Advances in Biology Laboratory Education
Publication of the Association for Biology Laboratory Education
Volume 41, Article 20, 2020

Inquiry-based Problem Solving: Analysis of CO_2 Uptake by *Spirulina* sp. in Varying Water Temperatures

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Microalgae are efficient consumers of carbon dioxide. However, due to excess carbon emissions in the atmosphere, microalgae can create harmful algal blooms (HABs) that cause health-related problems to organisms. The purpose of this experiment was to measure the efficiency of microalgae when consuming carbon dioxide in varying environments. This experiment was designed for undergraduate biology students with previous knowledge of introductory chemistry and biology. This activity was structured around experiential learning and inquiry based problem solving using climate change data.

Keywords: microalgae, biochemistry, environment, HABs, and *Spirulina sp.*

Introduction

Algae are photosynthetic eukaryotic organisms that are essential to any aquatic ecosystem (Anderson et al. 2002). They belong to a very large and diverse group, ranging in size from microscopic diatoms to giant kelp. All plants are thought to have evolved from a single charophyte algae about 500 million years ago (Anderson et al. 2002). Algae are complex organisms, however they can be deadly. When water temperatures begin to warm up in late spring due to increased sun exposure, these algae divide by mitosis, rapidly increasing algal population numbers (Kanhaiya et al. 2011). This increase in algae population can lead to a phenomenon called an algal bloom. These blooms can either be toxic or non-toxic depending on the abundance of dangerous algae cells such as *Alexandrium catenella,* which can cause paralytic shellfish poisoning (Anderson et al. 2002). Harmful algal blooms (HABs) can cause a variety of health-related issues to both animals and humans as well as damage local economies (Anderson et al. 2002). Toxic algae can devastate aquatic ecosystems and local economies. This is why monitoring algal blooms are so important. Information about how, where, and why algal blooms occur can be detrimental to the environment. Algae convert carbon dioxide to oxygen via photosynthesis in both freshwater and marine environments (Sarmiento and Orr 1992). The experiment that is the main focus of this paper did just that. An assessment as to which bodies of water are more likely to create algal blooms was conducted by measuring pH and carbon dioxide. The information gathered in this experiment can help managers locate and monitor algal blooms as well as aid in determining which prevention strategies will work best in a particular location.

This laboratory assignment was developed to aid undergraduate biology students in understanding the efficiency of carbon upcycling in differing environmental settings, as well as how microalgae populations reduce carbon dioxide accumulation in water habitats. Students should have completed introductory biology and ecology courses before participating in this exercise.

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Student Outline

Objectives

- Successfully use a pH meter and probe
- Observe how temperature affects carbon dioxide uptake by *Spirulina sp.*
- Understand how microalgae populations reduce carbon dioxide accumulation

Introduction

The Environmental Protection Agency (EPA) defines algal blooms as overgrowths of algae in the water. Some of these blooms can release toxins into the water and potentially sicken or kill humans and animals (Anderson 2002). Even if these blooms are non-toxic, they can still be detrimental to the environment and local economies. Algal blooms can create dead zones in bodies of water, raise treatment costs for the bodies of water they impact, and financially hurt the industries that rely on the source for clean water (Kim et al. 2014). Bodies of water, especially our oceans are huge carbon sinks (Sarmiento and Orr 1992). When excess carbon dioxide makes its way into the water, the water becomes acidified and the temperature increases (Kumar et al. 2011). Algae, however, are great at taking up this excess carbon dioxide because they use it to photosynthesize (Kim et al. 2014). In this lab exercise, please imagine that you are a phycologist studying *Spirulina sp*. You decide that you want to travel from the northern east coast to the southern east coast to observe where algal blooms are more prominent. You plan your road trip from Bradford, Pa to Florida and take water samples in both locations, comparing them to your laboratory distilled room temperature water. Since Bradford is on the northern east coast, this represents your "cold water" sample and since Florida is on the southern east coast, this represents your "warm water" sample. Use the thermal map (available at https://www.ospo.noaa.gov/data/sst/contour/global_small.cf.gif) to identify the temperature range of the bodies of water in question (Bradford, Pennsylvania to Miami, Florida). Based upon this map, the range of temperatures is from 6 to 24 ℃. This experiment is designed for undergraduate college biology students who have successfully completed prerequisite courses in biology and chemistry. All materials that are necessary to successfully complete this experiment will be provided to you before you begin. The total time necessary to complete the experiment is from 45 to 60 minutes.

