Plant Cell Walls to Alcohols

Overview: This lesson looks at the break down of plant material to demonstrate the production of biofuels.

Keywords: Biofuel, biomass, lignocellulosic, pretreatment, hydrolysis, distillation, lignin, celluloses, hemicelluloses

Age / Grade Range: 9th-12th

Background: Biofuel is a source of renewable energy that can be used as an alternative to nonrenewable fossil fuels. There are several types of biomass that can be used to manufacture **biofuel** (any fuel that is made from biological organisms or their products); common categories include biodiesels and alcohols. Alcohols such as ethanol or isobutanol can be produced from starch-based or lignocellulosic sources. This lesson will address conversion of lignocellulosic biomass to alcohol.

Lignocellulosic biomass is a plant material feedstock that contains as its two primary constituents lignin and cellulose. The cells comprising plant tissue have cell walls, and upon plant death and desiccation the cell walls remain. (Liquid and organelles that were housed inside the cell wall during the cell's life dry up and their contribution to biomass is negligible.) Thus cell wall material makes up the bulk of plant biomass that is used for fuel production.

Plant cell walls are constructed of a matrix of cellulose and hemicellulose (starches) and lignin, a complex compound that is difficult for organisms to break down and digest. Celluloses and hemicelluloses are the raw material that is the goal for subsequent conversion to alcohols. Lignin essentially binds the cellulose and hemicellulose together and prevents access to these starches by other organisms. Thus lignin helps to protect the plant from predators and provides rigidity to the cell wall.

Just as in the natural world, it is the starches that are of most interest in current biofuel production processes, as they are readily broken down to release energy. The presence of lignin in biomass presents challenges in fuel production that are analogous to those faced by organisms trying to consume plants for their starchy components.

What follows is a discussion of the steps of lignocellulosic biomass conversion

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to alcohols.

Pretreatment is the separation and/or removal of lignin so that carbohydrates are more easily accessed during hydrolysis. Lignin acts to prevent access from chemicals or enzymes that break starches into their sugar components. Lignin is ecologically recalcitrant; it is not easily broken down by most organisms, primarily due to its chemical structure. It contains many aromatic rings which are difficult to break apart. Pretreatments can be physical (chipping, milling, grinding), physico-chemical (ex. steam explosion) chemical (ex. acid or alkaline pretreatment), biological (ex. microbial delignification). It is important that students take away from this lesson that there are many ways to pretreat and break down feedstock and that these techniques are still being developed. Considerations in choosing a pretreatment method include technical feasibility, economic feasibility, yield/efficiency (interested in sugar recovery and ability to be hydrolyzed and fermented) (Limayem and Ricke 2012, Chiaramonti et al. 2012) Steam explosion is seen as relatively cost-effective but destroys a portion of the sugar, generates inhibitory products and does not completely break apart the lignin-carbohydrate complex Acid hydrolysis typically has high cellulose conversion rates but creates inhibitory products. Additionally, acid concentration, temperature and reaction time must be weighed and at least one must be made more severe to achieve subsequent high carbohydrate recovery rates (Chiaramonti et al. 2012). microbial pretreatment has low energy and chemical requirements but high process time (Wan and Li 2012, Chiaramonti et al. 2012) Each has different applicability for different feedstocks (ex.: woody biomass is more difficult to pretreat than agricultural residues, higher lignin content (Limayem and Ricke 2012) Pretreatment is often the most costly step in the conversion process (Chiaramonti et al. 2012)

Hydrolysis is the breaking apart of carbohydrates (polymers) into their simple sugar components (monomers) Hydrolysis is generally either chemically (acid) or enzyme catalyzed Dilute acid hydrolysis generates chemicals that inhibit the fermentation process (Limayem and Ricke 2012) Concentrated acid hydrolysis require costly recovery of acids to be economically feasible, but has high recovery of sugars (Limayem and Ricke 2012) Enzymatic hydrolysis can be inhibited by products of pretreatment or by the sugar products of hydrolysis itself. (The self-inhibition effect can be reduced if hydrolysis is conducted simultaneously with fermentation so the

