Biomolecules and Biofuel

Lesson Author: Adapted from the Boyce Thompson Institute

By: Laura Penman Monroe Community College Rochester, NY

Target: Freshman/Sophomore College students, non-biology majors

Time Frame: One 110-minute lesson period in week 1, followed by a 30-minute period (can occur on day 1- 7…see procedural notes).

Project Overview: Students will learn about the four different classes of biological molecules, and they will use common biological indicators to discover that enzymes are a type of protein. Students will also investigate pH as a factor that affects enzyme function while conducting an experiment to convert Switchgrass cellulose to fermentable glucose. The cellulose to glucose model will provide a meaningful framework to discuss monomers, polymers, hydrolysis, and dehydration synthesis.

Lesson objectives:

- Reinforce the scientific method and experimental design
- Introduce students to the different classes of biological molecules and techniques used to identify them.
- Recognize the role of enzymes as reaction catalysts.
- Understand the relationship between monomers and polymers and the role of dehydration synthesis and hydrolysis in converting between these forms.
- Recognize enzymes are proteins whose function is sensitive to pH.
- Investigate substrate size as another factor that can influence enzymatic degradation.

Key Concepts: monomers, polymers, hierarchy of organization in living systems, dehydration synthesis, hydrolysis, effect of pH on enzyme function, renewable resources, types of biological molecules and indicators used to detect them.

New York Standards (if interested in adapting for secondary school curricula)

Standard 1: 2.3, 3.1, 3.3, 3.4,

Standard 4: 1.2h , 5.1c, 5.1f, 5.1g

Lab Safety

The major safety issues associated with this lab involve the use of HCl acid and NaOH to produce pH gradients. Students should wear gloves and eye protection when handling these chemicals. Benedict's solution requires the use of a hot water bath, and students should exert caution to avoid burns from steam, hot glass, or hot water.

Materials list:

Part I: Biochemistry of "Accellerase"

Student lab handout

The following supplies for each group of students:

Sudan IV to test for lipids + 3 test tubes + vegetable oil for pos. control
Biurets solution to test for proteins + 3 test tubes + 1% albumin for pos. control
Benedicts solution to test for sugar + 3 test tubes + 1% glucose solution of pos. control
Iodine to test for starch + 3 test tubes + 1% starch
Grease pencil to write on tubes
Test tube rack
Distilled water
Hot water bath
Droppers
"Accellerase 1550" from Genecor

Part 2: Optimizing "Accellerase" activity

Dried switchgrass
Scissors to cut dried switchgrass into 1cm long segments
Pelletized switchgrass
Triple beam balance and weigh-boats
Powdered switchgrass
50 ml conical centrifuge tubes with screw tops (8 per group of students)
Accellerase 1500 stock from Genencor
Urinalysis strips to measure glucose or a glucose meter with strips (8 strips per group)
Tube racks to accommodate tubes (1 per group)
Dropper bottles containing 1M HCl and 1M NaOH
pH strips
Sharpie markers for writing on tubes
Pipettes for adding Accellerase to tubes
Distilled water
Refrigerator for incubation (>3 days) or incubator for short term incubation (<1day)

Lesson Plan:

<u>Student Background</u>: Based on our college curriculum, organic molecules are introduced during the second week of the semester, shortly before they have been exposed to corresponding lecture material. As such, students will have little previous knowledge about organic chemistry, and this laboratory is designed to introduce them to important concepts they will encounter in the lecture portion of the course. Prior to this laboratory exercise, they will have been exposed to the scientific method and considerations of experimental design. They will also have been introduced to the hierarchical organization of life (atoms build molecules, molecules build cells, cells build multicellular organisms, etc.) Some instructors will have introduced students to basic chemistry concepts and the pH scale by this point, but most students will likely not be exposed to pH in this course before this lab.

<u>Pre-Laboratory Activity (15 minutes)</u>

Assigned either in class or as homework, the first three pages of the student handout address the need for alternative fuels and describe the variety of biochemical molecules.

