Enzymes and Bioenergy Activity

Objective

- To learn what enzymes are and their chemical functions in living tissue.
- To learn the function of cellulase and its limitations, specifically time-dependence.
- To learn how to produce sugars from non-traditional food sources.

Skill Level: High school	**Prep time:** 24 hours elapsed **Class time:** 50 minutes

Materials

Experiment 1:

Per Group:

- 2 250 mL beakers
- Hydrogen peroxide (3% solution)
- 1 raw potato
- 1 knife
- A few small rocks

Experiment 2:

Per Class:

- 1 test tube for class demonstration
- 2 re-sealable 2L bottles;
- 2 1000 mL beakers
- 2 Dropper bottles of Benedict's Reagent (approximately 50 mL each)
- 5 grams cellulase
- 250 mL rubbing alcohol (isopropyl alcohol)
- 2 hot plates (with test tube clamps for safe transfer from hot water bath to racks)
- Paper pulp (shredded recycled paper, blender, water)

Per Group:

- Paper pulp (shredded recycled paper, blender, water)
- Thermometer
- 4 test tubes, 1 for class demonstration
- Labels and markers

Next Generation Science Standards

Disciplinary Core Idea:

LSC1.C: Organization for Matter and Energy Flow in Organisms

Performance Expectation:

MS-LS1-k: Develop a model to support the explanation that within an individual organism food moves through a series of chemical reactions in which it is broken down and rearranged to form new molecules, to support growth, or to release energy.

HS-LS1-7. Use a model to illustrate that cellular respiration is a chemical process whereby the bonds of food molecules and oxygen molecules are broken and the bonds in new compounds are formed resulting in a net transfer of energy.

Practices	Crosscutting Concepts
☒ Asking questions / defining problems	☐ Patterns
☒ Developing and using models	☐ Cause and effect: Mechanism / explanation
☒ Planning / carrying out investigations	☐ Scale, proportion, and quantity
☐ Analyzing / interpreting data	☐ Systems and system models
☐ Math / computational thinking	☒ Energy / matter: Flows, cycles, conservation
☐ Constructing explanations / design solutions	☐ Structure and function
☐ Engaging in argument from evidence	☐ Stability and change
☐ Obtaining / evaluate / communicate	

Background

Introduction:

Currently, a major sector of the bioenergy industry concerns bioethanol production from corn. This is centered in the Midwest U.S. and yielded 14 billion gallons in 2011. Bioethanol is produced from fermentation of reduced sugars by microorganisms, typically the yeast *Saccharomyces cerevisiae*. Corn contains starches, which are dense, complex sugars called carbohydrates. When broken down, starches provide the reduced sugars for energy for *S. cerevisiae*. However, there have been many economic and political debates about using a "food source" like corn for fuel, which limits the price and production of ethanol. Since microorganisms need only the reduced sugars, not necessarily those from corn, the industry now faces a new questions: where can we readily acquire usable sugars for ethanol without using traditional food sources?

Plants are composed of many types of carbohydrates. Plant biomass is mostly composed from cellulose, the most abundant source of carbohydrate on Earth, and is often referred to as cellulosic.

Cellulosic sources such as wheat straw, corn stover, and grass straw have become potential new sources of biomass for the bioethanol industry. Cellulose also breaks down to reduced sugars. However, about a third of the energy is wasted because *S. cerevisiae* cannot ferment all of the reduced sugars that cellulose produces. Plant cellulose is also molecularly restricted because of other plant molecules, including lignin. The relationship between cellulose and lignin has been compared to the relationship between rebar and cement in reinforced concrete, where cellulose is the rebar (steel reinforcement bars) and lignin is the cement. Lignin molecules surround and structurally inhibit access to the cellulose in plants.

In order to make lignocellulosic sources (cellulosic sources with lignin) accessible to fermentation, complex processes are required for cellulosic bioethanol production. Figure 1 displays the typical process of producing bioethanol from these sources, which include pretreatment, hydrolysis and fermentation. Pretreatment usually involves acids or bases and/or high temperatures and pressures. Hydrolysis, the chemical breakdown of cellulose into reduced sugars, is usually done in one of two ways: acid breakdown or enzymatic reduction.

