Biofuel Bumper Crop: How Much Does Environmental pH Matter?

Target Grade Levels and Courses

Grades 7-12 Biology, Environmental Science, Chemistry, and Agriculture courses

Learning Standards Addressed

Next Generation Science Standards (NGSS) Performance Expectations

- Ask questions about the inputs, factors, and contributors of producing biofuels from Switchgrass to develop a general system model of alternative fuel production.
- Carry out an investigation to see how environmental factors such as pH affect percent germination of Switchgrass.
- Analyze and interpret data collected from the investigation to see if any patterns in pH and seed germination exist (how did environmental conditions affect growth and what do the findings suggest about acid rain?).
- Use evidence from analyzed data to communicate to others how humans might successfully engineer the large scale production of Switchgrass as an alternative fuel.

HS-LS1-1. Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.

HS-LS1-5. Use a model to illustrate how photosynthesis transforms light energy into stored chemical energy.

HS-LS1-6. Construct and revise an explanation based on evidence for how carbon, hydrogen, and oxygen from sugar molecules may combine with other elements to form amino acids and/or other large carbon-based molecules.

HS-LS2-1. Use mathematical and/or computational representations to support explanations of factors that affect carrying capacity of ecosystems at different scales.

HS-LS4-6. Create or revise a simulation to test a solution to mitigate adverse impacts of human activity on biodiversity.

HS-ESS3-1. Construct an explanation based on evidence for how the availability of natural resources, occurrence of natural hazards, and changes in climate have influenced human activity.

HS-ETS1-1. Analyze a major global challenge to specify qualitative and quantitative criteria and constraints for solutions that account for societal needs and wants.

Standards for Technological Literacy (STL)

http://www.iteaconnect.org/TAA/PDFs/xstnd.pdf

Standard 4-Cultural, social, economic, and political effects of technology
Standard 5-Effects of technology on the environment
Standard 15-Agricultural and related biotechnologies
Standard 16-Energy and power technologies

National Council for Agricultural Education (NCAE)

https://www.ffa.org/thecouncil/Documents/finalafnrstandardsv324609withisbn_000.pdf

LifeKnowledge® and Cluster Skills:

- Demonstrate appropriate health and safety procedures for AFNR occupations.
- Use tools, equipment, machinery and technology appropriate to work within areas related to AFNR.
- Utilize scientific inquiry as an investigative method.

Environmental Service Systems:

- Examine the relationships between energy sources and environmental service systems.

Biotechnology Systems:

- Demonstrate the application of biotechnology to Agriculture, Food and Natural Resources (AFNR).

Plant Systems:

- Prepare and implement a plant management plan that addresses the influence of environmental factors, nutrients and soil on plant growth.

Lesson Overview

Students will…

1. learn what biofuels are and the problems/issues to which biofuels are a response. Reviewing the background knowledge might be a short review for a more experienced class or a 10-minute **interactive lecture** for students unfamiliar with biofuels.
2. explore a promising biofuel source: Switchgrass. They learn about its recent history as an alternative energy source and its physical and chemical properties. **Interactive lecture, guided reading, and/or videos** would be effective learning activities here.
3. generate a list of variables that collectively define growing conditions (e.g., sun exposure, soil density, soil pH). Depending on students' ages and backgrounds, the teacher may need to remind students about, or introduce for the first time, a grade level-appropriate definition of acids and bases. Once soil pH is understood, teacher notes that, as more farmers start to grow Switchgrass, they need guidance about optimal pH. Next, students will act like scientists to try to give farmers good advice about optimal pH for this valuable crop.
4. complete lab in groups of 4. The lab consists of set-up and then two data collection periods, the first one 3-5 days after set-up and the second one 7-9 days after set-up.

Planning Ahead

This lab incorporates pH solutions. You will need to obtain or mix pH solutions 5, 6, 7, and 8. You may make pH solutions using ascorbic acid/lime solutions, or order prepared pH buffers from a biological supply company, such as Carolina Biological. You may also widen the scope by increasing the pH range investigated, but be aware of proper safety protocols for handling acids and bases.

Estimated Time

Setup - Day 1: 20 minutes for background information on biofuels and Switchgrass; 10 minutes for generating a list of variables that make up a plant's "growing conditions" and for setting up the experiment conceptually; 25 minutes for *physical* set up of lab (Background and brainstorm can be done on a separate day if needed)

Observation Day 1: (3-5 days after Setup): 20 minutes for Counting Germinated Seeds and Watering Seeds if needed

Observation Day 2: (7-9 days after Setup): 45 minutes for Counting Germinated Seeds and Lab Analysis and Conclusion

(The Conclusion section could be assigned as homework and discussed during the next class period, which would shorten the amount of in-class time required.)

