pretreatment” process which opens up the plant cell wall, exposes the cellulose and removes a large fraction of the lignin and hemicellulose. In our experiment, these treatments make it easier for the enzyme to break down the cellulose into glucose.

Interpreting results:

Probably the most fun, educational, but also challenging part of this investigation is summarizing all of the results and drawing conclusions. There are numerous factors affecting results, not including sources of experimental error. Students will be grappling with the same challenges as GLBRC scientists. Below is a list of important factors affecting biomass to ethanol conversion efficiency. Also included are some caveats and words of caution with interpreting data.

Some important factors:

- **Biomass particle size:** the smaller the particle size (more finely ground), the more effective cellulase enzymes will be at attaching to cellulose and breaking it down into sugar. This is a result of the surface area-to-volume relationship.
- **Biomass composition:** only the cellulose can be broken down into glucose and converted to ethanol in this experiment. The percent composition of cellulose relative to lignin and hemicellulose affects conversion rates. Also some plant cell walls are simply more “recalcitrant” or resistant to being broken down.

A light blue speech bubble icon with the word "Discussion" written inside in a dark blue font.

The following references providing information about the percent cellulose content of common biomass types:

- See: <http://ncsungrant.sdstate.org/uploads/publications/SGINC1-07.pdf>
- See: http://www.afdc.energy.gov/fuels/ethanol_feedstocks.html
- **Pretreatment method:** Different biomass types are more susceptible to certain pretreatment methods, such as boiling, than others.

Discussing the “Big Picture”

Cardboard produced the most ethanol, so why aren't we using cardboard and waste paper to make biofuels rather than switchgrass and corn stover? Many questions will come up about which biomass sources are the best options and why. This provides the opportunity to bring up other criteria to use when evaluating potential crops and feedstocks. The instructor should prompt students to brainstorm other criteria besides total ethanol production that should be considered in evaluating biomass feedstocks. Likely, students will come up with some of the same criteria studied by GLBRC researchers, such as:

- Net energy: How much energy goes into making the fuel compared to what you get out?
- Availability: Can we sustainably produce this biomass source in sufficient quantities?
- Cost: How expensive would it be to grow and produce large quantities of a fuel from a biomass resource?
- CO₂ emissions: How much CO₂ is released in the process of growing/harvesting biomass and then converting it to fuel?

As an example, how would cardboard measure up in some of these categories? A lot of energy went into processing wood into paper so converting paper into fuels might not be a net energy gain. How much cardboard is available? Even if all cardboard was converted into fuels, this would be a tiny fraction of the biomass needed to meet our transportation fuel needs. Have students work through a simple “pro/cons” or “cost/benefits” tabulation for different feedstocks. The analytical skills applied in this process are central to engineering. If time permits, students can do some internet research to find data to compare crops and feedstocks by different criteria. The GLBRC *Life Cycle Assessment* activities can serve as a good follow-up to help students consider the larger sustainability issues associated with producing biofuels.

Some caveats and tips:

- The Celluclast cellulase product contains detectable quantities of glucose, as discussed previously. Keep in mind that some of the observed increase in glucose levels comes from the enzyme itself. When hydrolysis is very effective, as with the cardboard example, this contribution is minor. With samples that are not as easily digested, however, this can make it appear that the biomass is converting into a small amount of glucose. Set up a control with enzyme and water to quantify how much glucose is coming from the cellulase. Note: We did not measure detectable background glucose in the powdered cellulase from Carolina Biological Supply.
- Standard baker's yeast also contains some sugar. This explains why the control samples that had "Lo" ethanol readings still produced some ethanol. Set up a control with yeast and quantify how much ethanol is coming from the yeast alone.
- Ethanol production results will not always track with glucose production. This is because there are many nasty, toxic compounds, such as lignin, in the digested biomass mixture that are harmful to yeast. Baker's yeast in particular has not evolved or been selected to grow and thrive in this environment.
- Microbial contamination of the samples can be an issue in this lab. Make sure to start with sterilized Falcon tubes.

Extensions and options:

Possible extensions:

- Proceed to distillation and combustion. This works best if ethanol concentrations are at least 1%. You can pool all samples in a container and distill the batch as a demonstration. Test and/or demonstrate the chemical energy in the ethanol through combustion. Simple ethanol-powered sterling engines are available at a reasonable price (~\$100) from Amazon.
- Allow students to modify additional variables such as boiling time, heat and pressure for pretreatment (pressure cooker), quantity or types of enzymes, duration of hydrolysis and fermentation, mixing during hydrolysis, etc.

