BioFuels: Cellulose Lab Teacher Guide

Driving Question: How is biomass processed to become a biofuel?

In this activity your students will:

1. Investigate how to prepare a biofuel source for conversion to a combustible product.
2. Understand that the refining process for biofuels requires an enzymatic reaction.

Background Information

Biomass can be used for energy production through combustion or direct burning. Raw plant and tree materials can also be refined for energy production. One method is to press seeds or plant materials and extract oils from them to create biodiesel. Another method involves cleaning used vegetable oil and converting it to biodiesel fuel. Another way to refine biomass is to use sugar extraction for cellulosic ethanol production. Cellulosic ethanol production is used as a biofuel or gasoline additive. Using plants for a source of oil, or sugar for cellulosic ethanol production is a way to reduce the waste and pollution involved in combustion-based energy production. This lab models the process of cellulosic ethanol production.

Plant cells have cell walls that contain cellulose. Cellulose is a polysaccharide, otherwise known as a complex sugar (also called dietary fiber). Many biofuels are derived from crops such as corn or switch grass. Organic waste products from clothing, paper, construction material manufacturing such as recycled paper, paper pulp, wood scraps, sawdust, rayon, or cellophane retain wood's cellular structure even after processing. Since these materials are often viewed as waste products, the biofuel process contributes to less waste disposal since lost fuel resources are recaptured. Researchers are also presently working to develop a process to harvest the potential energy sources in algae.

Humans cannot digest cellulose. Some animals and insects digest cellulose and then release the sugars, providing energy. Some organisms have special symbiotic microorganisms and/or digestive enzymes that breakdown the cell walls. Enzymes are proteins that encourage specific reactions and can help convert the starting substance (or substrate) into different products. Cellulase is one such enzyme. When plant fibers are exposed to cellulase it allows the sugars in the plants to be released. These sugars then ferment to become cellulosic ethanol, a form of alcohol that can be used as an alternative fuel source.

Student Information

Plants products can be used as a source for **biofuels**. All plant cells have cell walls that contain **cellulose**. Wood products such a paper, rayon, or cellophane retain wood's cellular structure. Cellulose is a polysaccharide, otherwise known as a complex sugar. Humans cannot digest cellulose. Cellulose is what we call dietary fiber.

Some animals and insects can digest cellulose and release the plant's sugars. These sugars provide energy. These animals may have special symbiotic microorganisms and/or digestive **enzymes** that breakdown the cell walls. **Enzymes** are proteins that help specific reactions. They can help make the starting substance turn into different products. **Cellulase** is one such enzyme. When plant fibers are exposed to cellulase, it allows the sugars in plants to be released. These sugars then **ferment** becoming **cellulosic ethanol**, a form of alcohol that can be used as an **alternative fuel source**. Presently cellulosic ethanol is used as an additive in gasoline.

Lab Description

This experiment demonstrates the effectiveness of cellulase, an enzyme that breaks down cellulose in paper pulp. In the experiment, students test for the presence of cellulase in different solutions. Two test

tubes will include paper pulp exposed to the enzyme cellulase. In the other test tubes, the paper pulp will be exposed to water and to rubbing alcohol; these test tubes do not contain cellulase.

Two test tubes of paper pulp will be exposed to the cellulase for different lengths of time – one overnight and the other during the students' experimental procedure. The cellulase reaction will release plant sugars from the cellulose found in the paper pulp. The cellulase requires ay least a few hours to release sugars from the cellulose. Benedict's reagent will be added to the four test tubes. The Benedict's reagent changes color in solution to reveal the presence of simple sugars such as glucose, lactose, and fructose in the test tubes.

Students will work in groups of 4.

Equipment List (minimum for one class with 32 students)

- Shredded paper – (use newsprint or recycled classroom waste)
- Blender
- 2 buckets (2 or 5 gallon)
- 8 metric ruler
- 8 wax pencils
- 36 test tubes-*Pyrex or equivalent* - approx 40 ml (4 tubes per student group and a demonstration set for the teacher)
- 9 test tube racks (1 per group+ demo set)
- 18 test tube clamps (2 per group + demo set)
- 2 – 1000 ml beakers (minimum) – *Pyrex or equivalent*
- 4 dropper bottles
- 8- 250 ml dispenser or wash bottles
- Cellulase (available through educational science supply distributors, 25 gram bottle. approximately $25-35 -*1 yr shelf life - store in cool, dry, dark location*)
- Benedict's Reagent-*qualitative* (available through educational science supply distributors – order 500 ml. Approximate cost $13/ liter -*2yr shelf life*)
- 1L (minimum) plastic storage or bottles with lids for cellulase mixture and pulp/cellulase fermentation.
- Rubbing alcohol (1 - 475 ml bottle)
- Water
- 2 Hot Plates
- Goggles/ Gloves

Step 1: Laboratory Preparation

DAY PRIOR (see chart on page 4 for quantity, timing, and storage notes)

1. Shred paper.

2. Fill bucket with warm water, 2 parts water to 1 part paper. (You may need to increase the water amount up to 4 parts water based on absorbency of paper product)

3. Soak for at least 3-4 hours in water. Materials can soak for up to 2 days prior to the next step if necessary. (Note: as fermentation continues sugar availability decreases.

4. Place .5 liter of mixture into a blender at a time. Pour into a holding bucket. Repeat until all paper is pulped. *The mixture should be a thin liquid, not as thick as a pulp mix for papermaking.*
    a. If no blender is available, have students shred and pulp by hand.