Key Background Information

Switchgrass Seed Biology

Scientists around the world are working to increase the efficiency of producing ethanol from plants. Current ethanol production from corn has become controversial due to its impact on food supply. Plant breeders have been studying the growth of plants native to North America that are not domesticated or depended on for food. The biomass from these plants can be converted to ethanol to produce a fuel source that does not compete with food production. One important biomass feedstock, Switchgrass (*Panicum virgatum* L.) is a perennial warm-season grass established by seed and native to most of North America. Within the species there are different varieties of Switchgrass because of the different conditions where the varieties are endemic and how the plants evolved to be successful in those environments. Switchgrass has been used as ground cover, forage for livestock, soil and water conservation, and wildlife habitat. In the United States, the Department of Energy (DOE) recommended Switchgrass as a model herbaceous biofuel crop because Switchgrass produces a high yield, contains high levels of cellulose, requires low energy input for production, grows in marginal lands, and can be a dedicated biofuel crop. The most immediate challenge to growing Switchgrass on a large scale basis is understanding seed germination rates. Scientists are asking questions like:

- In what conditions do seeds germinate best?
- Are some varieties of seeds better than others?

If you were a famer planting a biomass feedstock, what other questions might you have before planting?

Testing for seed quality is routinely performed on seed lots of all commercial seeds. A seed lot is a batch of seeds of a particular crop and variety that was produced in the same area and time harvested and handled in the same manner. Data from a standard germination trial of a seed lot provides the basis for labeling seeds for sale and this information is provided on a seed tag. The Rules for Testing Seeds is the official method for testing germination in the United States and is regulated by the national organization, AOSA, the Association of Official Seed Analysts.[1] The standard germination test is conducted under ideal environmental conditions for a particular species and is optimized for breaking dormancy to achieve the highest germination. For Switchgrass this process involves a stratification period where the seeds are grown at 5°C for 14 days before being moved to 30°C for 8 days followed by another 16 hours at 15°C. Switchgrass seeds are sown in the late spring or early summer in New York and in the Northeastern US, when soil temperatures are warm. Therefore,

[1] (Association of Official Seed Analysts, 2008)

Switchgrass seeds are not exposed to a moist chilling followed by warm conditions in the field. The result is that the germination reported on the seed tag overestimates field emergence. We anticipate that classroom germination tests in this will more closely mimic the spring/summer conditions (without cold treatment) and results will give insight into the impact of pH on seed germination. The lab that follows will give your students the opportunity to develop and apply their scientific inquiry knowledge.

Acid Rain Chemistry

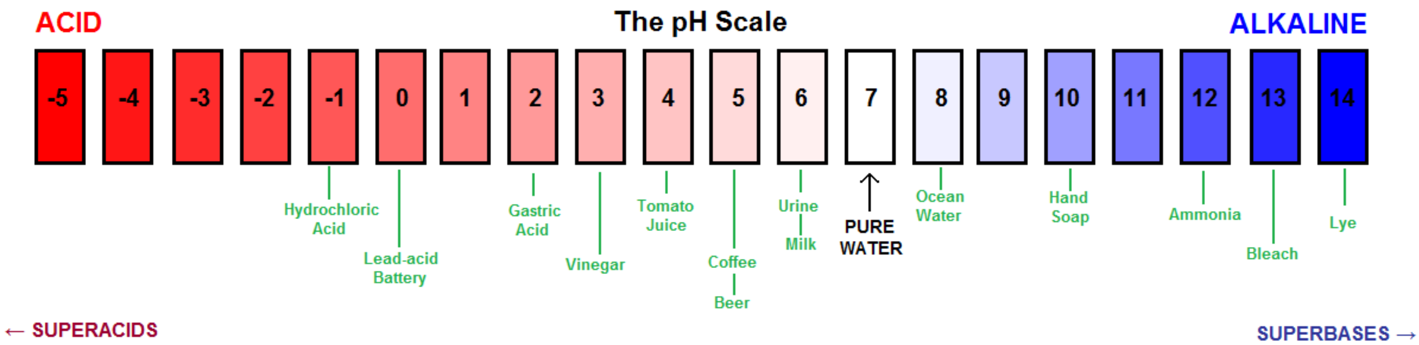
(Acid rain *per se* is not foregrounded in this lesson; however, once students see that low pH is not conducive to Switchgrass growth, it would be fruitful to talk about acid rain as one well-known [even among kids] source of low pH): Industrial development has enhanced human life in many ways. However, it is important to acknowledge its potential negative effects, perhaps especially in this era of rapid development in countries such as China and India. One such effect is air pollution from industrial byproducts. Air pollution is detrimental to the environment as it can be spread with even the slightest of winds. Emissions that contribute to air pollution include particulate matter, lead, sulfur dioxide, nitrous gas, carbon dioxide, and ozone (see the National Ambient Air Quality Standards [NAAQS] and the New Source Performance Standards [NSPS]).

