

## Algaculture & Biofuels: Experiments With Algae

### Objective:

1. Students will be able to identify the resources algae need to survive and grow.
  - Students will be able to describe how algae responds to light
  - Students will be able to describe how algae respond to a nitrogen source
  - Students will be able to describe how algae respond to presence of CO<sub>2</sub>
  - Students will be able to describe how algae respond to heat

**Description:** In this activity, you will conduct one or more experiments to learn about how algae survive and grow. You will take on the role of a scientist whose job is to determine the resources needed to successfully raise algae for biofuel production. You will need to determine how algae responds to light, heat, CO<sub>2</sub>, or nitrogen fertilizer in order to determine what resources will be needed for a successful algaculture system.

Each experiment will be accompanied by a list of materials, a procedure, and a series of questions you will need to answer. In conducting each experiment, you will use the scientific method. The steps of the scientific method are:

1. **Question:** What question are you trying to answer?
2. **Research:** What information already exists that may answer the question? How trustworthy is this information? What information is missing?
3. **Hypothesis:** What answer do you think you will come up with? Will it confirm or conflict with information you found in your research?
4. **Experiment:** Collect data to test your hypothesis. What kind of data do you need? How will you get it?
5. **Conclusion:** Analyze the data and determine whether your hypothesis was correct, partially correct, or incorrect, and why. Did you answer the question you set out to answer? Is more data needed?

## Algaculture & Biofuels: Exploration

### Experiments With Algae- Photosynthesis

#### Experiment 1: Photosynthesis

**Description:** In this experiment, you'll be investigating how microalgae respond to the presence of a light source. Microalgae are tiny microscopic plant cells, so they should behave similarly to other plants. You'll be determining whether this is true by testing two samples of algae under different lighting conditions; one with light, and one without. If algae cells respond to light as other plant cells do, they will use the sunlight in a process called photosynthesis. Photosynthesis is the process of converting light energy from the sun and carbon dioxide from the air into chemical energy which is stored in bonds between atoms in organic molecules such as carbohydrates (sugars), proteins, and lipids (oils/fats). Some waste products are generated, such as oxygen gas. In order to detect whether photosynthesis is occurring, we will need to determine whether the microalgae are using up carbon dioxide and generating oxygen as a waste product. We will do this by using a pH indicator. When the microalgae use up carbon dioxide and generate oxygen, the pH in the solution will change. By using a pH indicator, we will be able to see whether the pH has changed, and thus determine whether the carbon dioxide was used up and replaced with oxygen, which will tell us whether photosynthesis has occurred.

#### Materials:

- ✓ Algae beads
- ✓ Plastic spoon
- ✓ Small kitchen strainer
- ✓ Hydrogen carbonate pH indicator
- ✓ 2 small vials or cuvettes, approximately 5 mL, with caps
- ✓ Strong light source
- ✓ Opaque paper
- ✓ Clear tape

#### Procedure:

1. Write the question you are trying to answer with this experiment:

#### Question:

2. Use a computer, mobile device, library, or other resources to answer the following questions:
  - What is photosynthesis?
  
  
  
  
  
  
  
  
  
  
  - What resources are used up by plants in photosynthesis?

- What products and byproducts are produced by plants in photosynthesis?
  
  - Do microalgae use photosynthesis like other plants?
3. Considering the information you uncovered in your research, write the answer you expect to find to your original question.

**Hypothesis:**

4. Experimental Procedure:
- a) Remove algae beads from the test tube by pouring through the kitchen strainer.
  - b) Use the plastic spoon to place an equal number (approximately 10) of algae beads in two of the three vials/cuvettes.
  - c) Fill all three vials to the top with hydrogen carbonate pH indicator solution.
  - d) Cap each vial securely.
  - e) Place a small strip of tape on each vial, and use a marker or pen to write "Sample A" and "Sample B" on the two vials with algae beads, and "Sample C" on the vial with only the indicator solution.
  - f) In the Data Collection Table (below), note that Sample A will be in the "algae + light" treatment group and Sample B will be the "no light" treatment group. Sample C will be the "control" group.
  - g) In the Data Collection Table (below), note the before-test color of the pH indicator solution in each of the three samples. Use the provided numbered color chart.
  - h) Cut a strip of opaque paper that is as wide as the Sample B vial is tall
  - i) Wrap strip of paper around the Sample B vial, and secure it with tape so it doesn't fall off.
  - j) Place both samples 10 cm from the light source
  - k) Turn the light source on
  - l) Wait approximately 30 minutes
  - m) Turn off the light source
  - n) Remove the paper from Sample B.
  - o) In the Data Collection Table (below), note the color of the pH indicator solution in each sample. Use the provided numbered color chart.
  - p) In the Data Collection Table, subtract the "after" color from the "before" color to find the color change. A positive color change means CO<sub>2</sub> was used up by the algae.
  - q) Pour off the indicator solution through the kitchen strainer, rinse the algae beads with distilled water, place them back in the test tube, fill the tube with distilled water and cap it.

