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**Science Education Title** Biofuels: Producing Ethanol from Cellulosic Material

**Overview** In this experiment, we will use a cellulosic material (such as corn stalks, leaves, grasses, etc.) as a feedstock for the production of ethanol. The cellulosic material is first pretreated (ground and heated), digested with enzymes, and then fermented with yeast. Ethanol production is monitored using an ethanol probe. The experiment can be extended to optimize ethanol production by varying the feedstock used, pretreatment conditions, enzyme variation, yeast variation, etc. An alternative method of monitoring the reaction is to measure the carbon dioxide produced (using a gas sensor) instead of the ethanol. As a low tech alternative, glucose meters (found in any drug store) can be used to monitor the glucose during the process, if an ethanol probe or carbon dioxide gas sensor are not available.

With an increased emphasis on ‘inquiry-based learning”, scientific probes are becoming more popular. Handheld devices like the Vernier Lab Quest used in conjunction with a variety of probes (such as those for conductivity, dissolved oxygen, voltage, and more) allow for less focus on collecting data and/or making graphs and more on analyzing the data and making predictions. Another advantage is that these are small and lightweight and can be taken into the field for measurements.

**Principles** The United States is looking to wean itself off of fossil fuels, especially petroleum used in gasoline. Global climate change, dependence on foreign countries, and increased political instability around the world are only a few reasons why. One possible way to decrease dependence on petroleum as a transportation fuel is by using more ethanol. Currently, regular gasoline contains approximately 10% ethanol as an additive. Special flex-fuel vehicles can use E85 gasoline, which is 85% ethanol.

In Brazil, ethanol is made by using sugarcane as the feedstock. The main product of sugarcane is sucrose, which is a disaccharide of glucose and fructose. The glucose (C6H12O6) is separated, then fermented by yeast to produce ethanol and carbon dioxide. The chemical reaction is as follows:

C6H12O6 → 2 CH3CH2OH + 2 CO2  
 yeast

However, in the US, ethanol is made from corn. This requires more effort than making ethanol from sugarcane. To make ethanol from corn, first corn kernels need to be ground up, then the ground corn treated with enzymes to convert the starch in the corn to glucose. After this step, the process continues as in the sugarcane method above by using yeast to ferment the glucose into ethanol and carbon dioxide. The chemical reaction is as follows:

(C6H12O6)300-600 → C6H12O6 → 2 CH3CH2OH + 2 CO2  
 enzyme yeast

The production of ethanol from corn is problematic, however. For one thing, it takes some corn out of the food supply, thus driving up prices. It is also energy and fertilizer intensive to produce corn, decreasing its desirability as a transportation fuel alternative to petroleum. Therefore, scientists are increasingly turning to cellulosic material to make ethanol. These materials include wood, grasses, and non-edible parts of plants. These are more desirable as they do not impact food supply. However, in order to release the glucose from the cellulosic material, much more effort is needed, as the glucose from cellulosic material is bound up in cellulose, which is then wrapped with hemicelluloses and lignin. First the cellulose needs to be extracted from the hemicelluloses and lignin bindings. This is done through a pretreatment of grinding and acid hydrolysis. Then, the cellulose is treated with enzymes to break it up into its component glucose. Finally, the glucose can be fermented with yeast to produce ethanol and water. The process can be summarized as follows:

Cellulosic material → (C6H12O5)n → C6H12O6 → 2 CH3CH2OH + 2 CO2  
 pretreatment enzyme yeast

This is currently too energy intensive to make it a practical method for ethanol production. However, research is underway to make the process better.

**Procedure**

Sample Preparation:

1. Select cellulosic material to be used as feedstock. This can be corn stalks, grasses, leaves, pet bedding, or paper.

2. Using a coffee grinder, grind feedstock into a fine powder with no large pieces remaining.

3. Measure 1.0 g of feedstock and place in a 50 mL centrifuge tube. Label the tube with the feedstock chosen.

4. Label a second 50 mL centrifuge tube as “Control”. Do not put any feedstock in this tube.

Pretreatment:

1. Set up a 500 mL beaker with approximately 400 mL of water on a hotplate and heat to a gentle boil.

2. Add 25 mL of distilled water to the 2 centrifuge tubes. Swirl to mix. Put the cap on the centrifuge tubes loosely.

3. Put the centrifuge tubes in the beaker full of gently boiling water. Be sure that water from the water bath does not leak into the tubes. Let boil for 30 minutes.

4. Let tubes cool to room temperature.

Enzymatic Digestion:

1. Add 1 mL of cellulase enzyme to both tubes.

2. Put tubes in an incubator at 50°C for 24 hours.

Fermentation:

1. Add 1 .0 g of active yeast to each of the centrifuge tubes. Swirl to mix.

2. Loosely cap the centrifuge tubes. Do NOT cap tightly because CO2 will be generated, creating pressure in the tube. This could cause the cap to be ejected off the tube if some of the CO2 is not allowed to escape, reducing the pressure.

3. Place the centrifuge tubes in a rack and put in an incubator at 37°C for 24 hours.

4. Using an ethanol probe, measure the ethanol concentration in the control tube. (Figure 1)

5. Using an ethanol probe, measure the ethanol concentration in the sample tube.

Extension:

1. Students can vary the feedstock used to determine which biomass material produces the most ethanol.

2. Students can vary the enzyme used to determine which enzyme produces the most ethanol.

3. Students can vary other conditions (temperatures, time, etc.) to try to optimize the ethanol produced.

**Representative Results**

The % ethanol in the solution will be displayed on the handheld tablet screen using the software related to the brand of the ethanol probe used. (Figure 2)

Representative results of the percent ethanol produced by various feedstocks can be seen in table 1.

**Applications** The Energy Independence and Security Act of 2007 set into law a renewable fuel standard. It created a phase-in for renewable fuel volumes starting at 9 billion gallons in 2008 and ending at 36 billion gallons in 2022. Of that 36 billion, it was expected that 16 billion of that would come from cellulosic materials. For 2014, the original proposal was for 18.15 billion gallons of renewable fuel, 1.75 billion of that coming from cellulosic material. Unfortunately, based on the volume of cellulosic ethanol that is feasible to be produced currently, this number has had to be reduced to 17 million gallons according to a recent EPA proposal. Improving the process of creating ethanol from cellulosic material is currently a very hot area of research. In this experiment, students will be emulating the scientific practices that scientists in the top research labs are following. This experiment is also a great example of incorporating engineering practices into scientific research, as recommended by the Next Generation Science Standards.

**Legend:**

**Figure 1:** Ethanol probe measuring the ethanol concentration in the control tube.

**Figure 2:** Handheld tablet displaying % of ethanol.

**Table 1:** Representative results of the percent ethanol produced by various feedstocks