Questions to Answer before Beginning the Experiment:

1. According to the thermal map above, which location do you predict to have the highest concentration of algae and why? Will this increase or decrease carbon dioxide uptake?

2. According to the thermal map above, which location do you predict to have the lowest concentration of algae and why? Will this increase or decrease carbon dioxide uptake?

Questions to Answer after Finishing the Experiment:

1. What effect does the addition of carbon dioxide have on the pH of water? How does the addition of *Spirulina* change this?

2. According to the data, what did you observe among differing water temperatures? What temperature had the lowest pH fluctuation?

3. Were your predictions correct after performing the experiment?

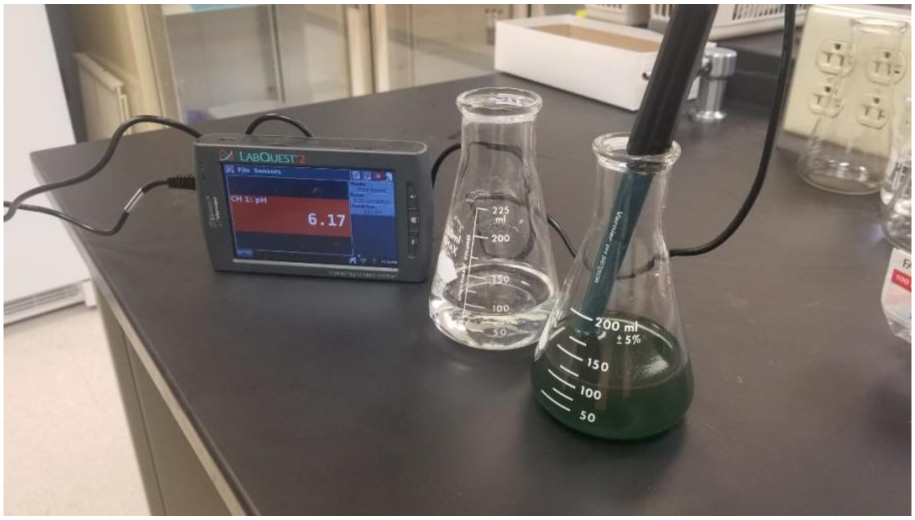

Figure 1. Two Erlenmeyer flasks: one control and one with *Spirulina sp.* pH meter probe inserted.

Procedure:

Step 1: For each group of 3 students, obtain two 250 mL flasks. One will be your control and the other will be your experimental. Fill each flask with 100 mL of your assigned water temperature (Bradford-cold, Miami-warm, and room temp). Add 10 mL of spirulina to your experimental flask. Calibrate your pH meter before inserting a probe into a flask. Refer to the pH meter handout on how to operate the meter.

Step 2: Record the initial pH of one of your flasks until a reoccurring number is established. Then, record this number in the data table below. Repeat this step for the other flask.

Step 3: Blow bubbles into one of your flasks using the straws provided for 1 minute. Record this reading on the data table. Repeat this step for the other flask.

Step 4: To observe how the algae are interacting with their environment. Set a timer for 15 minutes. Record the pH every 5 minutes.

Step 5: Plot your points on the board.

Step 6: Repeat steps 2-5 for your experimental.

Step7: Answer the post-procedure questions.

Table 1. Student data.

Time (minutes)	pH of control flask	pH of experimental flask w/ *Spirulina*
Initial		
Initial after blowing bubbles		
5		
10		
15		

Materials

- Three pH meters and probes (1 per group of 3 students)
- Six 250mL Erlenmeyer flasks (2 per group of 3 students)
- Six plastic straws
- 100mL Distilled water per Erlenmeyer flask
- Three 100mL graduated cylinders (1 per group of 3 students)
- Three 10mL syringes
- *Spirulina sp.*
- Latex gloves for each student
- Safety goggles for each student
- Aprons for each student
- Hot plate
- Refrigerator

Notes for the Instructor

An element of this lab that is helpful for student success is projecting the thermal map in color.