Data Collection Table

	Example	Sample A	Sample B	Sample C
Treatment Group	No light			
Color before test (Number)	4			
Color after 30 min. (Number)	5			
Color Change (After minus before)	1			

# pH Indicator

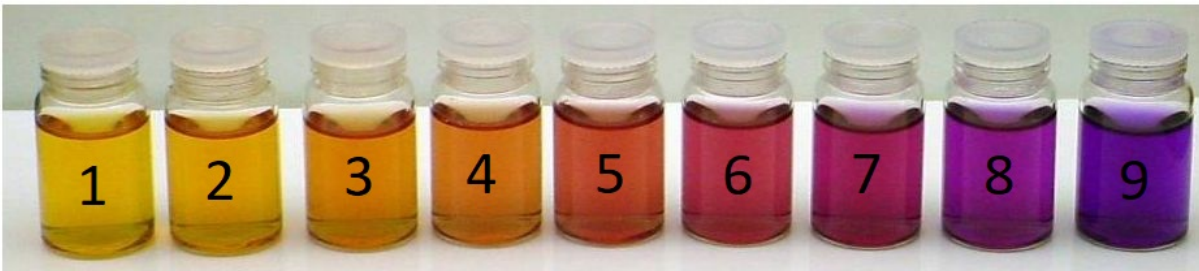


Increasing CO<sub>2</sub>  
in indicator



Atmospheric  
CO<sub>2</sub> level

Decreasing CO<sub>2</sub>  
in indicator



pH indicator is used to measure carbon dioxide levels in aquatic systems.

It is red in equilibrium with atmospheric air.

It becomes more orange/yellow with increased carbon dioxide levels.

It changes from red through magenta to deep purple as carbon dioxide is removed.

**Follow up Questions:**

1. For Sample A, did the concentration of CO<sub>2</sub> increase, decrease, or stay the same?
  - a. How do you know?
  
2. For Sample B, did the concentration of CO<sub>2</sub> increase, decrease, or stay the same?
  - a. How do you know?
  
3. For Sample C, did the concentration of CO<sub>2</sub> increase, decrease, or stay the same?
  - a. How do you know?
  
4. Based on these results, can you tell whether microalgae use photosynthesis when light is present?
  
  
  
  
  
  
  
  
  
  
5. Did photosynthesis take place with the algae in Sample A that were given light? How do you know?
  
  
  
  
  
  
  
  
  
  
6. Did this confirm or conflict with your hypothesis?
  
  
  
  
  
  
  
  
  
  
7. What other information would be interesting or useful to know?

**Algaculture & Biofuels:**  
**Experiments With Algae- Light Intensity and Photosynthesis**

**Experiment 2 – Light Intensity and Photosynthesis**

**Description:** In this experiment, you'll be investigating how microalgae respond to variations in light intensity. Since microalgae are microscopic plant cells, they use photosynthesis like other types of plant cells. Photosynthesis is the process of converting light energy from the sun and carbon dioxide from the air into chemical energy which is stored in bonds between atoms in organic molecules such as carbohydrates (sugars), proteins, and lipids (oils/fats). Some waste products are generated, such as oxygen gas. In order to detect whether light intensity affects microalgae photosynthesis, we will need to determine how much carbon dioxide is being used up by the algae under different lighting intensities. We will do this by using a pH indicator. When the microalgae use up carbon dioxide and generate oxygen, the pH in the solution will change. By using a pH indicator, we will be able to see how much the pH has changed under different light intensities, and thus determine how much carbon dioxide has been used up. If more carbon dioxide is used up in a given time period, it will mean that photosynthesis is happening more quickly.

**Materials:**

- ✓ Algae beads
- ✓ Plastic spoon
- ✓ Small kitchen strainer
- ✓ Hydrogen carbonate pH indicator
- ✓ 6 small vials or cuvettes, approximately 5 mL, with caps
- ✓ Strong light source
- ✓ Clear tape

**Procedure:**

1. Write the question you are trying to answer with this experiment:

**Question:**

2. Use a computer, mobile device, library, or other resources to answer the following questions:
  - What is photosynthesis?
  
  
  - What resources are used up by plants in photosynthesis?
  
  
  - What products and byproducts are produced by plants in photosynthesis?

- Will light intensity affect the rate of photosynthesis in microalgae?
3. Considering the information you uncovered in your research, write the answer you expect to find to your original question.

