Algae Balls

Introduction

Biofuel is an energy source derived from biological materials, most commonly, plants.


What is the biological process that plants use to create biofuels? ________________________________

What is the energy source for this process? ________________________________

Where do the carbon molecules contained in the biofuel come from? ________________________________

Over the past decade, interest and controversy about growing microalgae for biofuels has surged. These unicellular photosynthetic organisms appear to be an attractive alternative to fossil fuels because of their ability to grow solely on sunlight and carbon dioxide from the atmosphere without taking up valuable farmland. Algae growers harvest the lipids from their algae and convert the lipids into biodiesel. However, there are still significant hurdles in both engineering and biology before large-scale microalgae farms can become economically viable. An important component to their future success is a thorough understanding of algal metabolism and physiology.

What do you already know?? List five facts that you already know about photosynthesis.

1. ______________________________________________________________________________________
2. ______________________________________________________________________________________
3. ______________________________________________________________________________________
4. ______________________________________________________________________________________
5. ______________________________________________________________________________________

As you read below, underline anything that you listed above.

In this lab, you will measure the change in carbon dioxide concentration when algae are placed in light and the effect of light on photosynthesis. Photosynthesis is the process by which primary producers use CO$_2$ to make carbohydrates. Some of the carbohydrates produced are used by the algae for respiration. Other carbohydrates are used in other metabolic pathways to create carbon-containing compounds necessary for life. Some will be converted into the lipids harvested for biodiesel.

Chemically, you can summarize photosynthesis as follows:

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

This equation glosses over the many chemical intermediates on the path from carbon dioxide to glucose. In the **light-dependent reactions**, photons of light excite electrons within chlorophyll molecules. The excited electrons pass through the electron transport chain and ultimately wind up with the electron carrier NADP$^+$. This process generates ATP and NADPH, both of which are required to power the light-independent reactions, called the **Calvin-Benson cycle**, that convert CO$_2$ into carbohydrates. Each reaction in the Calvin-Benson cycle is catalyzed by a specific **enzyme**. Factors such as temperature, light, and the presence of pollutants, directly influence enzyme activity and affect how quickly these reactions can take place.

In this lab, you will measure photosynthesis using single-celled algae immobilized in balls of calcium alginate. Calcium alginate consists of negatively charged alginate molecules that can be cross-linked by positively charged calcium ions when in solution. This creates a gelatinous polymer that will be used to make the algae balls.

**Measuring pH**

As your algae photosynthesize, they will be using carbon dioxide and producing oxygen and glucose. But we can’t see these chemicals – so how do we know anything is actually happening?

There are machines that can analyze the composition of gases in a sample, but these tools are expensive and calibrating them can be very difficult. Instead, we will be using a technique called **titration** in order to watch in real-time as our algae balls cause an indicator solution to change color from yellow to purple, due their uptake of CO$_2$.

When carbon dioxide is dissolved in water, it changes the pH (a.k.a. acidity) of the solution by producing carbonic acid, H$_2$CO$_3$. The more CO$_2$ there is in the solution, the more H$_2$CO$_3$ is produced, which decreases the pH (lower pH means more acidic). Conversely, if CO$_2$ is taken out of the solution, less H$_2$CO$_3$ is around, and the pH increases.

*Draw the pH scale and label where acids and bases are indicated.*
There are a number of different chemicals that change color in response to changes in pH. These are called **chemical indicators**. The indicator that we will be using is called **hydrocarbonate**. This indicator is sensitive to changes within the pH range that we are measuring.

*In which tube do you think the concentration of H⁺ ions is higher? ________________________________*

*What evidence do you have to support your answer? _______________________________________________
______________________________________________________________________________________
______________________________________________________________________________________

There are two main ways to correlate the color of the hydrocarbonate indicator solution in your experiment to a specific pH value. You can estimate the unknown solution’s pH by comparing the color of the sample to the color of a set of standards of known pH values.