Each major contaminant has its own effects on the environment. Two of these contaminants, sulfur dioxide and nitrous gas, directly affect the agricultural industry. Primarily, these pollutants are a result of combustion from cars or other power-generation industries. Nitrous gas and sulfur dioxide will dissolve and react with the water in the clouds through acid-base interactions. The end products of these reactions are sulfuric acid and nitric acid, which are crucial components of acid rain. Acid rain has been known to be detrimental to crops as most crops are not resilient to acidic conditions. Due to the EPA emission standards set about by the NAAQS and the NSPS, the United States average acidity for acid rain is between 6.0-5.0. The lack of regulations in emerging and developing countries such as China create much more acidic rain in those countries. These acidity values tend to be between 5.5-3.7. This lab will simulate the effects of acid rain on germination, specifically Switchgrass germination rates.

pH

pH is a scale used to measure acid and bases. pH measures the hydrogen ion concentration. A pH of 7 represents a neutral substance; there are equal amounts of Hydrogen (H^+) and Hydroxide (OH^-) ions. Bases have a greater OH^- concentration and acids have a greater H^+ concentration.

Studies have shown that pH values lower than three and greater than eight can inhibit germination. Certain seeds germinate best at an optimal pH. For example, sunflowers and tomatoes prefer slightly acidic conditions, around a pH of 6. In this lab, bioenergy grass seeds are used to better understand the impact of the acidity from pollution on the environment, and to determine optimal pH conditions for biofuel grass germination.



source: Wikimedia commons

Additional Background Information:

http://www.epa.gov/acidrain/

http://www.biotechnologyforbiofuels.com/content/5/1/80

http://www.scientificamerican.com/article.cfm?id=native-plants-on-marginal-lands-to-reduce-food-versus-fuel-from-biofuels

http://hortsci.ashspublications.org/content/25/7/762.full.pdf

Key Scientific Vocabulary:

Acidic
having a pH less than 7

Basic (Alkaline)
having a pH greater than 7

Biofuel
fuel derived from biomass

Cellulose
the structural component in plant cell walls, a linear chain of several hundred to over ten thousand glucose molecules

Dependent Variable
the variable in an experiment being observed to see if it changes in response to changes in the independent variable

Endemic
unique to a defined geographic location

Ethanol
ethyl alcohol, a volatile flammable colorless liquid used as a biofuel additive for gasoline

Fossil Fuels
fuels formed by natural processes of buried dead organisms

Greenhouse gases
gases that can absorb and emit infrared radiation

Hypothesis
proposed explanation for a phenomenon

Independent Variable
the variable in an experiment being manipulated to determine its possible effects on another variable

Marginal Lands
land that is difficult to cultivate

Neutral
having a pH of 7

pH
a measure of the acidity or basicity of an aqueous solution

Radicle
the first part of a seedling to emerge from the seed during the process of germination

Renewable
naturally replenished

Seed
a small embryonic plant enclosed in a seed coat some stored food

Switchgrass *(Panicum virgatum L.)*
a perennial, warm-season bunchgrass used as a biomass crop for cellulosic ethanol, in phytoremediation projects, fiber, electricity, and heat production and for biosequestration of atmospheric carbon dioxide

Setting up the Lab

BTI will provide: (Per class of 32 students, 8 groups of 4)

- 64 Petri Dishes
- 64 White blotter paper rounds
- 64 Brown germination paper rounds
- 4 Humidity chambers
- 16 packets each of 4 varieties of switchgrass seed
- 32 Transfer pipets
- 32 toothpicks
- Parafilm (enough to cut 128 strips)

You will need to provide:

- 150 mL beakers
- Water
- Masking Tape
- Permanent marker
- pH solutions 5, 6, 7, 8

