Photosynthesis Lab with Aquatic Plants and Freshwater Algae

Overview

Photosynthesis is the process by which plants transform light energy into chemical energy for growth, reproduction, and metabolism of the organism. Students will perform two sets of experiments. The first set overviewing the fundamentals of photosynthesis including the role of chlorophyll, light, and carbon dioxide. The second set investigates the potential applications for bioproducts with algae. Students will work in small teams to perform each experiment. At the end of the experiments, the students will answer a series of questions to reinforce a deeper understanding of core principles of photosynthesis and connecting this experience with the bioeconomy The provided kit contains materials suitable for a classroom of 30 students to work in groups of 3. Questions are included in the kit as well as key background information, procedures, and pre-lab inquiries for students to think about while performing experiments.

Objectives

Students will

- Study the importance of chlorophyll, light, and CO_2 in photosynthesis
- Observe sugar production in the form of starch as a result of photosynthesis.
- Observe the impact of light, sugar, and other variables on the growth of algae.

Content Standards

This lesson is appropriate for high school biology/chemistry students and addresses the following Next Generation Science Standards:

Disciplinary Core Ideas/Practices/Cross-cutting concepts covered
North Carolina Essential Standards

Earth/Environmental	EEn.1.1.4; EEn.2.2.2; EEn.2.6.3; EEn.2.6.4; EEn2.7.3; EEn2.8.1; EEn.2.8.2
Physical Science	PSc.3.1.1; PSc.3.1.2
Chemistry	Chm.2.2.1; Chm2.2.2; Chm 2.2.3; Chm 2.2.4; Chm 3.1.1
Biology	Bio1.1.1.; Bio1.2.1; Bio2.2.1; Bio2.2.1, Bio2.2.2; Bio4.1.1; Bio4.2.1; Bio4.2.2

Next Generation Science Standards:
Grades 9-12, Disciplinary Core Ideas/Practices/Cross-cutting concepts:

DCI	HS-PS1 Matter and Its Interactions HS-ETS1 Engineering Design HS-ESS3 Earth and Human Activity HS-LS1-5 From Molecules to Organisms: Structures and Processes HS-LS2-3,5 Ecosystems: Interactions, Energy, and Dynamics HS. Matter and Energy in Organisms and Ecosystems
Practices	Asking Questions and Defining Problems Planning and Carrying Out Investigations Analyzing and Interpreting Data Using Mathematics and Computational Thinking Constructing explanations
Cross-cutting concepts	Patterns Energy and matter Stability and change Structure and function

Time Requirements for *Elodea*

Preparation

Order *Elodea* at least 2 weeks before requested delivery date and ensure arrival at least 3 days before lab day.

- Prep time 3 days before lab day: ~10 minutes
- Prep time 1 day before lab day: ~ 20 minutes
- Prep time on lab day: ~45 minutes

Class time

Total time for all 3 experiments takes about 60 minutes assuming students read manual beforehand and answer any pre-lab questions. Activities should be completed with the guidelines provided.

- Activity 1: 10 minutes
- Activity 2: algae- 45 mins
- Activity 3: 20 minutes

After Class Time

Questions can be completed in about 30 minutes including discussion. Students are encouraged to compare their pre-lab assumptions to the actual results.

Materials (algae activities)
Per Group:
• Algae samples- Ulothrix from Carolina Biological supply
• Deionized, spring or well water
• 8 oz. clear plastic soda bottles or mini waterbottles (enough for each student or small groups)
• Miracle Grow™ Soluble All Purpose Plant Food
• Coffee filters
• Scissors
• Small rubber bands
• Luxi Meter app for Apple or Android smartphone or light meter
• Gram scale
• 500 ml graduated cylinders(one for each group)
- Bioenergy Education Initiative Growing Algae for Fuel
1/4 to 1/2 tsp (1-2 grams) of sugar weekly.
500 ml beakers

Materials(aquatic plant activities)
Included in the kit:
Order form for *Elodea* shipment
36 microscope slides and coverslips
2 bottles of potassium-iodine solution
7 mL pipet
Isopropyl alcohol
Bromothymol blue
25 vials with caps
Teacher's manual

Needed, but not supplied:
Rulers
Test tube racks
Colored pencils
Microscopes
Timers
100-mL graduated cylinders
Permanent markers
Electronic balance
Weight boats or paper
Scoopula
Stir rod
Paper towels

Safety
Ensure that students understand and adhere to safe laboratory practices when performing any activity in the classroom or lab. Demonstrate the protocol for correctly using the instruments and materials necessary to complete the activities, and emphasize the importance of proper usage. Use personal protective equipment such as safety glasses or goggles, gloves, and aprons when appropriate. Model proper laboratory safety practices for your students and require them to adhere to all laboratory safety rules.