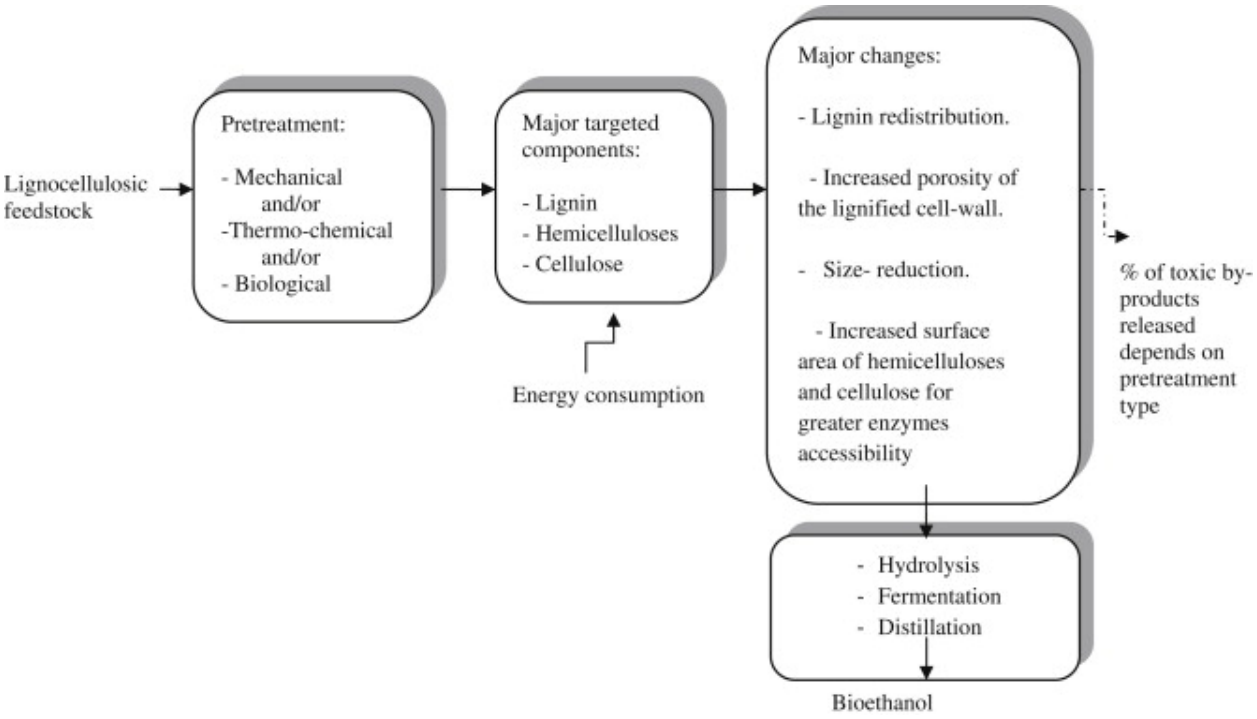

Figure 1. The major steps and outcomes of a lignocellulosic bioethanol production process. Reference

Ultimately, cellulosic ethanol needs be cheaper and available in large quantities to compete with corn ethanol. However, currently thermo-chemical processes are not feasible. These processes require large amounts energy (a very undesirable trait considering the end result is fuel), sometimes produce toxic residues, and can be time-dependent.

One proposed solution that has helped many industries become more efficient is the use of

biological catalysts that require low use of energy and chemicals. These biological catalysts, called enzymes, govern almost all life-sustaining reactions and are found in every living organism. Enzymatic bioconversion has been highlighted as a potential solution to pretreatment and hydrolysis, so that raw plant material can quickly be converted to usable sugars for fermentation. Current research is ongoing to discover, manipulate, and produce enzymes that work faster and more efficiently.

Background:

Enzymes are complex molecular proteins that catalyze many biological reactions. They are an integral component to sustaining life through the transfer of energy. Enzymes are responsible for basic life functions, such as the synthesis of DNA, as well as complex multi-organ processes, such as the transfer of CO_2 from bodily tissues to the blood and then to the air. Enzymes are capable of this because of their catalytic power and specificity.