**Hypothesis:**

4. Experimental Procedure:
- a) Remove algae beads from the test tube by pouring through the kitchen strainer.
  - b) Use the plastic spoon to place an equal number (approximately 10) of algae beads in all five vials/cuvettes.
  - c) Fill all five vials (containing algae beads) to the top with hydrogen carbonate pH indicator solution.
  - d) Cap each vial securely.
  - e) Place a small strip of tape on each vial, and use a marker or pen to label each sample (Sample A – Sample E).
  - f) In the Data Collection Table (below), note which treatment group each sample is in. A treatment group will be the distance from the light source, starting with 10 cm, and then every five cm up to 35 cm.
  - g) In the Data Collection Table (below), note the before-test color of the pH indicator solution in each of the five samples. Use the provided numbered color chart.
  - h) Place one sample 10 cm from the light source, another at 15 cm, another at 20 cm, and so on until all five samples are placed at 5 cm increments from the light source.
  - i) Turn the light source on
  - j) Wait approximately 30 minutes
  - k) Turn off the light source
  - l) In the Data Collection Table (below), note the color of the pH indicator solution in each sample. Use the provided numbered color chart.
  - m) In the Data Collection Table, subtract the “after” color from the “before” color to find the color change. A larger color change means more CO<sub>2</sub> was used up by the algae.
  - n) Pour off the indicator solution through the kitchen strainer, rinse the algae beads with distilled water, place them back in the test tube, fill the tube with distilled water and cap it.

Data Collection Table

	Example	Sample A	Sample B	Sample C	Sample D	Sample E
<b>Treatment Group</b> (cm from light source)	5 cm					
<b>Color before test</b> (Number)	5					
<b>Color after 30 min.</b> (Number)	9					
<b>Color change</b> (After minus before)	4					

# pH Indicator



Increasing CO<sub>2</sub>  
in indicator



Atmospheric  
CO<sub>2</sub> level

Decreasing CO<sub>2</sub>  
in indicator



pH indicator is used to measure carbon dioxide levels in aquatic systems.

It is red in equilibrium with atmospheric air.

It becomes more orange/yellow with increased carbon dioxide levels.

It changes from red through magenta to deep purple as carbon dioxide is removed.



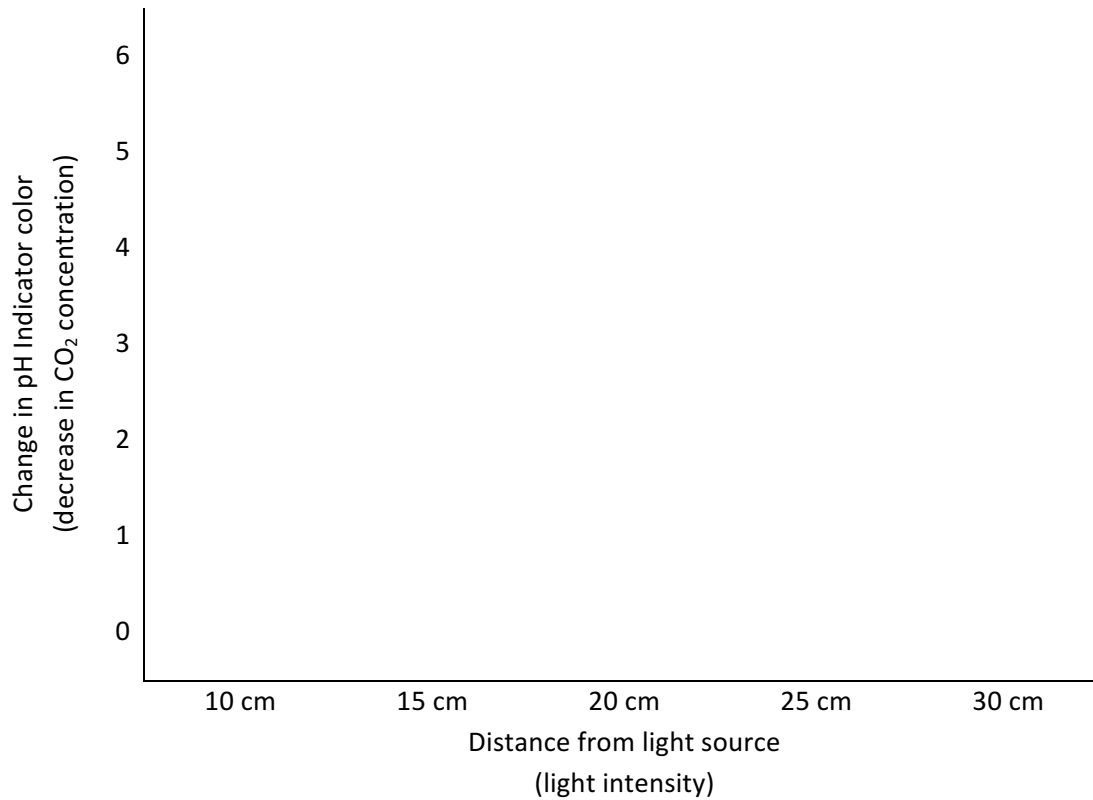
**Follow up questions:**

1. Using the blank chart below, plot the CO<sub>2</sub> changes as a function of light intensity (distance from the light source). Draw lines connecting the points to form a line graph.