Bromothymol blue and potassium iodine can stain skin and clothing so gloves and protective clothing should be worn when handling these substances. Isopropyl alcohol is poisonous if ingested and highly flammable. Keep away from heat sources or open flames. Refer to MSDS for proper disposal of these substances. Glass pipets, tubes, microscope slides, and coverslips are fragile and may break when handling. Ensure student handle these materials with care and any breakage should be cleaned up promptly and carefully.

Background Information
Photosynthesis is one of the most important biological processes on earth, and without it, life would not exist as we know it. Photosynthetic organisms transform light energy into chemical energy for cellular growth and other vital functions. In early Earth, our atmosphere was made up of mostly a dense, hot mixture of CO_2 and nitrogen, resulting in a barren landscape inhospitable to life. As the chemical composition of our atmosphere changed over millions of years, the environment became more hospitable to microorganisms. Photosynthesis dominated as the method of energy production and cellular growth mechanism due to the high concentration of CO_2 in the atmosphere. As these organisms started taking in CO_2 and water, they were expelling massive quantities of oxygen into our atmosphere, changing the chemical composition of the atmosphere to what we know today.

Algae has a pretty simple cellular makeup including a nucleus, cytoplasm, pyrenoids, chloroplasts, and a cell wall. The nucleus is the part of the cell that holds the genetic information. Chloroplasts are the photosynthetic organelles, where light energy is converted to chemical energy found near the cell wall. The pyrenoids are micro-compartments found in the chloroplasts, where photosynthesis occurs. Cytoplasm is the viscous liquid that fills the cell, made up of salts, proteins, and other nutrients. The cell wall is the protective outer membrane that holds the cell together.

$$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6H_2O + 6O_2$$

The generalized equation above expresses the overall inputs and outputs of photosynthesis. In plant cells, specifically the chloroplasts, the process is much more complicated. In the first set of reactions called the light-dependent reactions, light energy excites chlorophyll and releases an electron to the electron transport chain that ultimately reduces $NADP^+$ to NADPH. In result, the positive energy gradient (proton gradient) is used by an enzyme called ATP synthase to produce ATP that provides energy for further reactions. The chlorophyll is regenerated with electrons through the photolysis (light induced splitting) of a water molecule, which then releases oxygen. In the second set of light-independent reactions called the Calvin cycle, CO_2 is captured from the atmosphere and converted into various sugars including glucose and starch driven by the energy from the ATP generated by the first set of reactions.

The gas exchange mechanism between CO_2 and oxygen is a vital part of keeping our atmosphere, and everything in it, healthy and long lasting. When you think about where this process is occuring, your first thought would be trees, right? While terrestrial plants do account for about 28% of the oxygen produced in our ecosystem, the majority (70%) is produced from aquatic plant species and microorganisms such as kelp, phytoplankton , and algae. Therefore, the oceans are key at mitigating the potential harmful effects of global warming, an ever-increasing issue in our society.

Algae are a tiny but mighty force, although they are small, the energy that they have the potential to produce can have a big impact. Most species of microalgae can be used to produce a large amount of oil, which can be converted into biodiesel, a common source of biofuel. In fact, algae has up to 60 times more bioenergy potential than ethanol producing corn. So not only can algae help keep our planet healthy, but it can also serve a key role in the bioeconomy. Algae can be used for bioenergy, as previously stated, but may also be used as feed for certain species of fish which drives an entire other industry.

Preparation

11 days before lab day: Algae subculturing (detailed procedure) (Teacher only)

a. Half a bottle of medium
b. 5 pipettes(15 mL) worth of sample algae
c. Repeat with 2 bottles for every 3 experimental units(student bottles)
d. **Shake bottles every day**

Three days before lab day:

e. Place 4 *Elodea* sprigs in conditioned or spring water and store in a dark, cool environment.
f. Allow to sit for 48 hours

One day before lab day:

g. Pull 20 leaves from the light-deprived sprigs and place in a small container or clean petri dish.
h. Fill container with 70% isopropyl alcohol.
i. Cover container and keep in the dark environment for 24 hours
j. Repeat with light exposed sprigs and store in an area with ambient light. Keep away from direct exposure of sunlight to ensure the alcohol does not evaporate.
k. Algae planning hypothesis + setup

During lab:

l. Remove light and dark leaves from alcohol and place in cool tap water.