Each enzyme governs only a single reaction or set of reactions. They are identified by their "-ase" suffix and are often named by the substrates they act upon (e.g. cellulase breaks down cellulose). This specificity is determined by an enzyme's structure, in which the active site, the site for catalytic activity, is arranged only to break a certain bond from a certain molecular structure. Enzymes carry the energy created from the breaking of one bond into the creation of other bonds, and catalyze biological reactions efficiently. An enzyme's main function is to convert energy from one form to another.

Enzymes are found in every living organism and like all proteins are synthesized from DNA. Researchers study the enzymes of plants, animals, and microbes for their functions and value. Enzymes are usually grown in large quantities in microorganisms, which are inexpensive and grow very quickly. Microbial genomes can be altered so that the production of the desired enzyme is increased. *Aspergillus niger*, a fungus, produces over 40 commercial enzymes because of its unique bulk quantity attributes. Enzymes useful to the bioenergy industry, such as cellulases and liginases, would have to be mass-produced for cellulosic bioethanol to be successful.

Enzymes go beyond bioenergy, as life would cease to exist without them. In order for any organism to be self-sustaining, its cells must carry out multiple processes at one time, as well as be self-controlled, so that chemical equilibriums are maintained. The enzyme catalase, which is highlighted in the living tissue experiment below, converts hydrogen peroxide (H_2O_2) into water (H_2O) and molecular oxygen (O_2). Hydrogen peroxide is produced in small amounts as a by-product of energy conversion by mitochondria. Hydrogen peroxide is toxic to cells and would ultimately kill the cell if it built up. Catalase protects the cell by removing the harmful hydrogen peroxide waste product. Catalase is called a protective enzyme because of this function.

Engage

Which enzymes will be more efficient than the conventional process to simultaneously create and break down cellulose to useable sugars for microorganisms to ferment? Where might scientists look to find these enzymes (plants, fungi, in the stomachs of plant eating animals)? Research into cellulases and liginases has shown that these enzymes reduce or remove the need for pretreatment, thus dramatically increasing the efficiency of the cellulosic ethanol process. However, these enzymes are time-dependent and expensive. Although energy efficiency is important, efficient use of time is also very important, especially when considering industrial-scale operations and competitive pricing.

Explore

Experiment Questions:

- Does the potato or the rock contain the enzyme catalase? How can you tell?
- What reaction is occurring at the surface of the potato? Why would this produce bubbles? Why is the reaction maintained on the surface?
- Cellulase breaks down cellulose. Which ingredient(s) provided the cellulose in the experiment? How could you tell?
- What were the colors of four test tubes before and after heating? Were any darker than the others? If so, why might this be?

Procedure: Living Tissue Experiment

1. Fill each beaker with approximately 100 mL of Hydrogen Peroxide (3% solution)
2. Dice one slice of raw potato, set aside for later.
3. Wash the small rocks with water, dry and set aside for later.
4. Add the diced potato to the first beaker and the dried rocks to the second beaker. Make sure to label.
5. Note whether or not bubbles are formed on and released from the surface of the potato. If so how fast are these bubbles created?
6. Note whether or not bubbles are formed on and released from the surface of the rock. If so

how fast are these bubbles being created?

Procedure: Cellulase Experiment

24 Hours Prior to Experiment (done the night before):

1. Shred paper and fill two buckets (minimum 2L) with 2 parts warm water, 1 part paper (increase water to account for absorbency) and let soak for 3-4 hours.
2. Incrementally place the water-paper mix in the blender and pulse until mixture is a thin liquid; fill halfway two re-sealable 2L-bottles with the paper pulp mixture. Set aside one of the bottles and label "Paper Pulp".
3. Prepare 0.5% Cellulase solution. Stir 5 grams cellulase into 1 L water.
4. Add 500mL of 0.5% cellulase solution to second bottle of paper pulp mixture, re-seal the bottle to aid enzymatic function and label this bottle "Overnight Cellulase".
5. Set aside the remaining 500 mL of 0.5 % cellulase solution.