Introduction: Motivating and Background Information (40 minutes on day 1)

-Use the Power Point presentation "Biomolecules and Biofuels" provided, or create your own. Slides 1-30 are for use on day 1. The remaining slides are for day 2.

Students will be reminded that atoms joined together with covalent bonds share electrons and form molecules. They will be informed that important biological molecules tend to be large, complex structures with carbon skeletons. They will be introduced to the major classes of biological molecules, (carbohydrates, proteins, lipids, and nucleic acids). Many of these large molecules are called **<u>polymers</u>** because they are assembled from smaller subunit molecules called **<u>monomers</u>**. This is a good opportunity to describe organic molecules as a source of fuel for animals (and automobiles). One type of promising biofuel is ethanol, which is produced through yeast fermentation of sugars. Another type of biofuel is biodiesel, which relies on conversion of fats and oils into fatty acids.

They will be introduced to the concept of enzymes (but not told what kind of biological molecule they are—that will be one of the objectives of their experiment). They will be told that organisms can break down or assemble large molecules through the process of hydrolysis or dehydration synthesis, respectively. This lab will focus on the degradation of cellulose polymers into glucose. Glucose is a sugar, and a plant can use that glucose as a monomer to build larger molecules as a source of energy, to store energy, or to build structural components (such as cellulose) through dehydration synthesis. Some bacteria and fungi can break down cellulose into glucose molecules through hydrolysis—which is a good thing. Without these decomposers, we would be swimming in dead leaves and navigating around stacks of fallen logs.

In order to produce ethanol, we need an inexpensive and abundant source of sugar. Students will be reminded that cellulose is an important structural component of cell walls. One way to produce liquid fuel is to break down the cellulose polymer into its glucose monomers, and then use yeast in order to ferment the glucose into ethanol.

Students will be told that a special chemical called "Accellerase" was discovered that can convert cellulose into glucose. (The "Accellerase" is actually an enzyme mixture, but that will not be revealed until the students have investigated its chemical nature themselves). This "Accellerase" was obtained from bacteria and fungi, so we know it is a biological molecule. One of their tasks is to determine whether it is a carbohydrate, protein, or lipid.

Lab Procedure Day 1:

1. Walk students through the experimental procedure of Part I—identifying the type of biological molecule that "Accellerase" is. Ask them to identify the variables of the experiment as well as to identify the positive and negative controls.

2. Students determine the type of biological molecule that "Accellerase" is. You are encouraged to "divide up the labor." Each student in a group of four can be responsible for completing one test. Remind students that the water bath produces steam and that hot water can also cause burns. Once students have completed all parts of the experiment, check their tubes to ensure that they have completed all tests before cleaning up. After all groups have been "checked off", confirm with the class that "Accellerase" is a protein and that it is a special kind of protein called an <u>enzyme</u>. Enzymes speed up certain chemical reactions and are sensitive to some environmental conditions.

3. Describe how a variety of factors might maximize glucose production by "Accellerase". If we want an inexpensive source of sugar (and ethanol), then we want to optimize the effectiveness of "Accellerase". This lab will look at the effects of pH and substrate size.

3. Have students work in groups of two to four. One third of the class will use the cut switchgrass leaves, another third will use pelletized switchgrass, and the other third will use the powdered switchgrass. All groups will be investigating the effect of pH. Guidelines are provided in the student instructions to help them approximate the appropriate pH (3, 5, 7, and 9). Remind the students that acids and bases can be harmful and should be handled carefully.

4. Circulate around the room as the students set up their experimental treatments and help them to interpret instructions as needed.

5. If you are meeting with the class again the following day, samples can be placed in an incubator (37 degrees C) to speed up enzyme activity. If you plan to take measurements on day 2 or day 3, samples can be placed on the countertop. If you are taking measurements on day 4 or later, samples should be placed in the refrigerator.

6. Guided by the student worksheet, students will work on answering questions pertaining to Part I of this lab and will generate their hypotheses for Part 2 of the lab.

DATA TABLE FOR PART I.