- Track energy and matter inputs and outputs for this process, such as energy for grinding, heating, etc. and consider whether the process is sustainable. What changes could be made to improve efficiency?
- For advanced chemistry classes, do stoichiometry to determine actual vs maximum theoretical yield for each step. Discuss ways to improve conversion efficiency. Compare class data to results from published studies using same biomass.

Extending the learning:

1. Read and discuss a research story about Dr. Donna Bates, GLBRC scientist currently investigating fermentation in [A Modern Scientist-Engineer in the World of Fermentation](#).
2. Extend the learning with the [Data Dive: Boosting Yeast's Appetite for Sugars](#) by having students learn about how scientists are using directed evolution techniques to create mutant yeast strains that can ferment all of the sugars in plant biomass, not just the glucose.
3. Conduct the [Fermentation in a Bag](#) investigation comparing simple sugars like sucrose, glucose, and xylose as the food source for yeast.
4. Conduct the [Fermentation Challenge](#) investigation in which students test how changing different variables such as pH, temperature or yeast strain can affect fermentation rates.
5. Extend the learning with [Life Cycle Assessment](#) activities by having students walk through the energy and matter inputs and outputs associated with each step of biofuel production.
6. Have students play the [Bioenergy Farm board game](#) so they can explore the sustainability challenges of growing biofuel crops.
7. Use the [Investigating Fuel Sustainability](#) activity to have students investigate and analyze the carbon footprints of biofuels vs gasoline or electricity.
8. Use the [Biofuels vs Fossil Fuels Unit](#) to trace matter and energy through photosynthesis, fermentation, and combustion with hands-on activities.

Appendix

Video Resources:

CB2E Tutorials - Three short videos that help clarify the lab procedure for CB2E. The first of these gives an overall summary/structure of the lab. The second and third give tips and tricks for using blood glucose meters and classroom-grade ethanol sensors (respectively) in a classroom lab setting.

<https://youtu.be/249JMD150so>

<https://youtu.be/NjQIEYgoYJE>

<https://youtu.be/8iNAWPpy7xS8>

Exploring Life Cycles of Fuels: This video created by GLBRC and Into the Outdoors compares how biofuels and fossil fuels impact the carbon cycle.

<https://www.glbrc.org/education/classroom-materials/exploring-life-cycles-fuels>

Converting Biomass to Liquid Fuels - Excellent 5-minute summary of the difference between corn and cellulosic ethanol and process currently used to make cellulosic ethanol.

http://www.nrel.gov/learning/re_biofuels.html

What is cellulose and how is it used to make ethanol? A short, simple GLBRC video illustrates how plant cellulose can be converted into sugars and then ethanol.

<http://vimeo.com/10378252>

The Biofuels Story: GLBRC Prezi to introduce why and how we make biofuels and some of the key sustainability concerns.

<https://www.glbrc.org/education/classroom-materials/biofuels-story-prezi>

See our website for additional educational videos:

<http://www.youtube.com/user/GLBioenergy/videos>

Text/Online Resources:

U.S. Department of Energy Office of Science. Biofuels for Transportation (2007):

FAQ-style pages with overview material such as “What is biomass?,” “How is ethanol produced from cellulosic biomass?” and “Can one gallon of ethanol displace one gallon of gasoline?” Links to many other quality resources are available from the Department of Energy.

<http://genomicsgtl.energy.gov/biofuels/index.shtml>

U.S. Department of Energy: Biofuels Basics (2013):
Information aimed at general audiences about the production steps involved in making bioethanol and other biofuels. Links to many other quality resources are available from the Department of Energy.

<http://www.energy.gov/eere/bioenergy/biofuels-basics>

Standards:

AAAS PROJECT 2061 (1993):

- 1B - The Nature of Science: Scientific Inquiry
- 3A - The Nature of Technology: Technology and Science
- 4E - The Physical Setting: Energy Transformations
- 5E - The Living Environment: Flow of Matter and Energy
- 8B - The Designed World: Materials and Manufacturing
- 12C - Habits of Mind: Manipulation and Observation
- 12D - Habits of Mind: Communication Skills
- 12E - Habits of Mind: Critical Response Skills

See page 3 for Next Generation Science Standards (2013) alignment.

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