Alternatively, a more accurate method is to use a machine called a **spectrophotometer**. This machine works by passing a beam of light through a solution and measuring how much light of a particular wavelength is absorbed by the solution. For this experiment, we would measure the absorbance of our indicator solution at a wavelength of 550 nanometers. High absorbance corresponds to high pH, while low absorbance corresponds to low pH.

http://www.vernier.com/products/sensors/spectrometers/visible-range/svis-pl/

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**Change in color of the hydrocarbonate indicator solution in response to changes in pH**

![Image of hydrocarbonate indicator solution changes in color from low pH to high pH.](image-url)
Algae Balls
Lab Protocol

Materials: Check your workstations to make sure all supplies are present before beginning the lab.

<table>
<thead>
<tr>
<th>Student Workstation:</th>
<th>Common Workstation:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• 6 graduated transfer pipettes</td>
<td>• Tap water</td>
</tr>
<tr>
<td>• 2 small beakers or cups</td>
<td></td>
</tr>
<tr>
<td>• Tea strainer or mesh filter</td>
<td></td>
</tr>
<tr>
<td>• Plastic spoon</td>
<td></td>
</tr>
<tr>
<td>• 6 2.0 mL microcentrifuge tubes</td>
<td></td>
</tr>
<tr>
<td>• Sodium alginate (2%)</td>
<td></td>
</tr>
<tr>
<td>• Calcium chloride (2%)</td>
<td></td>
</tr>
<tr>
<td>• 1X hydrocarbonate indicator solution (orange)</td>
<td></td>
</tr>
<tr>
<td>• concentrated algae solution</td>
<td></td>
</tr>
<tr>
<td>• dH2O</td>
<td></td>
</tr>
<tr>
<td>• Permanent marker</td>
<td></td>
</tr>
<tr>
<td>• Tap water</td>
<td></td>
</tr>
</tbody>
</table>

Procedure:

Preparing Algae Balls

1. Check your station to make sure you have all materials.

2. Use a marker to label the following:

   • 2 beakers or cups “calcium chloride”
   • 1 beaker or cup “water”
   • 1 plastic transfer pipette “alginate”
   • 1 microcentrifuge tube “algae mix”
   • 1 microcentrifuge tube “water mix”
   • 1 microcentrifuge tube “algae/light”
   • 1 microcentrifuge tube “algae/dark”
   • 1 microcentrifuge tube “water/light”
   • 1 microcentrifuge tube “water/dark”

   Check to make sure you have correctly labeled everything and put these aside until you need them

3. Add approximately 20 mL of calcium chloride to one of the cups labeled calcium chloride.

   ! Tip: Alginate is very goopy. Pipette slowly and stir carefully to avoid trapping air bubbles in your solutions.

4. Using the “alginate” pipette, transfer 1.0 mL of alginate into the “algae mix” tube. Set the “alginate” pipette aside, you will use it again later.

5. Using a clean transfer pipette, add 1.0 mL of the concentrated algae solution to the “algae mix” tube. Use the pipette to gently mix the alginate and algae until the mixture is an even green color
6. Using the same pipette (from step 5), carefully suck up from the “algae mix” tube. Slowly squeeze the bulb of the transfer pipette until a drop appears at the tip of the pipette. Let the drop fall into the “calcium chloride” beaker. You should see the drop form a green sphere or algae ball. Continue until you have used up all the algae mix.

⚠️ Tip: While you’re making the balls, gently swirl the beaker of CaCl₂ so the algae balls don’t clump together.

7. Allow the algae balls to harden in the calcium chloride for 5 minutes.

8. After hardening, pour the algae balls and calcium chloride through a tea strainer into the other beaker labeled “calcium chloride”. Remove the tea strainer and set both “calcium chloride” beakers aside, they will be reused to make the water balls.

9. Label a clean beaker “water”. Hold the tea strainer containing the algae balls over the beaker and gently rinse the algae balls with tap water.