	Sugar Test	Starch Test	Protein Test	Lipid Test
Name of Indicator				
Heat required?				
Materials in negative control				
Color of negative control				
Materials in positive control				
Color of positive control				
Color of material in tube "3" Experimental tube				

Day 2 Lesson.

1. Students will measure glucose levels with the glucose strips or with a glucose meter. Students will discuss the pH results with their partners and draw a conclusion about the effects of pH on the activity of this particular enzyme ("Accellerase"). They will record data and summarize their conclusion in the accompanying student handout.

2. A review of graphing may be required if students do not have much experience with graphing. Students will be expected to graph their data to summarize the effects of "Accellerase" upon switchgrass degradation and the impact of pH on Accelerase function.

3. Discuss the findings of the pH portion of the experiment with the class.

4. Refer back to the bio-indicator data: Inform students that "Accellerase" is made up of enzymes. What kind of biological molecule is an enzyme?

5. Demonstrate (via raw egg and hydrochloric acid demonstration or video) that proteins can be denatured and lose their function by exposure to acids. This is why pH is so tightly controlled by living things (homeostasis).

6. Once students recognize that the enzyme has an optimal pH (and pH limits), student groups will compare data to determine if the cut, pelletized or powdered form of switchgrass produced more glucose.

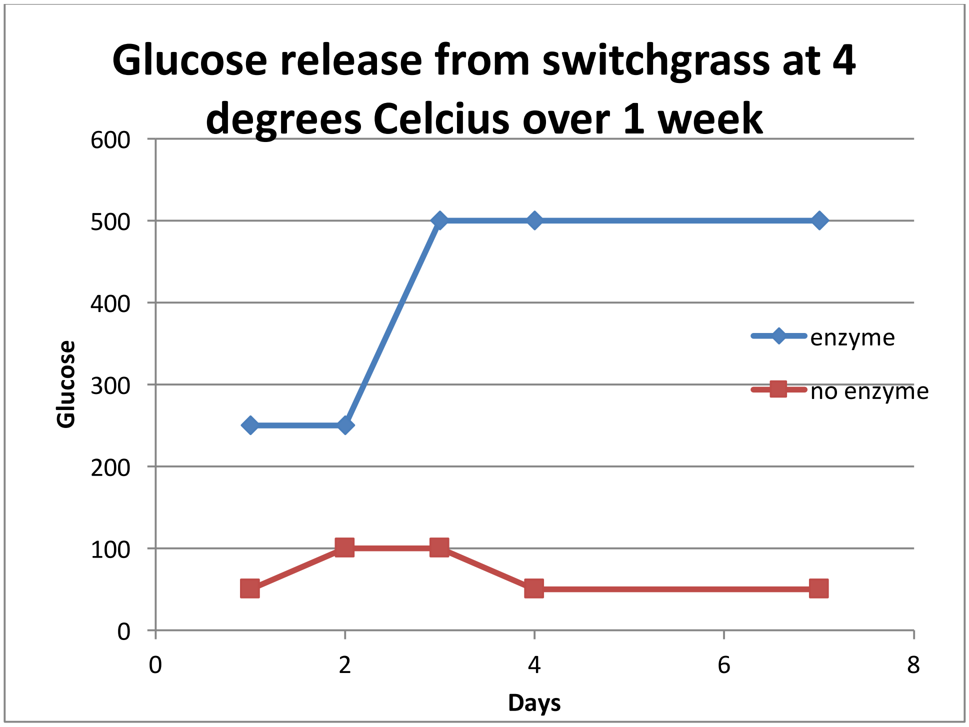
Data table for Part2

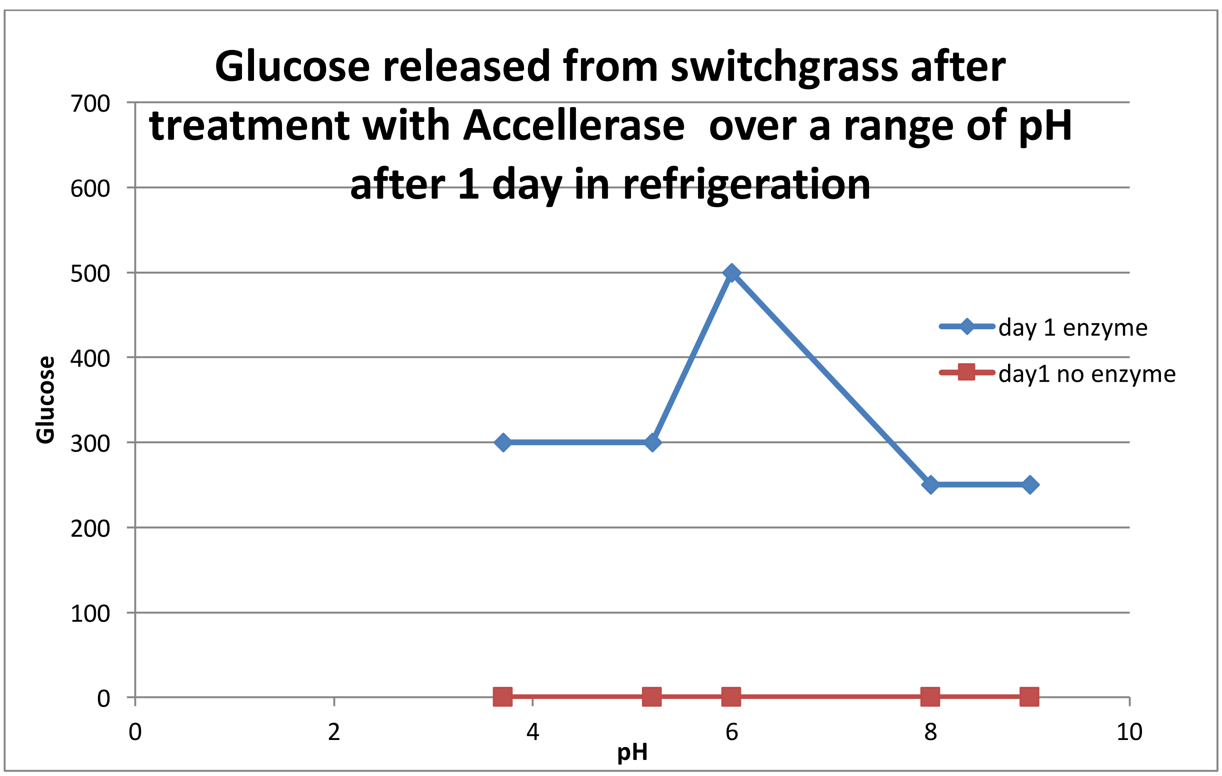
	pH 3	pH 5	pH 7	pH 9
Accellerase				
No accellerase				

