U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

ENERGY EDUCATION AND WORKFORCE DEVELOPMENT

Cell Wall Chemistry of Biofuel

Grades: 9-12

Topic: Biomass

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Owner: ACTS

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A Pre-treatment Model for Ethanol Production Using a Colorimetric Analysis of Starch Solutions

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GRADE LEVEL/SUBJECT:
9-12th Grade
Environmental Science/Chemistry

Curriculum Standards (from National Science Education Standards (www.nap.edu/readingroom/books/nses<http://www.nap.edu/readingroom/books/nses>)

Science Content Standards: 9-12

CONTENT STANDARD A: **Science as Inquiry**
As a result of activities in grades 9-12, all students should develop

- Abilities necessary to do scientific inquiry
- Understandings about scientific inquiry

CONTENT STANDARD B: **Physical Science**
As a result of their activities in grades 9-12, all students should develop an understanding of

- Structure of atoms
- Structures and of properties in matter

- Chemical reactions

CONTENT STANDARD C: **Life Science**
understanding of the cell

CONTENT STANDARD E: **Science and Technology**
As a result of their activities in grades 9-12, all students should develop
- Abilities of technological design
- Understandings about science and technology

CONTENT STANDARD F: **Science in Personal and Social Perspectives**
As a result of activities in grades 9-12, all students should develop understanding of
- Natural resources
- Environmental quality
- Science and technology in local, national, and global challenges

CONTENT STANDARD G: **History and Nature of Science**
As a result of their activities in grades 9-12, all students should develop understanding of
- Science as a human endeavor
- Nature of scientific knowledge

TEACHER'S OVERVIEW:

This module focuses on the production of sugar (glucose and maltose) from cornstarch. The first lesson from this module relates glucose production from cornstarch to ethanol fuel production from corn stover. Another lesson uses a calculator based colorimeter interface from the Vernier® Company to quantify the hydrolysis of starch to sugar by salivary amylase. In this lesson saliva is added to a starch solution containing a couple of drops of iodine. Light initially doesn't pass through this solution. If the absorption decreases after the addition of the saliva, this means more light is passing through and the starch is being hydrolyzed (broken down into maltose and glucose). The third lesson again uses colorimetry but this time to measure starch hydrolysis by dilute (1% volume to volume) sulfuric acid. Finally, we offer suggestions for using starch hydrolysis and colorimetry as a basis for student designed experiments.

Learning Objectives:

Students will

- Recognize the environmental and economic benefits of ethanol as a fuel additive

- Identify ethanol as a product of sugar fermentation
- Know that photosynthesis produces complex carbohydrates (polysaccharides)
- Understand that hydrolysis is a technique used by chemists to break apart polysaccharides into saccharides that can be fermented
- Demonstrate that starch can be hydrolyzed by salivary amylase
- Demonstrate appropriate safe laboratory behavior and technique while mixing chemicals
- Follow correct procedures for using a colorimeter
- Document observations and data in an organized appropriate laboratory format
- Analyze and interpret the results of the colorimetric data and observations
- Communicate their results orally

TIME ALLOTTED:

Five 45minute class periods, one for each of the following topics

- Background information and discussion
- Sulfuric acid tests
- Spit test
- Self-directed investigation
- Discussion of results and conclusions
- Evaluation

VOCABULARY

Ethanol	Corn stover	Hydrolysis
Cellulose	Hemicellulose	Carbohydrate

Polysaccharide	Starch	Saccharide
Glucose	Enzyme	Salivary Amylase
Cellulase	Colorimeter	Cuvette
Blank sample	Test Sample	Concentration
Absorbance	Wavelength	Nanometer
Fermentation	Renewable resource	Non-renewable Resource

RESOURCES/MATERIALS:

Protective eye wear
Vinyl gloves
Lab apron
Graduated cylinder
250 mL beaker
Stirring rod
Distilled water
Four to eight 15mL test tubes and stoppers per group
Labels for glassware
Waterproof pen
Notebook
Mass balance
Weighing paper
Vernier LabPro and cords Order Code: LABPRO Price: $220
Vernier Colorimeter and cuvettes Order Code: COL-BTA Price: $110
http://vernier.com/
Kimwipes
Disposable pipettes Carolina Biological Supply Product Code 73-6984
3.0 mL capacity Price: $4.10 Pack of 100
http://carolina.com
TI Graphing Calculator (preferably TI-83 Plus Silver Edition), or a computer
Corn Starch (grocery item)
Iodine Tincture (pharmaceutical item and there are hazards for Iodine Tincture, please know and follow all safety measures)

PREREQUISITE KNOWLEDGE:

Students should have used the scientific method in previous student-created experiments. In addition, they should know lab safety rules. Students need also be familiar with photosynthesis and using either the Vernier Labpro® or TI CBL equipment with either a computer or TI calculator.