Safety

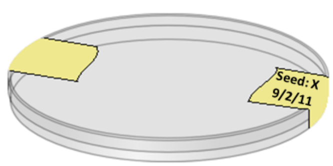
- Wash or sanitize hands prior to touching blotter paper
- Safely handle pH solution by wearing gloves and goggles
- Wear gloves while handling the germination plates, and wash hands after handling to avoid contamination
- Safety Data Sheet (SDS) for pH solutions should be provided

Procedure: Day 1- Preparation

1. Before starting the lab, have students wash or sanitize hands and wear gloves. This will reduce the chances of contamination in the germination chambers.
2. Form groups of 4 students. Each group needs 8 seed packs, 8 petri dishes, 8 blotter paper rounds (white), 8 germination paper rounds (brown), 4 pipettes, 4 toothpicks, pens, parafilm, and about 12" of masking tape. *Each student will set up two petri dishes with **one** of the acidity solutions (pH 5, 6, 7 or 8). Each group member will test the same seed line (one seed line per group of 4 students).*
3. Each group member should do the following:
   a. Use a permanent marker to label their petri dishes with today's date, your period, your group's **seed line name**, and the pH of your solution. You may label on both the lid and the bottom of the dish. Students should use "A" and "B" to distinguish their two dishes from each other. *Example: 6/27/15, 2nd period, Carthage-A, pH 6*

b. Touching only the very edge of the paper, place one blotter paper round (white) in your petri dish. If necessary, use a toothpick to gently push it down into the dish.
c. Touching only the very edge of the paper, place one germination paper round (brown) on top of the blotter paper you just placed in the dish.
d. Using the transfer pipettes, add just enough solution (about 7-10 mL) into each dish to completely cover the paper in your pH solution. Allow the solution to stand for ten seconds. Then pick up the plate, carefully tilt it to one side, and use your pipette to remove any excess solution that has not been absorbed by the paper **<u>and discard in the sink</u>**.
e. Carefully open your seed packet and hold it parallel to the dish. Gently squeeze the sides of the envelope so it opens, and shake the seeds out of the envelope onto the petri dish. Be sure to rotate the petri dish while shaking out the seeds so that they are evenly distributed around the paper. If seeds are clumped in one area or are touching, use your toothpick to spread them out around the plate. It is recommended to arrange the seeds in a grid pattern, to allow for easier counting. Once you are done spreading the seeds, immediately place the lid back on the petri dish.
f. Use two pieces of tape to secure the lid to the bottom plate of the petri dish (see Figure 1). Use strips of Parafilm to seal the outside of the petri dish. Repeat steps 3a-3e with your second dish, using the same pH solution.
g. Store the petri dishes in a humidity chamber to keep the dishes from drying out.
h. Hypothesize which dish will result in the best germination.

Figure 1



<u>Procedure: Observation Day 1 (3-5 days after Preparation)</u>

1. Each group obtains its 8 petri dishes, pens and (optional) magnifying glasses.
2. Take your individual petri dishes, and start by recording in the Data section of the lab handout any observations about changes in the seeds, the brown germination paper, any mold growth (tiny black dots), etc.
3. Carefully (you don't want to disturb the seeds!) undo one of the pieces of tape and open the petri dish, keeping the lid attached on one side.
4. Counting Germinated Seeds:
   a. For this experiment, let's define "germinated" as having a root emerged through the seed coat by 1 millimeter (Figure 2).

b. To keep track of which seeds you have counted, use a pen or marker to place a dot on the blotter paper next to the seed you've counted as germinated.
c. Record the number of seeds that have germinated in the Data section. Count the total number of seeds and record that number in the data section.

Figure 2

5. If the petri dish has dried out, you may need to water your seeds. Gather a 150mL beaker containing about 50 mL of your pH solution and a pipette. If the brown germination paper is still moist, you do not need to add water. To water your seeds:
   a. Fill your pipette with your pH solution.
   b. Hold the pipette against the side of the petri dish, aim for a gap between the side of the dish and the brown germination paper, and slowly squirt the solution into the dish.
   c. Let the solution stand for 10 seconds then gently tilt the petri dish and use your pipette to remove any excess liquid from the dish.
   d. Place the lid back on the dish and use the same pieces of tape to seal the dish.
6. Reseal the sides of the petri dishes with Parafilm strips and place dishes back in the humidity chamber.
7. Using your data, calculate the percent germination for your petri dishes. Share with your group. *Note that most seed packets will not have exactly 25 seeds and the total number of seeds should be counted, in addition to the number germinated.*

 # seeds germinated / total # of seeds x 100 = % germination

Procedure: Observation Day 2 (7-9 Days after Preparation)