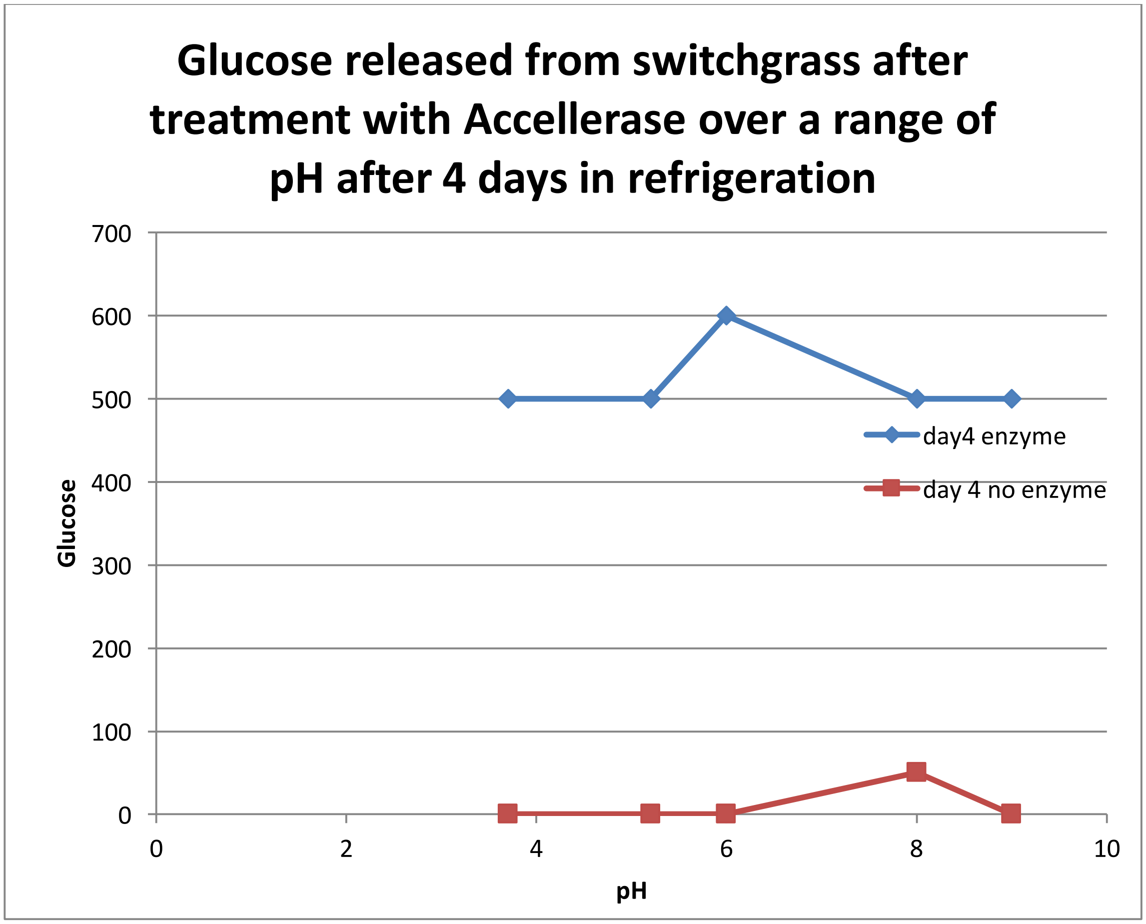
Conclusion & Discussion:

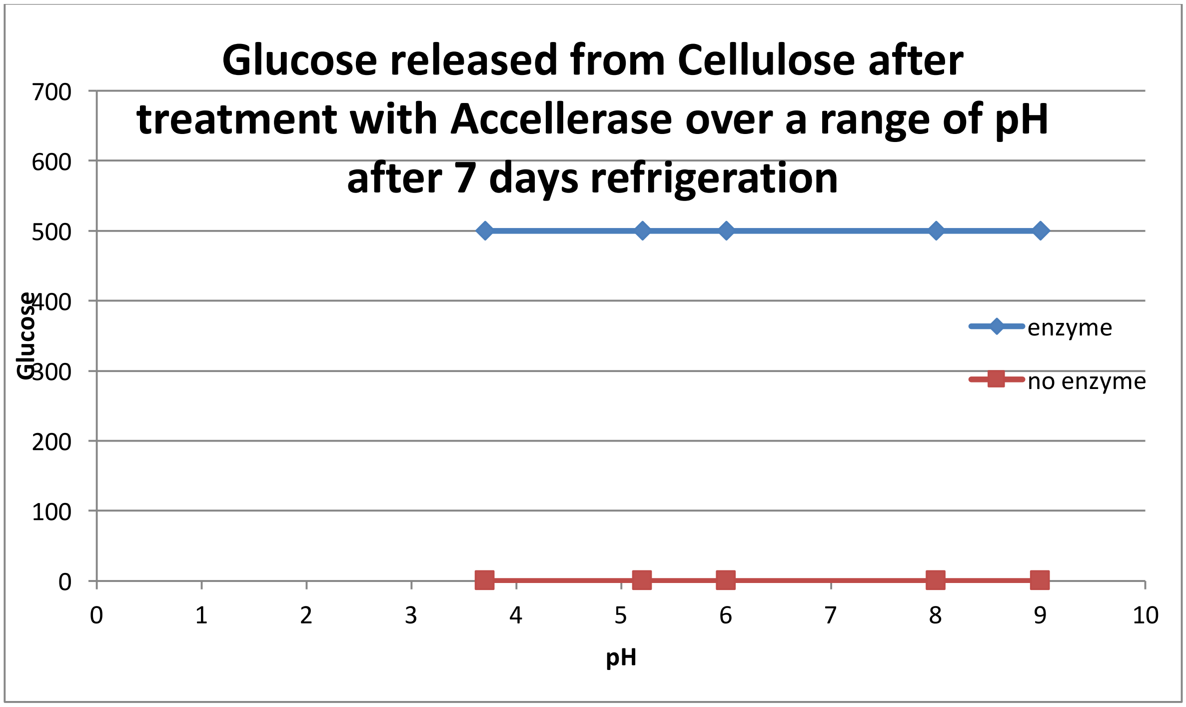
After students have completed the activity, host a class discussion where students share their results. Post results from each group and have the class draw conclusions on both the effects of pH and the effects of switchgrass pre-treatment. Assign remaining questions in student packet.