Table 1.

*Change in CO<sub>2</sub> concentration as a function of light intensity*

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2. Can you see a trend between light intensity and how much CO<sub>2</sub> was used? What trend, if any, do you see?
3. Based on these results, what can you say about the effect of light intensity on the rate of photosynthesis in microalgae? Did this confirm your hypothesis? What other information might be useful or interesting?

**Algaculture & Biofuels:  
Experiments With Algae- Light Wavelengths (colors)**

**Experiment 3 – Light Wavelength (Color)**

**Description:** In this experiment, you'll be investigating how microalgae respond to different light wavelengths (colors). Since microalgae are microscopic plant cells, they use photosynthesis like other types of plant cells. Photosynthesis is the process of converting light energy from the sun and carbon dioxide from the air into chemical energy which is stored in bonds between atoms in organic molecules such as carbohydrates (sugars), proteins, and lipids (oils/fats). Some waste products are generated, such as oxygen gas. In order to detect whether light wavelength affects microalgae photosynthesis, we will need to determine how much carbon dioxide is being used up by the algae under different light wavelengths. We will do this by using a pH indicator. When the microalgae use up carbon dioxide and generate oxygen, the pH in the solution will change. By using a pH indicator, we will be able to see how much the pH has changed under different light wavelengths, and thus determine how much carbon dioxide has been used up. If more carbon dioxide is used up in a given time period, it will mean that photosynthesis is happening more quickly.

**Materials:**

- ✓ Algae beads
- ✓ Plastic spoon
- ✓ Small kitchen strainer
- ✓ Hydrogen carbonate pH indicator
- ✓ 4 small vials or cuvettes, approximately 5 mL, with caps
- ✓ Strong light source
- ✓ Clear tape
- ✓ Strips of red, green, and blue light filter film.

**Procedure:**

1. Write the question you are trying to answer with this experiment:

**Question:**

2. Use a computer, mobile device, library, or other resources to answer the following questions:
  - What is photosynthesis?
  
  
  - What resources are used up by plants in photosynthesis?
  
  
  - What products and byproducts are produced by plants in photosynthesis?

- Will the wavelength (color) of light affect the rate of photosynthesis in microalgae?
3. Considering the information you uncovered in your research, write the answer you expect to find to your original question.

**Hypothesis:**

4. Experimental Procedure:
- a) Remove algae beads from the test tube by pouring through the kitchen strainer.
  - b) Use the plastic spoon to place an equal number (approximately 10) of algae beads in all four vials/cuvettes.
  - c) Fill all four vials with algae beads to the top with hydrogen carbonate pH indicator solution.
  - d) Cap each vial securely.
  - e) Place a small strip of tape on each vial, and use a marker or pen to label each sample (Sample A – Sample D).
  - f) In the Data Collection Table (below), note which treatment group each sample is in. A treatment group will be the color of the light: Red, Green, Blue, or Control (no color filter).
  - g) In the Data Collection Table (below), note the before-test color of the pH indicator solution in each of the four samples. Use the provided numbered color chart.
  - h) Cut a strip of each color filter material that is as wide as a vial is tall, and just long enough to wrap around a vial once without overlapping or leaving a gap.
  - i) Wrap one vial in red film, one in green film, and one in blue film, securing each with a small strip of tape.
  - j) Place each vial 10 centimeters from the light source and close to the other vials.
  - k) Turn on the light source.
  - l) Wait approximately 30 minutes
  - m) Turn off the light source
  - n) In the Data Collection Table (below), note the color of the pH indicator solution in each sample. Use the provided numbered color chart.
  - o) In the Data Collection Table, subtract the “after” color from the “before” color to find the color change. A larger color change means more CO<sub>2</sub> was used up by the algae.
  - p) Pour off the indicator solution through the kitchen strainer, rinse the algae beads with distilled water, place them back in the test tube, fill the tube with distilled water and cap it.

## Data Collection Table

	Example	Sample A	Sample B	Sample C	Sample D
<b>Treatment Group</b> (Filter color or Control)	Red				
<b>Color before test</b> (Number)	4				
<b>Color after 30 min.</b> (Number)	6				
<b>Color change</b> (After minus before)	2				

# pH Indicator

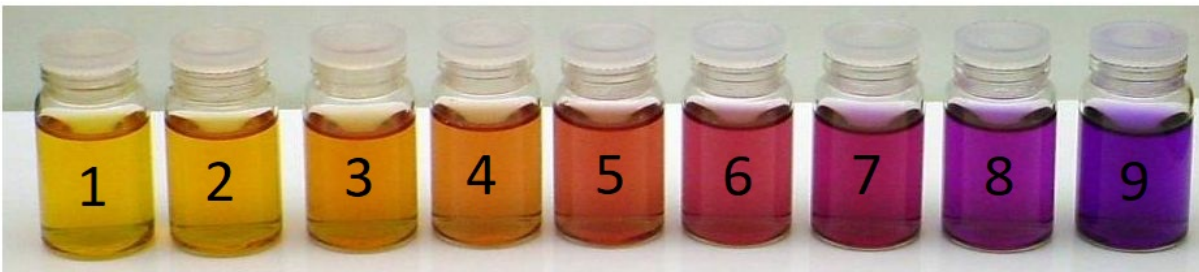


Increasing CO<sub>2</sub>  
in indicator



Atmospheric  
CO<sub>2</sub> level

Decreasing CO<sub>2</sub>  
in indicator



pH indicator is used to measure carbon dioxide levels in aquatic systems.