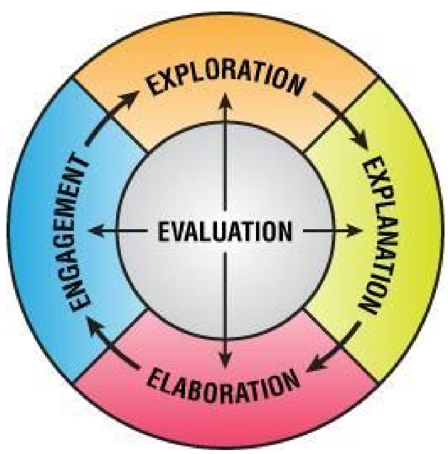
m. Dilute bromothymol blue solution using a 1:5 dilution.
n. Blow air gently into the solution using a straw until the solution turns yellow-green. Be sure to not suck in the solution.
o. Cut off 12 cm long sprigs for Activity 2 and 8 cm long sprigs for Activity 3 and place in spring or conditioned water.

Day 1: algae planning hypothesis + setup (45 mins)
Day 2: Elodia → L&D + Elodea pH(activity 3) (45 mins)
Day 3: Lecture + Elodea pH observation (Activity 3)
Day 4: Elodea leaves → Isopropyl(activity 1) (15 min)
Day 5: Algae Draining + microscope leaves and algae(activity one) (45 mins)

- One group goes to elodia scope(10-15 mins), and 9 make and look at algae slides(10-20 mins), as groups finish up with algae they then go to the drain station(10 mins)
- Once the group at the elodia station finishes, they move on to algae and then draining and the draining station goes to elodia one group at a time as they finish

Day 7: Algae mass and write up (45 mins)

Guiding the lesson using the 5E Learning Cycle



Engage (10 mins)
1. How much solar energy do plants take in per day in a 48 m^2 area?
2. Explain the importance of photosynthesis and the impact it has on everyday life.
3. Give brief demonstration of procedure

Explore (30 minutes)
1. Students will follow general procedure for each activity in order.
2. Teacher should check-in with every group during each activity and start probing questions related to observations.

Explain (10 minutes)

1. Students will explain amongst themselves why each step was taken in the procedure.
2. Students should think: What are the products of this process?

Elaborate (5 minutes)
1. Students will answer questions provided and may ask questions to the teacher.
2. The teacher must not directly say the answer but rather answer their question with a guiding question.
3. Students should give at least 2 examples of how humans can utilize this process and the effect it may have.

Evaluate (5 minutes)
1. Discuss results and if any answers are wrong, probe questions to lead to correct answer.

Differentiated Learning and Extension Activity ideas

- Each activity is very visual and hands-on.
- A portion of the activities can introduce a mathematical process that may help some students understand more clearly what is happening during the experiment.

Answers to Questions in the Student Guide

Supplemental Resources
- Carolina BioKits™: Photosynthesis

Name ______________________ **Student Guide**
Date _______________________

Overview:

Photosynthesis is the process by which plants transform light energy into chemical energy for growth, reproduction, and metabolism of the organism. Students will perform three experiments that address the roles that chlorophyll, light, CO_2, and oxygen play in photosynthesis. Students will work in small teams to perform each experiment. At the end of the experiments, the student will answer a series of questions to reinforce a deeper understanding of the core principles of photosynthesis followed by a short discussion with the instructor. The provided kit contains materials suitable for a classroom of 30 students to work in groups of 3. Questions are included in the kit as well as key background information, procedures, and pre-lab inquiries for students to think about while performing experiments.

Background:
Photosynthesis is one of the most important biological processes on earth, and without it, life would not exist as we know it. Photosynthetic organisms transform light energy into chemical energy for cellular growth and other vital functions. In early Earth, our atmosphere was made up of mostly a dense, hot mixture of CO_2 and nitrogen, resulting in a barren landscape inhospitable to life. As the chemical composition of our atmosphere changed over millions of years, the environment became more hospitable to microorganisms. Photosynthesis dominated as the method of energy production and cellular growth mechanism due to the high concentration of CO_2 in the atmosphere. As these organisms started taking in CO_2 and water, they were expelling massive quantities of oxygen into our atmosphere, changing the chemical composition of the atmosphere to what we know today.