5. Prepare 5% Cellulase solution. Mix 5-gram cellulase solution to 1 liter water.

6. Set aside 1 liter of pulp for aged solution component (vary amount reserved based on class count and size)
    a. Introduce 500 ml 5% cellulase solution to 1 liter of pulp the evening prior to class.
    b. Cover mixture after adding cellulase solution to aid in the enzymatic fermentation process.
    c. Label this solution: **Overnight**
    d. NOTE: Do not ferment longer than 1 day. As fermentation continues sugar availability decreases and time needed for reaction increases.

DAY OF LABORATORY (see chart on page 4 for quantity, timing, and storage notes)

1. Fill 3 250ml dispenser bottles per group with the following solutions
    a. 5% cellulase solution
    b. Rubbing alcohol
    c. Water

2. Fill 1 dropper bottle per group with Benedict's reagent.

3. Set up workstations.

    a. Students Stations
        i. Students will work in groups of four.
        ii. 4 test tubes
        iii. 2 test tube holders
        iv. 1 wax pencil
        v. Lab investigation sheets

    b. Hot plate stations
        i. Set up 2 hotplates.
            1. This experiment does not release dangerous fumes. A fume hood is not necessary. However, if you have fume hoods use these areas to demonstrate proper lab techniques.
        ii. Fill 1000 ml beaker with 500 ml water.
        iii. Heat water to 40-50 degrees Celsius.

    c. Pulp supply stations
        i. Display and label the following pulp supply containers.
            1. 1 pulp solution: **overnight**
            2. 1 pulp solution: **pure pulp**
        ii. Item for transfer of pulp to test tubes (may be disposable pipettes, graduated cylinders, spoons, etc.)

Preparation Schedule Overview (enough solution for 5 classes of 30 students)

Item/ When	Amount (increase amount if preparing for multiple classes)	Day Prior	Before Class	Storage/ Comments
Raw Paper Pulp Soak	2 liters or 1/2 gallon	X		Stock for Paper Pulp solutions below*
Cellulase/ Paper Pulp Aged Solution*	1 liter of raw paper pulp – divided from original. Mix 2 parts pulp: 1part Cellulase solution	X		Cover in plastic container.
Pure Paper Pulp Solution*	1 liter of raw paper pulp – divided from original	X		Store in bucket or plastic containers.
Cellulase Solution 5%	1 liter (This amount may provide for extra stock solution) 5g cellulase: 1000 ml water	X		500 ml is used for Aged paper solution. Place remainder in dispenser bottles.
Benedict's Reagent	4 dropper bottles		X	Share 1 dropper bottle per 2 groups
Rubbing Alcohol	4 dispenser bottles		X	Share 1 dropper bottle per 2 groups
Water	4 dispenser bottles		X	Share 1 dropper bottle per 2 groups

Step 2: Conducting the Experiment

Guide the students through the following steps. A modified version of these instructions will be on the student lab sheet.

Students work in groups of 4.

1. Mark test tubes 1-4 on the white label space.

2. Store test tubes in test tube racks to keep upright.

3. Measure and mark at **3 cm** and **6 cm** from the bottom of the test tube.

4. Have students put goggles on.

5. Fill **tube 1** with **overnight cellulase/ pulp solution** to **6 cm** mark.

6. Fill **tubes 2, 3, and 4** with **pure pulp** solution to **3 cm** mark.
    a. Fill **tube 2** to **6 cm** mark with **5% cellulase solution**.
    b. Fill **tube 3** to **6 cm** mark with **rubbing alcohol**.
    c. **Tube 4** is the **control tube**. Fill to **10 cm** mark with water.
    d. **Gently swirl solutions in each tube. DO NOT SHAKE.**

7. Instruct students to complete the **first column** in the **"What is in your test tube?"** data table. Describe the solution you placed in the test tube. What was added to the paper pulp?

8. Ask students to predict which solution(s) will show that sugars were released from the cellulose after heating. Instruct them to write their predictions in the **Prediction Section** on their investigation sheet.

9. Add **10 drops** of **Benedict's reagent** to each test tube.
    a. **Gently swirl solutions** in each tube, again. **DO NOT SHAKE.**

10. Fill out **"What was the color of the solution before heating?** on your data chart.

11. Carefully heat the test tubes by suspending in a hot water bath at about 40-50 degrees Celsius for **5 minutes**.

12. Remove test tubes from hot water bath using test tube clamps. Return tubes to test tube rack to cool.

13. Complete lab investigation sheet.
    a. Fill out **"What was the color of the solution after heating?" on your data chart.** Note any color change in the test tubes.
    b. If sugar is present the solution, it will change color to green, yellow, orange, red, brick red, or brown. The color is determined by the sugar present.

14. After tubes cool, empty liquid to drain and flush. Empty any solids to the trash.

15. Wash, rinse, and dry test tubes. Dispose pulp by flushing or place in compost.

Prediction Section

1. Which solution do you think will release the sugars from the cellulose pulp? **Why?**

Ask students to write predictions on their investigation sheets and share with class.

Data Collection

1. Review table and investigation sheet with class.

Sample table with answers

Test Tube	What is in the test tube?	What was the color of the solution before heating?	What was the color of the solution after heating?	Was sugar present in the solution?
1	*Overnight in cellulase solution*	*Grey/Blue*	*Possible colors: green, yellow, orange, red, brick-red or brown*	*yes*
2	*Cellulase added just before heating*	*Grey/Blue*		*maybe*
3	*Rubbing alcohol*	*Grey/Blue*	*Grey/Blue*	*no*
4	*Water*	*Grey/Blue*	*Grey/ Blue*	*no*

Step 3: Analysis and Conclusions

Review the **BioFuels: Cellulose Lab Investigation Sheet** with your students. You will find answers on the *Biofuels: Cellulose Lab Assessment*.