It is red in equilibrium with atmospheric air.

It becomes more orange/yellow with increased carbon dioxide levels.

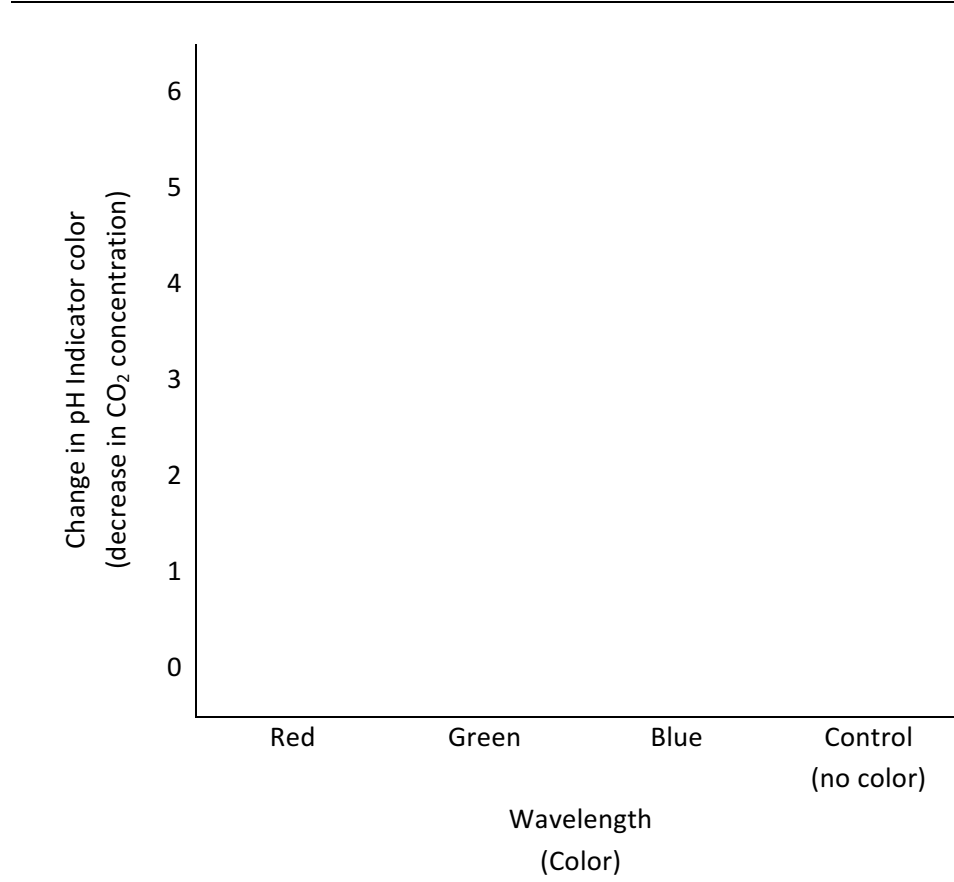
It changes from red through magenta to deep purple as carbon dioxide is removed.

**Follow up questions:**

1. Using the blank chart below, plot the CO<sub>2</sub> changes as a function of wavelength (color). Draw vertical columns to each point to make a bar graph.

Table 1.

*Change in CO<sub>2</sub> concentration by light wavelength*



2. Can you tell whether the color of light affected the rate of photosynthesis? How can you tell?
3. Based on these results, what can you say about how microalgae respond to different colors of light? Did this confirm your hypothesis? What other information might be useful or interesting?

**Algaculture & Biofuels:**  
**Experiments With Algae- Nutrition Enrichment**

**Experiment 4 – Nutrient Enrichment**

**Description:** In this experiment, you'll be investigating how microalgae growth rates are affected by the presence of different amounts of nutrients. Microalgae are microscopic plant cells, and like other plant cells they require various nutrients in order to survive and grow. These nutrients are used to store energy from the sun, build cell structures, and various other purposes. The primary nutrients needed to sustain algae growth are nitrogen and phosphorus, but trace amounts of various other minerals and metals are also needed. In this activity, you'll experiment with different concentrations of a common all-purpose fertilizer (which contains nitrogen, phosphorus, and many other nutrients) to determine how much of these nutrients are needed to maximize algae growth.