MAIN ACTIVITIES:

Day one: Introduce students to concepts related to the significance and production of Ethanol as a renewable resource and fuel. (See teacher background information.)

Day two: Students hydrolyze a solution of cornstarch and distilled water in saliva (salivary amylase) and make comparisons using the colorimeter.

Corn starch does not dissolve in water. Therefore, it will be necessary to frequently and vigorously mix it at strategic times during this inquiry, such as before decanting or placing in the colorimeter. It may be necessary to vigorously mix the cuvettes during their colorimetric analysis.

Also, salivary amylase hydrolyzes starch over time. Consequently, it may be valuable for students to prepare their mixtures with starch solution one day before collecting their colorimetric data.

Colorimetric analysis should be performed at a wavelength of 635 nm, at this wavelength the color change from the addition of iodine does not interfere with the effects of salivary amylase on the starch. (You may wish to have students check the absorption of just water with a couple of drops of iodine in it. At 635 nm the absorption should be zero. Ask the students why. *Answer: the iodine solution is reddish yellow. This means the solution absorbs other colors but reflects reddish yellow. The wavelengths of yellow to red range from about 570 nm to 700 nm. 635 nm falls right in the middle of that range.)*

- In a 100 mL graduated cylinder prepare a stock sample of 0.5g of cornstarch in 100mL of water. (Individual student groups will need less than 10 mL of this sample.)
- Calibrate the colorimeter with 3 mL of distilled water in a cuvette
- Prepare and analyze a blank sample cuvette of 3 mL of distilled water and one drop of iodine
- One student, who hasn't eaten in a while, collects about 10 mL of saliva
- Prepare and analyze one test sample cuvette by pipetting 1.5 mL of stock solution and 1.5 mL of saliva and record colorimetric data
- Prepare and analyze a second test sample cuvette by pipetting 1.5 mL of stock solution and 1.5 mL of saliva and adding one drop of iodine and record colorimetric data. (The absorbance should decrease with time in this sample. This shows that the starch is changing, but it doesn't show that glucose is formed. A Benedict's solution test could be done as a demonstration at this point.)

Day three: Acid hydrolysis of cornstarch and colorimetric analysis of the acid solution and saliva. Repeat the steps given for day one only substitute

1% sulfuric acid for distilled water. Prepare a 1% sulfuric acid solution by adding 1 ml of concentrated sulfuric acid to 99 ml of distilled water.

- In a 100 mL graduated cylinder prepare a stock sample of 0.5g of corn starch in 100mL of water1% sulfuric acid. (Individual student groups will need less than 10 mL of this sample.)
- Calibrate the colorimeter with 3 mL of 1% Sulfuric Acid in a cuvette
- Prepare and analyze a blank sample cuvette of 3 mL of 1% Sulfuric Acid and one drop of iodine
- One student, who hasn't eaten in a while, collects about 10 mL of saliva
- Prepare and analyze one test sample cuvette by pipetting 1.5 mL of stock solution of 1% sulfuric acid and 1.5 mL of saliva and record colorimetric data
- Prepare and analyze a second test sample cuvette by pipetting 1.5 mL of stock solution of 1% sulfuric acid and 1.5 mL of saliva and adding one drop of iodine and record colorimetric data.

Group Homework for Inquiry Lab: Students create an experiment involving starch hydrolysis and colorimetry. Students write the title, purpose, materials, and methods for their experiment.

Some possibilities for further inquiry include testing the affect of temperature on the amylase in saliva, seeing how temperature affects the rate of starch hydrolysis, testing individual differences in the amounts of amylase in each others' saliva, testing dog saliva (if a student has a "drooly" dog), or seeing if exercise affects the amylase concentration in saliva.
These are only suggestions. You may wish to encourage students to come up with their own questions.