Algae, has a pretty simple cellular makeup with a nucleus, cytoplasm, pyrenoids, chloroplasts, and a cell wall. Nuclei are the part of the cell that hold the genetic information. The pyrenoids are micro-compartments found in the chloroplasts, where photosynthesis occurs. Cytoplasm is the viscous liquid that fills the cell, made up of salts, proteins, and other nutrients. The cell wall is the protective outer membrane that holds the cell together.

$$6CO_2 + 12H_2O \rightarrow C_6H_{12}O_6 + 6H_2O + 6O_2$$

The generalized equation above expresses the overall inputs and outputs of photosynthesis. In plant cells, specifically the chloroplasts, the process is much more complicated. In the first set of reactions called the light-dependent reactions, light energy excites chlorophyll and releases an electron to the electron transport chain that ultimately reduces $NADP^+$ to NADPH. In result, the positive energy gradient (proton gradient) is used by an enzyme called ATP synthase to produce ATP that provides energy for further reactions. The chlorophyll is regenerated with electrons through the photolysis (light induced splitting) of a water molecule, which then releases oxygen. In the second set of light-independent reactions called the Calvin cycle, CO_2 is captured from the atmosphere and converted into various sugars including glucose and starch driven by the energy from the ATP generated by the first set of reactions.

The gas exchange mechanism between CO_2 and oxygen is a vital part of keeping our atmosphere, and everything in it, healthy and long lasting. When you think about where this process is occuring, your first thought would be trees, right? While terrestrial plants do account for about 28% of the oxygen produced in our ecosystem, the majority (70%) is produced from aquatic plant species and microorganisms such as kelp, phytoplankton , and algae. Therefore, the oceans are key at mitigating the potential harmful effects of global warming, an ever-increasing issue in our society.

Algae are a tiny but mighty force, although they are small, the energy that they have the potential to produce can have a big impact. Most species of microalgae can be used to produce a large amount of oil, which can be converted into biodiesel, a common source of biofuel. In fact, algae has up to 60 times more bioenergy potential than ethanol producing corn. So not only can algae help keep our planet healthy, but it can also serve a key role in the bioeconomy. Algae can be used for bioenergy, as previously stated, but may also be used as feed for certain species of fish which drives an entire other industry.

Procedure

Day 1:

I. Activity 1: Prepare algae samples with Sugar
   1. Make the fertilizer-water mix. In a one gallon container of distilled water, add 1 teaspoon of Miracle Grow™ Water Soluble All Purpose Plant Food. Shake the container in a circular motion to dissolve the fertilizer crystals. One gallon contains enough mix to make about 16 algae growing bottles.
   2. Next, students should fill their bottles about ¾ full with the fertilizer water mix (approximately 6 ounces or 177 mL).
   3. Have the students add 0.75 grams of sugar to the sugar variable bottles
   4. The students should then shake the algae samples to homogenize them as much as possible and add 15 mL of the medium with algae in it to their bottles
   5. Have students place the caps on their bottles, then shake the bottle and thoroughly mix the contents.
   6. Students should then remove the cap from the bottles. Have them place 2”x 2” pieces of coffee filter paper over the tops of their bottles and secure them with small rubber bands. This filter will allow for air transfer. **Save the caps.**

II. Activity 2: Filtering algae samples.
   1. Have students weigh the coffee filter they will be using to separate the algae. Record this number.
   2. Place the coffee filter over the top of a 16 ounce (473 ml) drink cup or 600 mL graduated cylinder and secure it with a rubber band around the lip.
   3. Slowly pour the algae culture into the coffee filter until the filter is full. Wait until the water drains through. Continue adding the algae culture until all of it is filtered. The green slime on the filter is algae. The filter process is slow and can take up to an hour.
   4. Carefully remove the filter paper and set it on a paper towel.
   5. Using a graduated cylinder, have students measure the volume (in milliliters) of liquid they recovered in the cup. Record this number. Discard this liquid and shake out whatever is remaining in the cylinder. Since they won’t be using this liquid for growing any more algae, you do not need to worry about contamination between cultures. Students or groups can share cylinders.
   6. Allow the filters with the algae to dry. You can do this by placing them in an oven at very low heat for a few hours or by leaving the filter on a counter over a couple of days.