One challenge that was faced when performing this experiment was the use of pH meters. It is vital that students follow the handout provided on how to operate the pH meter, and that the buffer does not enter the water samples. If this occurs the data will be skewed from the addition of a buffer. To avoid this from happening, explicit pH meter instructions can be provided to the students, as well as a beaker with water to wash the probe, and a verbal reminder to rinse the pH meter before taking the measurements for the experiment.

Students who are assigned to the group that uses the hot plate should be required to wear protectant gloves and eyewear.

Example data sets (Table 2) and graphs (Figure 2) are located in the Appendix. The data collected by students showed that when *Spirulina sp.* were present in the water, regardless of temperature, the pH had a less drastic drop, as opposed to when the water did not contain algae; however, water temperature also affected how quickly the pH recovered to a normal levels after excess CO_2 was added. The room temperature control with *Spirulina sp.* never decreased in pH, even with the introduction of CO_2 to the environment. The experimental trial that contained warm water and *Spirulina sp.* only dropped 0.02 in pH and fully recovered to the initial pH after fifteen minutes. Finally, the experimental trial that contained cold water with *Spirulina sp.* had the greatest drop in pH compared to the other trials containing microalgae and did not recover to the initial pH after fifteen minutes.

When microalgae were not present in the water, the pH showed a drastic drop and did not recover to the initial pH after 15 minutes. This shows that microalgae are beneficial in stabilizing ecosystems from extreme fluctuations in pH when CO_2 is added. The microalgae, *Spirulina sp.*, was found to upcycle CO_2 most efficiently under room temperature and warm water conditions.

Cited References

Anderson DM, Gilbert PM, Burkholder JM. 2002. Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences. Estuaries. [accessed 2019 Sept 24]; 25(4b): 704-726. https://doi.org/10.1007/BF02804901

Kim K, Choi J, Ji Y, Park S, Do H, Hwang C, Lee B, Holzapfel W. 2014. Impact of bubble size on growth and CO2 uptake of *Arthrospira* (*Spirulina*) *platensis* KMMCC CY-007. Bioresource Technology. [accessed 2019 Sept 24]; 170: 310-315. https://doi.org/10.1016/j.biortech.2014.08.034

Kumar K, Dasgupta CN, Nayak B, Lindblad P, Das D. 2011. Development of suitable photobioreactors for CO2 sequestration addressing global warming using green algae and cyanobacteria. Bioresource Technology. [accessed 2019 Sept 24]; 102(8): 4945-4953. https://doi.org/10.1016/j.biortech.2011.01.054

NOAA. Sea Surface Temperature (SST) Contour Charts. [accessed 2019 Dec 5]. Available from https://www.ospo.noaa.gov/data/sst/contour/global_small.cf.gif

Sarmiento JL, Orr JC. 1992. A perturbation simulation of CO2 uptake in an ocean general circulation model. Journal of Geophysical Research. [accessed 2019 Sept 24]; 97(C3): 3621-3645. https://doi.org/10.1029/91JC02849.

Acknowledgments

We would like to thank Dr. Denise Piechnik, Dr. Mary Mulcahy, and Dr. Francis Mulcahy for the pH meters and assistance with them. Thank you very much to all of the students who participated in this study and helped us to collect the data. Funding to support this project was provided by the Faculty Development Grant at the University of Pittsburgh at Bradford.

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Kelsey Andersen is a senior at the University of Pittsburgh at Bradford majoring in biology. She hopes to attend graduate school in 2020 for reproductive health to work in a hospital lab.

Cheyenne Brabham has been a student at the University of Pittsburgh at Bradford since 2014 and plans to graduate in April of 2019. She will graduate with a B.S. in biological sciences and plans to attend dental hygiene school in Buffalo, NY.