1. Repeat steps 1-4 from Observation Day 1. To prevent double-counting from the dots you drew you can carefully move the counted seeds from the germination paper side of the petri dish to the upside-down lid by using a toothpick or forceps.
2. Once you have finished counting germinated seeds you may discard your seeds, and germination and blotter papers in the garbage and return the other supplies to your instructor. Petri dishes can be cleaned and reused, if desired.
    - You can use the germinated seeds to start a switchgrass garden at your school or at home by planting them in shallow soil.
3. Using the data you collected, calculate the percent germination for your petri dishes.
4. Students analyze the data and use their findings to draw conclusions about pH and Switchgrass production.
5. Add class data to provided spreadsheet and send to BTI researchers at pgrp-outreach@cornell.edu

Possible Extensions of Lesson

1. Create bar graphs to compare the class average percent germination with their group's percent germination results
2. Create a scatterplot to see if there is a trend (exponential/linear/etc.) for the relationship between percent germination and acidity content of moisture.
3. Count seeds every day and use additional data to create line graphs to show percent germination over time
4. Measure shoot and root lengths for the different acidity conditions and hypothesize why the observations occurred.
5. Compare Day 3 and Day 7 percent germination and hypothesize about how pH relates to germination rates.
6. Experiment to see which Switchgrass varieties are more resilient to acidity.
7. Plant germinated switchgrass seeds to start a bioenergy garden.

Teacher's Corner

1. One suggestion for providing background knowledge for students is to create a learning activity in which students can develop a *model* (perhaps overly-generalized at this point) of the entire alternative fuel *system*, at least in terms of biofuels that can be produced on a large *scale* from native crops grown on marginal lands. The systems approach focuses on all the inputs, industries, groups, and people involved from the start to the final products. Students can see where their investigation of pH and acid rain is situated in this complex system and why it matters.

2. Devote time in the lesson for student reporting of interpreted data. This notion is listed in the extensions section of the lesson plan. For students to make sense of

what they performed in lab, they need a time in class to grapple with the data and try to find *patterns* or *trends*. What *caused* the *observed effects*? What aspects of their *experimental design* allowed them to detect these trends? How are pH and percent germination related to one another based on the findings of individuals groups and the entire class?

Student Worksheet

Group's Seed Line Name:_______________________________________

pH =   Student Name:_____________________

	Observation Day 1		Observation Day 2	
	Dish A	Dish B	Dish A	Dish B
# Seeds Germinated				
Total # Seeds				
% Germination (# Germinated/ Total #)				

Class Analysis

Percent Germinated on Observation Day 1=
(Number of Seeds Germinated / Total Number of Seeds on Petri Dish) * 100

Seed Variety	**Percent Germinated on Observation Day 1**			
	pH 5	**pH 6**	**pH 7**	**pH 8**

Percent Germinated on Observation Day 2=
(Number of Seeds Germinated / Total Number of Seeds on Petri Dish) * 100

Seed Variety	**Percent Germinated on Observation Day 2**			
	pH 5	**pH 6**	**pH 7**	**pH 8**

Conclusion

Trials and Accuracy

1. How do your group's percent germination results compare to the class average for Observation Day 1? Are they higher, lower, the same? (Hint: Be sure to discuss all 4 pH solutions.)

2. How do your group's percent germination results compare to the class average for Observation Day 2? Are they higher, lower, the same? (Hint: Be sure to discuss all 4 pH solutions.)

3. Which data set, the class' or your individual group's, do you think is a more accurate representation of the true percent germination for each of these seed varieties? Why?

4. Why is it important for scientists to conduct multiple trials when they do an experiment?

pH

1. Did germination results differ between the different pH environments? Which pH resulted in the best germination? The worst?

2. Do these results support your hypothesis about germination percentage and pH of moisture content?

3. Why do you think the results appeared as they did?

Environmental Applications

1. Based on the class data set, would you expect the same trends between acidity and germination percentage using a different seed such as watermelon seeds? Explain your reasoning.

2. In this experiment, the seeds were germinated under room temperature conditions with the varying amounts of acidity in the moisture content. What other factors should scientists consider and test for to determine the optimal growing conditions for Switchgrass seeds?

3. Major air pollutants include: carbon monoxide, hydrocarbons, and particulates. When nitrogen oxides and sulfur dioxide combine with water vapor, acid rain is created. After observing the effect of acid rain on seed germination, what other impacts on the environment might acid rain have?

4. Select one of the factors you identified in the last question and design an experiment that you or another scientist could do to test the effect this factor has on percent germination. Be sure to identify what the independent and dependent variables would be for the experiment?