Show the remaining slides of the provided "Biomolecules and Biofuel" Powerpoint, if desired. (Slides 31 onward). Several slides of this PowerPoint refer back to biomolecule chemistry, and asks students to declare whether images shown of various potential energy sources would be best converted to ethanol (sugar/carbohydrate-based) or biodiesel (oil/fat-based).


Glucose release from switchgrass at 4 degrees Celcius over 1 week
Glucose
600
500
400
300
200
100
0
0
2
4
6
8
Days
enzyme
no enzyme


Glucose released from switchgrass after treatment with Accellerase over a range of pH after 1 day in refrigeration
Glucose
700
600
500
400
300
200
100
0
0
2
4
6
8
10
pH
day 1 enzyme
day1 no enzyme


Glucose released from switchgrass after treatment with Accellerase over a range of pH after 4 days in refrigeration
700
600
500
400
300
200
100
0
Glucose
0
2
4
6
8
10
pH
day4 enzyme
day 4 no enzyme


Glucose released from Cellulose after treatment with Accellerase over a range of pH after 7 days refrigeration
700
600
500
400
300
200
100
0
Glucose
0
1
2
3
4
5
6
7
8
9
10
pH
enzyme
no enzyme

Extensions:

1) Follow up Ethanol Production:

Collect the "Accellerase" digestion products from all groups and all tubes and place into a fermentation bucket. Neutralize with HCl or NaOH as appropriate (a pH of 7 is desired, but yeast are able to grow and produce ethanol in a variety of pH environments). Be certain that the fermentation bucket (or carboy), lid, and airlock has been sterilized beforehand (by bleach, perioxide, or autoclave). Although water can be used in the airlock, inexpensive alcohol may reduce the opportunity for bacterial contamination.

Add yeast to the digestion product, stir with a sterile instrument, and set aside in a visible (but safe) location.

Once bubbles are no longer visible in the airlock, it is generally a sign that fermentation has stopped. At this time (or later if it suits the class schedule better) the fermented glucose product can be collected. A hydrometer can be used to estimate the alcohol concentration (best to sterilize it before use to avoid contamination).

A further extension would be to distill the alcohol (time and equipment-intensive) and subsequently ignite a small sample to demonstrate that it has properties of ethanol.

This extension would be useful when describing microorganisms and their use in biotechnology.

2) Investigation of other factors upon enzyme activity

Possibilities include temperature and enzyme concentration.

3) Investigation of other substrates

Students can select another cellulosic substrate for investigation, following the same basic procedure as used in Part 2 of the experiment. Theoretically, they will have already narrowed down on an ideal pH range.

Algae biodiesel --- compared to corn ethanol

http://www.oilgae.com/algae/pro/eth/eth.html

http://news.cnet.com/8301-11128_3-9966867-54.html

http://www.nytimes.com/gwire/2009/08/21/21greenwire-ethanol-producers-warily-eye-algaes-bloom-35329.html

http://www.cheg.uark.edu/5005.php

Switchgrass

http://www.scientificamerican.com/article.cfm?id=grass-makes-better-ethanol-than-corn