Whitney Colosimo has been a student at the University of Pittsburgh at Bradford since 2016 and plans to graduate in April of 2019. She will graduate with a B.S. in the biological sciences with a minor in chemistry and plans to attend graduate school for a medical degree.

Kassi Kloss has been a student at the University of Pittsburgh at Bradford since August 2016 and plans to graduate in December 2019. She will graduate with a B.S. in the biological sciences and plans to attend graduate school for an M.S. in Wildlife and Fisheries.

Cheyenne Staufenberger has been a student at the University of Pittsburgh at Bradford since August 2015 and plans to graduate in April 2019. She will graduate with a B.S. in the biological sciences and plans to move to South Carolina to pursue a career in ecology at SREL.

Sarah E. Ruffell teaches microbiology and focuses her research in the areas of science education and phycology. As of 2020, she has begun a new position at the University of Waterloo and would be happy to answer any questions you have at her new email sruffell@uwaterloo.ca.

Appendix A: Sample Student Data

Table 2. Class data showing the changes in pH with and without *Spirulina sp*. at different water temperatures.

Room Temperature Control	**Without Algae**	**With Algae**
initial	6.27	6.23
initial after bubbles	4.68	6.25
5 minutes	4.59	6.27
10 minutes	4.53	6.26
15 minutes	4.52	6.26
Warm Temperature (Miami)	**Without Algae**	**With Algae**
initial	6.65	6.30
initial after bubbles	6.63	6.40
5 minutes	6.45	6.28
10 minutes	6.45	6.29
15 minutes	6.45	6.31
Cold Temperature (Bradford)	**Without Algae**	**With Algae**
initial	6.74	6.30
initial after bubbles	4.88	6.07
5 minutes	4.66	6.17
10 minutes	4.58	6.18
15 minutes	4.52	6.18

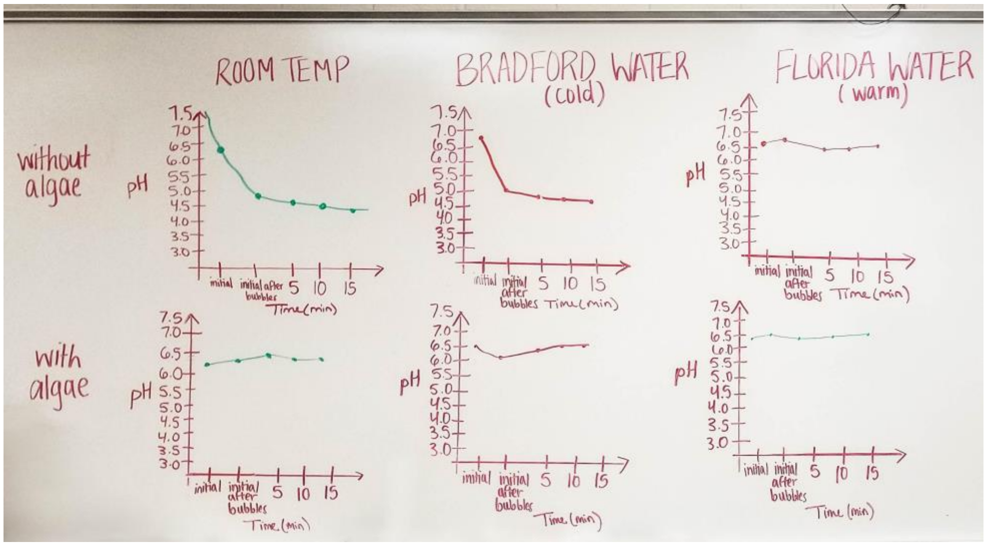

Figure 2. Data collected in class on the effect of algae (*Spirulina sp.)* on pH at different water temperatures.

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Citing This Article

Andersen K, Brabham C, Colosimo W, Kloss K, Staufenberger C, Ruffell SE. 2020. Inquiry-based problem solving: Analysis of CO_2 uptake by *Spirulina* sp. in varying water temperatures. Article 20 In: McMahon K, editor. *Advances in biology laboratory education*. Volume 41. Publication of the 41st Conference of the Association for Biology Laboratory Education (ABLE). https://doi.org/10.37590/able.v41.art20

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