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sugars produced by hydrolysis are continuously fermented so sugar level doesn't become inhibitory.) (Limayem and Ricke 2012)

Fermentation is the conversion of simple sugars to alcohol according to the equation: sugars → alcohol + CO2 The yeast species used determines product (i.e.: some yeast species will produce ethanol while others will produce isobutanol)

Distillation is a final step of separation of alcohol from other components such as water

Next Generation Science Standards & Common Core:

HS-PS1.A Structure and Properties of Matter
HS-PS1-B Chemical Reactions
HS-PS4: PS3.D Energy in Chemical Processes
HS-LS2: ETS1.B Developing Possible Solutions
HS-ESS3.A Natural Resources
HS-ESS3.C Human Impacts on Earth Systems
HS-ESS3.D Global Climate Change
HS-ESS3. ETS1.B Developing Possible Solutions

Goals:

- What are the steps in the process of converting lignocellulosic biomass to biofuel?
- What part of the plant is used?
- How is stored energy transferred from plant to fuel?
- What are some of the challenges and complexities associated with the conversion process?

Objectives:

Students will understand:

- where the energy in plants comes from
- the basic steps of the conversion process.
- that there are many ways to accomplish each step and that the research surrounding these various techniques is ongoing.
- that pretreatment is the most difficult step in lignocellulosic biomass conversion to fuel.

Materials:

- visual of a plant tissue that shows cell walls
- visual of plant cell wall cross-section showing lignin binding cellulos and hemicellulose
- scrap of paper for each student
- approximately 10 sugar cubes for each student or each group of students
- mud (or eggless cookie dough or peanut butter)

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- hot water
- small (about 32 oz.) container
- cocoa krispies or other cereal that changes the milk color
- another piece of scrap paper for each student
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Set up:

- Prepare visuals
- Coat sugar cubes in mud for the Explore activity. Ensure that the mud has sufficient time to dry. Ensure that the mud does not easily flake off. If it does, use a different material to represent lignin.
- Obtain a means of heating water.
- Write down on pieces of paper the chemicals and stages associated with the conversion process for the Evaluate activity

Classroom Time:

2.5 Hours

Introduction (Engage):

Ask students to name some plant materials that they have heard of that are used to produce biofuels, prompting if necessary. (corn, wood, switchgrass, sugar)

Ask students what kind of energy is stored in plant biomass, and how it got there.

Show students an image of a plant tissue that shows multiple cells with distinctly visible cell walls.

Ask what part of the plant cell is used to generate biofuels.

Tell students the three primary components of plant cell walls (cellulose, hemicellulose, lignin)

Show a cross-section of a plant cell wall that depicts cellulose, hemicellulose and lignin.

Tell students that they will be acting out the different components of the plant cell wall of a typical hardwood tree consisting of about 25% lignin and 75% cellulose and hemicellulose:

- Assign approximately ½ of the students to be cellulose, ¼ hemicellulose and ¼ lignin. Have each student make a nametag with an H, C or L as appropriate.
- Have the celluloses form lines of 4-6 students and link arms. Have the hemicelluloses do the same, but in lines separate from the cellulose lines.

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- Have the hemicellulose and cellulose lines stand parallel to each other.
- Have the lignins surround the hemicellulose and cellulose lines. Then have the lignin students hold hands over and through the cellulose/hemicellulose lines, so that the lignins' arms are reaching through and across the lines.
- The visual should be of lines of cellulose and hemicellulose surrounded by a lignin web.

Remind students that this is an approximation of the compounds that make up a plant cell wall.

Ask students why plants might form lignin? Why might this be a good investment of their energy?

Tell students that biofuel production uses the celluloses and hemicelluloses and not the lignin.