**Materials**

- ✓ Live microalgae culture (chlorella, ankistrodesmus, etc.)
- ✓ All-purpose water-soluble fertilizer granules (MiracleGro or equivalent)
- ✓ Several clean containers or beakers of equal size
- ✓ Non-chlorinated water (spring water from a natural source or aquarium water)
- ✓ 6 x 100 mL beakers or other similarly sized clear containers
- ✓ Strong light source
- ✓ Clear tape

**Procedure:**

1. Write the question you are trying to answer with this experiment:

**Question:**

2. Use a computer, mobile device, library, or other resources to try to answer the following questions:
  - What resources are needed by plant cells to promote growth?
  
  
  - Where do microalgae get nutrients?
  
  
  - What concentration of nutrients is required to maximize algae growth?

3. Considering the information you uncovered in your research, write the answer you expect to find to your original question.

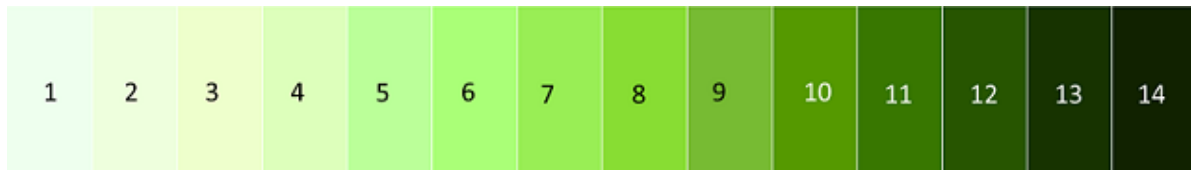
**Hypothesis:**

4. Experimental Procedure:

- a) Using five of the beakers or other containers, mix 100 mL of fertilizer/non-chlorinated water mixture in each of the following concentrations
  - Regular strength (follow package directions)
  - Double strength
  - $\frac{1}{2}$  strength
  - $\frac{1}{4}$  strength
  - $\frac{1}{8}$  strength
    - Note: This can be done by mixing a larger amount (~250 mL) of double-strength mixture, then adding 100 mL of this to the first container, 50 mL to the next container, 25 mL to the third container, and so on. Then fill each container the rest of the way to 100 mL with non-chlorinated water.
- b) Fill the 6<sup>th</sup> container with 100 mL of non-chlorinated water only (no fertilizer)
- c) Using the clear tape and a marker, label each container with a sample letter (A-F) and the fertilizer concentration (Double, regular, half, etc.). Label the water-only sample as "Control."
- d) Add 5 mL of live algae culture to each of the container, including the water-only beaker.
- e) Cap each vial securely.
- f) In the Data Collection Table (below), note which treatment group each sample is in. A treatment group will be the fertilizer concentration, or Control (no color fertilizer).
- g) In the Data Collection Table (below), note the before-test color of the algae/fertilizer/water mixture in each of the samples. Use the provided numbered color chart.
- h) Loosely cover each container with plastic wrap so that air can get in and out, but so that dust and other contaminants are kept to a minimum.
- i) Place each container 30 centimeters from the light source and close to the other containers.
- j) Turn on the light source.
- k) Allow several days for algae growth to take place
- l) Turn off the light source
- m) In the Data Collection Table (below), note the color of the algae/fertilizer/water mixture in each sample. Use the provided numbered color chart.
- n) In the Data Collection Table, subtract the "after" color from the "before" color to find the color change. A larger color change means more algae growth took place.
- o) Discard the algae mixture and clean and dry the containers and all other materials

**Data Collection Table**

	Example	Sample A	Sample B	Sample C	Sample D	Sample E
<b>Treatment Group</b> (Fertilizer concentration or Control)	0.125X					
<b>Color before Test</b> (Number)	2					
<b>Color after Test</b> (Number)	12					
<b>Color change</b> (After minus before)	10					