Day four: Students perform experiments of their choosing or design. (See our list of possibilities in the Group Homework for Inquiry Lab section above.)

Day five: Discussion and Evaluation

EVALUATION POSSIBILITIES:

- Use a lab rubric to evaluate the experiment. Students could be assessed on participation, safe lab techniques and proper methodologies.
- A written lab report could be evaluated by the teacher or by student groups.
- Use a rubric or score student presentations on the results and conclusions from the experiments they created.
- Have students write an essay summarizing the environmental and economic impacts of ethanol blended gasoline.

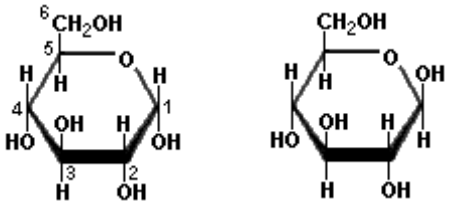
- Have students summarize the basic ideas behind colorimetry and how the colorimeter showed the hydrolysis of starch.

TEACHER BACKGROUND

Ethanol is a renewable energy source often added to gasoline. In this country some gasoline blends contain 10% - 12% ethanol. Other countries, like Brazil, have higher percentages of ethanol in their automotive fuels. The presence of ethanol in gasoline reduces the consumption of this nonrenewable resource. It also reduces pollution as ethanol combustion produces far fewer pollutants than the burning of gasoline. Another advantage of using ethanol for fuel is that it does not increase the level of carbon dioxide in the atmosphere (unlike gasoline). Ethanol comes from plants that absorbed CO_2 from the atmosphere for photosynthesis. The amount of CO_2 released during combustion of ethanol equals the amount used in photosynthesis, so there is not net atmospheric gain.

Ethanol is produced from the fermentation of sugars by microorganisms, typically yeast. Plant starch is commonly used as a source of sugar (mainly glucose) for fermentation. In the United States corn is used almost exclusively for the fermentation of sugar into ethanol. As the demand for ethanol as a fuel additive continues to rise, the amount of corn used for fuel will also rise. This raises an ethical issue pitting rich auto owners who want cheaper gas against poor people who need cheaper food. So a different, non-edible source of sugar is needed. One such source is corn stover. Corn stover is everything that is left of the corn plant after the kernels have been removed; cobs, stem, leaves, etc. Corn stover is approximately 45% cellulose, 30% hemicellulose, and 15% lignin. The remaining 10% is comprised of a variety of other materials.

The sugars are found in the cellulose and hemicellulose. Unfortunately, it is much more difficult to get sugars from corn stover than from cornstarch (this is why we used cornstarch in this education module rather than corn stover). Both starch and cellulose are polymers of glucose. The difference is that starch is comprised of repeating monomers of α - glucose while cellulose is made from chains of β- glucose. Can you spot the difference below?



α - glucose β - glucose

The β - glucose has a hydroxyl group on the first carbon on the same side as CH_2OH. This small difference accounts for the great differences between starch and cellulose (this could be a good tie-in to evolution). It is the reason why starch can be hydrolyzed (split apart by the addition of a water molecule) into glucose and maltose by amylase (found in saliva) but cellulose cannot. Cellulose requires cellulase to hydrolyze it into fermentable glucose.

The commercial production of ethanol from corn stover involves a dilute sulfuric acid and heat pretreatment. This hydrolyzes the hemicellulose into (among other things) fermentable pentoses (5-carbon sugars). Prior to pretreatment, the hemicellulose is a huge obstacle to enzymatic cellulose hydrolysis. After the hydrolysis of hemicellulose, cellulase is now able to break cellulose into fermentable glucose.

The trick is to get just the right acid concentrations and heat conditions. Too hot and/or too acidic and the sugars degrade and can't be fermented. But if it

isn't hot or acidic enough, not all the hemicellulose is hydrolyzed and the cellulase can't do its thing.

In this education module, starch is good substitute for corn stover. It shows biological hydrolysis (amylase) with a quantifiable method. It also shows that amylase is much more effective in breaking down starch than is 1% sulfuric acid (a fact that might surprise students). Amylase actually comprises less than 1 % of the volume of saliva. It is usually over 99% water.

For more information visit www.nrel.gov.

For more information of the colorimetry portion of this module, refer to the literature accompanying the Vernier® Colorimeter.