Day of Experiment:

1. Set up workstations
    a. Student stations should have 4-5 people and need 4 test tubes per group.
    b. Hot plate stations
        i. Set up 2 hot plates
        ii. Fill 1000 mL beaker with 500 mL of water and heat to 50^{o}C on each hot plate.
    c. Supply station
        i. Display and label the following supply containers.
            1. Pulp solution: "Overnight Cellulase"
            2. Pulp solution: "Paper Pulp"
            3. Reactant solution: 250 mL of 0.5% cellulase solution
            4. Reactant solution: 250 mL of rubbing alcohol
            5. Reactant solution: 250 mL of water
2. In student stations
    a. Number test tubes 1-4 and place in racks to keep upright. Use goggles.

b. Mark on each test tube a 3 cm and 6 cm line from the bottom.

c. Fill test tubes by having groups take turns at the supply station.

   i. #1 – Fill with "Overnight Cellulase" to 6 cm mark.

   ii. #2 – Fill to 3 cm mark with "paper pulp" and then to 6 cm mark with 0.5% cellulase solution.

   iii. #3 – Fill to 3 cm mark with "paper pulp" and then to 6 cm mark with rubbing alcohol.

   iv. #4 – Fill to 3 cm mark with "paper pulp" and then to 6 cm mark with water. (this is the control)

   v. Gently swirl solution in each tube, do not shake.

d. Add ten drops of Benedict's reagent to each test tube and gently swirl. Note the color of each solution before proceeding.

e. Benedict's reagent identifies the presence of reduced sugars in a solution by changing color when heated. Predict which test tube(s) will change color.

f. Carefully heat the test tubes by suspension in water baths at 50^{o}C for 5 minutes. Carefully place the test tubes back in their holding racks.

g. Discuss the Experiment Questions in student groups first and then with the whole class.

3. Disposal

   a. Empty liquid to drain and flush, empty any solid in trash.

   b. Wash, rinse and dry test tubes. Flush pulp in drain or place in compost.

Explain

- What other reactions within the human body or in living tissue are controlled by enzymes? If having trouble, look in chemistry, anatomy or biology textbooks.
- Which reactions might be controlled by enzymes for bioenergy purposes, especially when considering cellulosic ethanol?
- Do you think cellulase is a time-dependent enzyme? What might this mean for the

Bioenergy industry?

- Will the presence of more cellulase break down cellulose faster? Why or why not?
- What is a limiting factor? What are limiting factors in this experiment? What are limiting factors for cellulosic ethanol production?
- Research other industries that use enzymes to create products or control their processes (if having trouble look at pharmaceutical production). Where are enzymes being used? Why?

Elaborate

- If you were to cut the potato in half without taking the skin off would the reaction happen on the surface of the skin? If so would more or less bubbles be created? Do you think the skin of the potato contains the enzyme catalase?
- If you could isolate the enzyme catalase into a powder and add it to hydrogen peroxide what would happen? Does this theoretical example resemble the second experiment?
- What is a ligninase? Why is it being researched for Bioenergy? When investigating ligninases why is it important that the enzyme only work on the lignin, not the cellulose?
- After pulping the paper, try boiling the pure paper pulp solution for 5 minutes before adding in the cellulase solution (to either the overnight container or the in-class mixture) and continue with the procedure as normal. Was there a difference in the end results? Were more sugars released? If so, why might this be?
- After pulping the paper, add low molarity HCL acid to the solution and boil for 5 minutes before adding in the cellulase solution (to either the overnight container or the in-class mixture) and continue with the procedure as above. Was there a difference in the end results? Of all the methods tried, which released the most sugars? Discuss what these "extra" measures do for overall cellulosic ethanol process, both negative and positive.

Resources

Additional Resources (click links)

- BioFuels: Cellulose Lab Teacher Guide (Click links)
- How Enzymes Work Video

- A high level summary of the role of enzymes in the pharmaceutical industry

Resources Used:

- Ethanol Production News
- Cellulose Biomass for Ethanol Production
- Berg, Jeremy M., John L. Tymoczko, and Lubert Stryer. *Biochemistry*. 7th ed. New York: W.H Freeman and, 2012. Print.