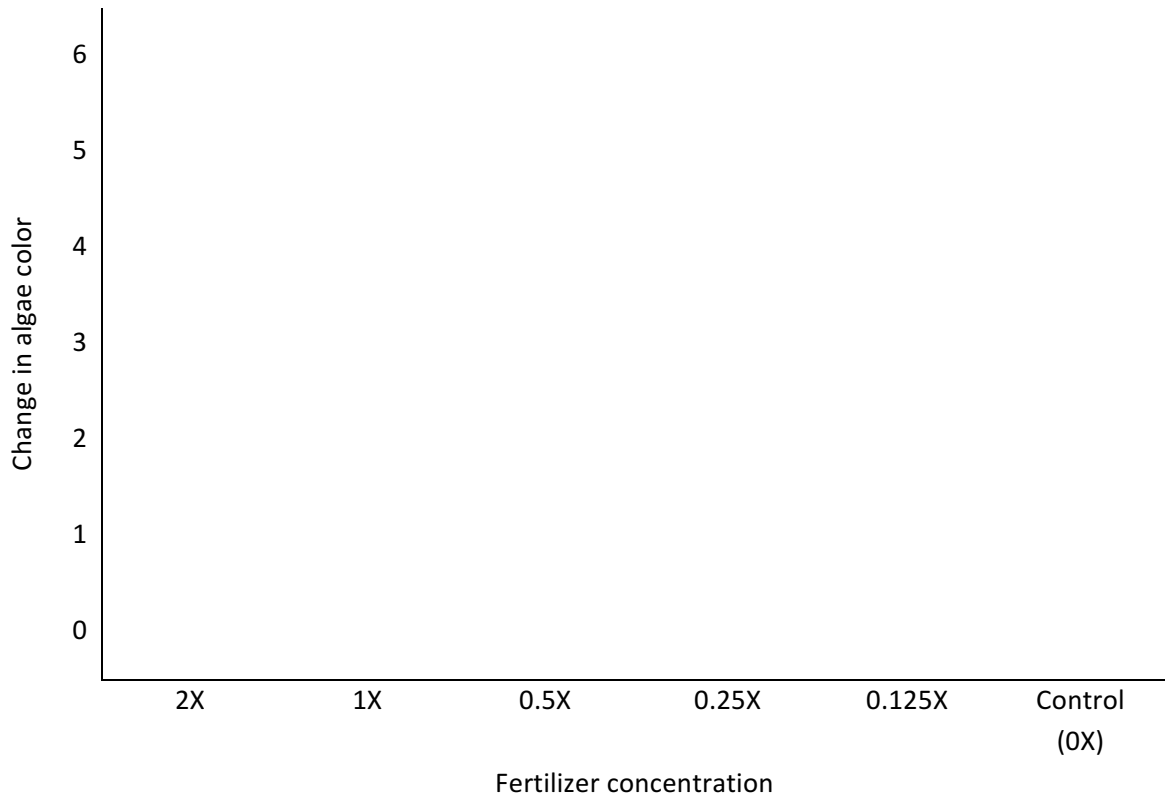
**Follow up questions:**

1. Using the blank chart below, plot the CO<sub>2</sub> changes as a function of fertilizer concentration (color). Draw lines to connect each point to form a line graph.

Table 1.

*Algae growth as a function of fertilizer concentration*

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2. Can you tell whether the concentration of fertilizer affected algae growth? How can you tell?
3. Based on these results, was your hypothesis confirmed, not confirmed, or do you need more information? What other information might be useful or interesting?
4. Is there an ideal concentration of fertilizer (one that produces the most algae growth)? If so, what is it, and how do you know?

**Algaculture & Biofuels:**  
**Experiments With Algae- Plant Growth and Carbon Dioxide**

**Experiment 5 – Carbon Dioxide Availability**

**Description:** In this experiment, you'll be investigating how microalgae growth rates are affected by the availability of carbon dioxide. Microalgae are microscopic plant cells, and like other plant cells they require carbon dioxide for photosynthesis. Photosynthesis uses carbon dioxide from the atmosphere, water, and energy from the sun to create sugar molecules where energy is stored, and oxygen gas as a waste product. Carbon dioxide makes up only about 0.04 percent of the air we breathe, but this is more than enough to support all the photosynthesis taking place in all the plant life on earth! In this activity, you'll experiment supplying algae with different amounts of air to find out how algae respond to increased or decreased levels of carbon dioxide.

**Materials**

- ✓ Live microalgae culture (chlorella, ankistrodesmus, etc.)
- ✓ Non-chlorinated water (spring water from a natural source or aquarium water)
- ✓ 3 small soda bottles (20 oz. or less)
- ✓ All-purpose water soluble fertilizer granules (MiracleGro or equivalent)
- ✓ 1 aquarium air pump/air stone (small enough to fit inside your container)
- ✓ 1 electric drill and a small set of bits
- ✓ Strong light source
- ✓ Clear tape

**Procedure:**

1. Write the question you are trying to answer with this experiment:

**Question:**

2. Use a computer, mobile device, library, or other resources to try to answer the following questions:
  - What resources are needed by plant cells to promote growth?
  
  
  - Where do microalgae acquire carbon dioxide needed for photosynthesis?
  
  
  - What byproducts are produced from photosynthesis?

3. Considering the information you uncovered in your research, write the answer you expect to find to your original question.