10. Find the “algae/dark” and “algae/water” microcentrifuge tubes

11. Using the plastic spoon, evenly divide the algae balls between the two tubes.

STOP You are now going to repeat the process using water instead of algae.

Preparing Water Balls

1. Use the “alginate” pipette to transfer 1.0 mL of alginate into the microcentrifuge tube.

2. Using a clean transfer pipette, add 1.0 mL of water to the “water mix” tube. Use the pipette to gently mix the alginate and water until thoroughly mixed.

3. Using the same pipette (from step 2), carefully suck up the “water mix”. Slowly add drops to the cup containing calcium chloride.

⚠️ Important: It is very difficult to see the water balls. Count your drops!

4. Continue until you have used up all the “water mix.” Gently swirl the beaker to prevent the water balls from fusing together.

5. Allow the water balls to harden in the calcium chloride for 5 minutes.

6. After hardening, pour the water balls and calcium chloride through a tea strainer into the other “calcium chloride” beaker. Remove the tea strainer and set the beaker aside.

7. Hold the tea strainer containing the water balls over the “water” beaker and gently rinse the water balls with tap water.

8. Label one microcentrifuge tube “water/light” and another tube “water/dark.”

9. Using the plastic spoon, evenly divide the water balls between the two tubes. Try to have approximately the same number of water balls and algae balls in all the tubes.
Once you’ve made both algae and water balls, follow the instructions below:

**Determining the pH of the experimental microcentrifuge tubes**

1. Use a clean transfer pipette to fill all four tubes with orange 1X indicator solution.

2. Close the tubes and gently invert them to mix. Let them sit for 3 minutes to allow the indicator solution to equilibrate.

3. Compare the color of the indicator in each tube to known standards or to the color chart provided by your teacher. Estimate the pH to the nearest tenth and record the pH values in the data table below.

4. Place one algae tube and one water tube under a light source for approximately 30 minutes.

5. Place one algae tube and one water tube in the dark for approximately 30 minutes.

6. When the 30-minute light incubation is complete, measure the pH of each sample and record the pH values in the data table.

7. Put the tubes back under the light source and incubate overnight. Record the pH after 24 hours.

**Table 1. Data Table: Change in pH as determined by hydrocarbonate indicator**

<table>
<thead>
<tr>
<th>Time</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Algae/ Light</td>
</tr>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td></td>
</tr>
</tbody>
</table>
Algae Balls
Pre-Lab Questions

Directions: After reading through the introduction and protocol for the Algae Balls lab, answer the questions below.

1. Briefly summarize the process of photosynthesis.

2. Explain how the indicator is used to measure the process of photosynthesis. (Hint: your answer to question #1 might be useful)

3. What is the purpose of an experimental control?

4. Which tube(s) will be your control in this experiment? Explain how the tube(s) you chose acts as a control.

5. Describe what you think will happen in each tube during the 30-minute incubation. What about after 24 hours?

Table 2: Result Predications

<table>
<thead>
<tr>
<th>Time</th>
<th>Predictions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Algae/ Light</td>
</tr>
<tr>
<td>30 min</td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td></td>
</tr>
</tbody>
</table>
Algae Balls  
Post-Lab Questions

**Directions:** After completing the Algae Balls lab, answer the questions below.

1. Did the results match your prediction from the pre-lab questions?

2. In general, you would assume that increasing the amount of light increases the rate of photosynthesis. Explain how you measured the increase in photosynthesis in this experiment.

3. Predict what you think would happen to the color of the indicator in the algae/dark if you put it in the light for 24 hours. Predict what change you might see in the indicator in the algae/light tube if you put it in the dark for 24 hours. Explain your predictions.

4. If the algae were boiled before making algae balls, and you repeated the experiments, would you expect that the results obtained would be the same? Explain your answer.

5. Imagine you could design your own experiment using algae balls to test some aspect of photosynthesis. Form a hypothesis and describe how you would design your experiment.