Ask students what difficulties might be encountered in accessing and using cellulose and hemicellulose in biofuel production.

Activity (Explore):

This activity will allow students to act out symbolic representations of the steps of the biomass conversion process.

Students can work independently or in groups of two to five.

Provide each student or group of students with ten sugar cubes coated in mud. The mud should be dried onto the sugar cubes. (If this is not feasible, try peanut butter or (eggless) cookie dough.) The sugar cubes represent the cellulose and hemicellulose components (bunches of individual sugar molecules bonded together) of plant cell walls and the mud represents the lignin. Tell students that they are engineers (or scientists) and that they have been assigned the task of separating the lignin from the other constituents. They will need to save each separated component for future use. (The task of separating the sugar cubes from the mud should prove somewhat difficult!)

Once the "lignin" has been removed, tell students that they will now demonstrate the next part of the process, hydrolysis. Tell students that this step is the breakdown of the bonded sugar compounds into individual sugar molecules. This will be represented by dissolving the sugar cubes in hot water.

CAUTION: This step involves hot liquid.

Provide each student or group with a container of hot water. Have them place

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their sugar cubes in the hot water and stir if necessary to dissolve the cubes. The dissolution represents the breakdown of the cellulose and hemicellulose compounds.

Have students note that the cloudiness or quantity of mud in their water indicates how well they succeeded in separating the lignin from the starches! Once the sugar cubes are dissolved, tell students that the next step of the process is fermentation, or the conversion of sugars to alcohols. This step will be represented by the addition of yeast organisms (cocoa krispies) to the slurry. The gradual color change that should ensue represents the gradual process of fermentation carried out by these organisms.

Referring to the activity in the "Explore" section, explain the steps of the conversion process.

Ask students which step was the most difficult.

Explanation

Pretreatment:

- Ask students to recall why it is necessary to pretreat lignocellulosic biomass
- Tell students some of the ways in which biomass can be pretreated. Compare at least two forms of pretreatment in terms of their advantages and disadvantages. Let students know that these technologies are still under development.
- Ask students to explain how the first step of the activity was similar to and different from the actual removal of lignin

Hydrolysis

- Remind students that hydrolysis is the breaking up of long chains of sugars (starches – in this case cellulose and hemicellulose) into their single sugar components.
- Tell students that hydrolysis can use enzymes or chemicals that break the bonds between individual sugar molecules.
- Ask students how hydrolysis was represented in the activity, and how this representation was accurate or inaccurate.

Fermentation

- Tell students that fermentation is the process by which an organism such as a yeast takes in sugars, uses the energy contained within them, and changes them into alcohols.
- Ask students to consider why it might be necessary to have individual sugar monomers for the next step in the process, fermentation by a yeast.
- Tell students that different yeasts can have different alcohol outputs (i.e. ethanol or isobutanol)

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- Ask students how fermentation was represented in the Explore activity.

Recall to students that they have not done anything with the lignin byproduct that they created during the "Explore" activity. Note the quantity of lignin generated, and that wood can be comprised of 10-25% lignin.

Elaboration: Have students brainstorm a list of possible uses for such lignin.

In groups of 3-4, have students write a brief proposal for a potential use of lignin byproduct, addressing how lignin would be a good candidate for the proposed use, possible markets, and transportation of the lignin product.

Evaluation: Assign each student a part of the conversion process – either a chemical compound (ex: lignin, simple sugar, alcohol, whole biomass tissue) or a process (ex: fermentation, acid hydrolysis) and have the entire class line up in the appropriate order. For an added challenge, have them do so without talking. As an additional component, have the class decide how to creatively act out each step in the process (for example, hydrolysis might be represented by a long "chain" of students representing cellulose being "cleaved" by a student "enzyme" swinging down an arm between the other two students).

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This work was supported by an Agriculture and Food Research Initiative Competitive Grant no. 2011-68005-30416 from the USDA National Institute of Food and Agriculture.