III. Activity 3: Carbon dioxide

1. Dilute bromothymol blue at a 1:5 ratio (20 mL of BB and 80 mL of water)
2. Blow into the balloon and insert tubing into the mouthpiece. Secure with rubber band and pinch tubing to keep air from flowing out.
3. Slowly allow air to flow through the tube into the solution until solution turns yellow/orange.
4. Place an 8 cm long sprig into a plastic vial and fill until overflowing with the diluted bromothymol blue solution, then cap.
5. In a separate vial, fill with the diluted bromothymol blue solution until overflowing and cap.
6. Place in sunlight or light bank.
7. Record the color of the contents of the vials before and after, for each sample and the blank.

Day 2

IV. Activity 1: Chlorophyll and Chloroplasts

1. Obtain two microscope slides and mark one with “LD” for light-deprived and “LE” for light-exposed in the lower corners of the slides.
2. Obtain a leaf from either the light-deprived or light exposed samples then place on the microscope slide.
3. Place a couple drops of DI water on the leaf and cover with a coverslip.
4. Place under the microscope and analyze under high power.
5. Sketch a picture of what you see, especially around the main vein.
6. Repeat with the remaining sample.
7. With a different leaf repeat steps 1-6 except instead of DI water, place 3 drops of the potassium iodine solution on sample and let sit for 10 minutes before analyzing.

V. Activity 2: Algae observation

1. Pull a small sample of the algae provided and place on microscope slide.
2. Add 2 drops of water onto the slide and cover with a coverslip.
3. Change magnification to at least 20 times magnification.
4. Sketch in detail what is shown in the microscope.
5. With the word list provided, try and label each part of the algae cell.

VI. Activity 3: Determining Total Yield and Concentration of Algae

Part 1: Total mass of algae

1. Determine the dry weight of the algae on the filter paper.
2. When the filter paper containing the collected algae has completely dried, weigh it again.
3. Then, subtract the weight of the unused filter paper from the weight of the used filter paper. This will give you the weight of the collected algae.

For example, if the initial weight of the filter paper was 0.25 grams, but after filtering and drying, the filter weighed 3 grams, the weight of the algae grown would be 2.75 grams (3 g – 0.25 g = 2.75 g). The total dry weight yield of the algae would be 2.75 grams.

Part 2: Find the concentration of algae per liter

.

1. Divide the dry weight of your algae (in grams) by the volume (in ml) of algae water you collected in your cup, and multiply that by the number by 1,000 ml.

For example, if you recovered 2.75 g dry weight of algae and you collected 414 ml (14 oz.) of algae water in your cup, you would divide 2.75 g by 414 ml and multiply by 1,000. (2.75 g ÷ 414 ml) x 1,000 = 6.64 g/ml.

Chart these numbers across the class and compare the outcomes. Were there differences in algae growth? Ask students to hypothesize why the differences occurred. Graph change in density/time comparison

Questions :

1. Did algae with or without sugar work?

2. Why do you think this was the case?

3. Some very high yielding algae is found to produce biodiesel at a rate of 136,900 L/ha/year. If the US uses an average of 142.86 billion gallons of gasoline annually and there are 3.78 liters in a gallon, how many hectares of algae would we need to grow to power the cars of the US for a year using only biodiesel?

4. Where does photosynthesis occur in a plant cell? Which molecule is necessary to absorb light needed to fuel photosynthesis?

5. Explain the differences between monosaccharides, disaccharides, and polysaccharides. Explain the relationship between glucose and starch molecules, and their functions in plants.

6. What did this experiment reveal about the role of light and chlorophyll in the process of photosynthesis?

7. Compare color of two solutions with the pH indicator. What happened?

8. What is the role of carbon dioxide in photosynthesis?

9. What would happen if the vials were placed in a dark room?
10. What is the purpose of the vial with no *Elodea*?

11. What acid/base is forming/consumed related to carbon dioxide?

Observations

Light with Water	**Light with Iodine**
Dark with Water	**Dark with Iodine**
Algae	**Word Bank** Pyrenoids Chloroplasts Cell wall Nucleus Cytoplasm

Day 2 Checklist

____Elodea Microscope and Sketches

____Algae Microscope and Sketches

____Weighing out dried algae mass

____Determine yield and concentration (g/L) of algae

____Answer questions