**Hypothesis:**

4. Experimental Procedure:
  - a. Fill each bottle about  $\frac{3}{4}$  full of non-chlorinated water.
  - b. Add an equal amount of fertilizer granules (a small pinch, about  $\frac{1}{8}$  teaspoon) to each bottle, then close the bottles tightly and swirl until the fertilizer has completely dissolved in the water.
  - c. Using the clear tape and a marker, label each container with a sample letter (A-C).
  - d. Remove the caps on two of the bottles, and drill the following holes in the two caps:
    - **Bottle 1:** Drill two holes of equal size that are just large enough to snugly fit the plastic tube from your air pump through the cap.
    - **Bottle 2:** In the top of the second cap, drill 6-8 smaller holes
  - e. Add 5 mL of live algae culture to each bottle.
  - f. Prepare each bottle as instructed below:
    - **Bottle 1** (two large holes in the cap): Thread the plastic tube from your air pump through the cap and down into the water so that the end nearly touches the bottom. You may need to weigh the end of the tube or use a small air stone. The second hole will allow air to escape as more air is pumped in. Once you have the air tube installed, cap the bottle securely with the air tube in place, then turn on the pump. If water spills out when the pump is on, you may need to pour a small amount of water out until the water doesn't splash out.
    - **Bottle 2** (with several smaller holes drilled in the cap): Close it securely using the cap with several small holes.
    - **Bottle 3** (with no holes in the cap): Cap the bottle securely so that no air can get in or out.
5. In the Data Collection Table (below), note which treatment group each sample is in. There are 3 treatment groups representing 3 levels of CO<sub>2</sub> availability: Pumped air, Passive (air holes, no pump), and Control (sealed bottle, no outside air exchange).
6. In the Data Collection Table (below), note the before-test color of the algae/fertilizer/water mixture in each of the samples. Use the provided numbered color chart.
7. Place each container 30 centimeters from the light source and close to the other containers.
8. Turn on the light source.
9. Allow several days for algae growth to take place.
10. Turn off the light source.

11. In the Data Collection Table (below), note the color of the algae/fertilizer/water mixture in each sample. Use the provided numbered color chart.
12. In the Data Collection Table, subtract the “after” color from the “before” color to find the color change. A larger color change means more algae growth took place.
13. Discard the algae mixture and clean and dry the containers and all other materials

**Data Collection Table**

	Example	Sample A	Sample B	Sample C
<b>Treatment Group</b> (Forced, Passive, or Control)	Passive			
<b>Color before Test</b> (Number)	2			
<b>Color after Test</b> (Number)	5			
<b>Color change</b> (After minus before)	3			



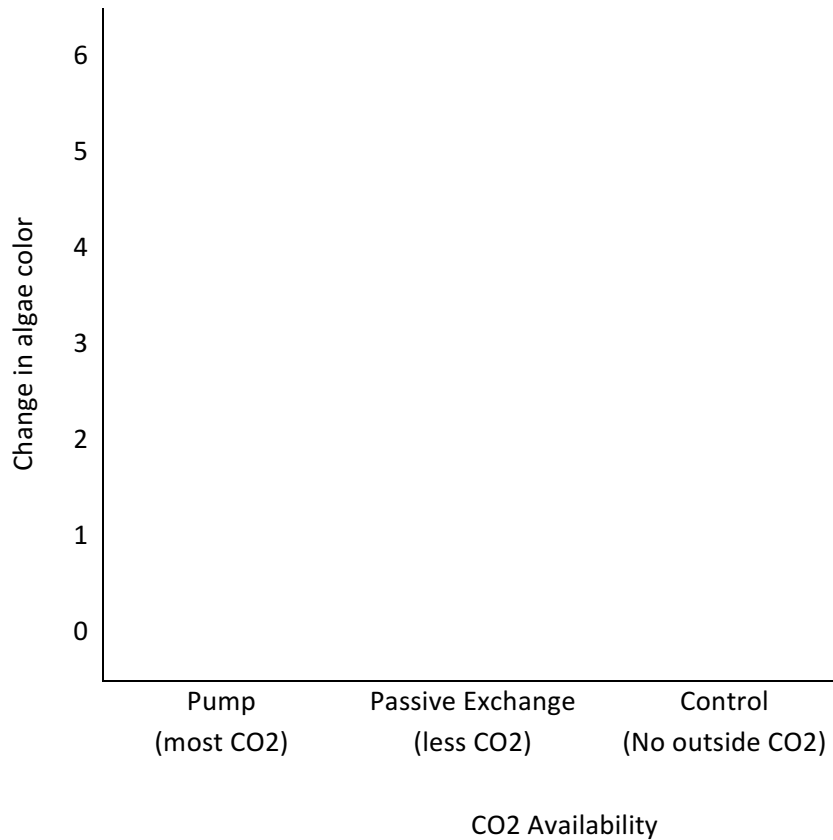
**Follow up Questions:**

1. Using the blank chart below, plot the CO<sub>2</sub> changes as a function of fertilizer concentration (color). Draw vertical columns to each point to form a bar graph.

Table 1.

*Algae growth as a function of CO<sub>2</sub> availability*

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2. Can you tell whether the availability of CO<sub>2</sub> affected algae growth? How can you tell?
3. Based on these results, was your hypothesis confirmed, not confirmed, or do you need more information? What other information might be useful or interesting?
4. Which level of CO<sub>2</sub> availability produced the most algae growth? Why do you think you got this result?