

**Project title:** Calcium Carbonate Production by Coccolithophorid Algae in Long Term,  
Carbon Dioxide Sequestration

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## Abstract

Predictions of increasing levels of anthropogenic carbon dioxide (CO<sub>2</sub>) and the specter of global warming have intensified research efforts to identify ways to sequester carbon. A number of novel avenues of research are being considered, including bioprocessing methods to promote and accelerate biosequestration of CO<sub>2</sub> from the environment through the growth of organisms such as coccolithophorids, which are capable of sequestering CO<sub>2</sub> relatively permanently.

Calcium and magnesium carbonates are currently the only proven, long-term storage reservoirs for carbon. Whereas organic carbon is readily oxidized and releases CO<sub>2</sub> through microbial decomposition on land and in the sea, carbonates can sequester carbon over geologic time scales. This proposal investigates the use of coccolithophorids — single-celled, marine algae that are the major global producers of calcium carbonate — to sequester CO<sub>2</sub> emissions from power plants. Cultivation of coccolithophorids for calcium carbonate (CaCO<sub>3</sub>) precipitation is environmentally benign and results in a stable product with potential commercial value. Because this method of carbon sequestration does not impact natural ecosystem dynamics, it avoids controversial issues of public acceptability and legality associated with other options such as direct injection of CO<sub>2</sub> into the sea and ocean fertilization. Consequently, cultivation of coccolithophorids could be carried out immediately and the amount of carbon sequestered as CaCO<sub>3</sub> could be readily quantified. The significant advantages of this approach warrant its serious investigation. The major goals of the proposed research are to identify the growth conditions that will result in the maximum amount of CO<sub>2</sub> sequestration through coccolithophorid calcite production and to evaluate the costs/benefits of using coccolithophorid cultivation ponds to abate CO<sub>2</sub> emissions from power plants.

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## **Introduction**

The objective of this project is to determine the efficacy of using coccolithophorid  $\text{CaCO}_3$  production in  $\text{CO}_2$  removal technology. This project will determine the methods and biological and chemical conditions needed to optimize the native ability of coccolithophorid algae to sequester  $\text{CO}_2$  in the form of  $\text{CaCO}_3$ . This project will identify the parameters necessary to produce coccolithophorid blooms and the factors required to obtain maximum calcification rates. The initial task of the research is to identify the species, cell strain and the specific growth conditions (e.g., temperature, light intensity, nutrient concentrations) that maximize population growth rates and rates of calcification. The second task of the project is to quantify the amount of  $\text{CO}_2$  sequestration by coccolithophorids when cells are grown under high-calcifying conditions in a custom-manufactured experimental vessel. We have now begun Task 2 and describe our preliminary results in this report.

## **Experimental**

We conducted our first closed-vessel experiment to investigate the impacts of coccolithophorid growth on the  $\text{CO}_2$  system parameters. We inoculated 7 liters of F/50 media with a small volume of *Emiliania huxleyi* cell strain CCMP 371. The culture was stirred continuously to keep cells uniformly suspended. This vessel was placed in a temperature- and light-controlled environmental chamber. We have run this initial experiment for 26 days thus far, at a temperature of  $18.5^\circ\text{C}$ , and a light/dark cycle of 16 hours light/8 hours dark.

Throughout the experiment, temperature and electromotive force (EMF in mV) were recorded every hour using the data acquisition program we developed in our lab. Daily samples of the culture were taken for measurements of cell density, total alkalinity (TA), and total dissolved inorganic carbon ( $\text{TCO}_2$ ). Subsamples for these measurements were taken without introducing additional atmospheric  $\text{CO}_2$ . The sample volume removed for analyses was replaced with argon from a mylar reservoir. Figure 1 shows the experimental vessel with the pH and temperature probes, the sampling port outfitted with a 3-way stopcock, and the mylar reservoir filled with argon.

## **Results and Discussion**

Partial results of this experiment are graphically depicted in Figure 2. Cell density increased over time showing exponential growth followed by stationary phase in which cell numbers leveled off. Values of pH (calculated from EMF measurements) similarly increased with population growth. When cell density appeared to reach stationary phase at  $5.7 \times 10^6$  cells  $\text{ml}^{-1}$ , we spiked the culture with additional nutrients. Subsequently, cell density reached  $9.8 \times 10^6$  cells  $\text{ml}^{-1}$ . The highest density of discarded coccoliths during the experiment was  $3 \times 10^8$  coccoliths  $\text{ml}^{-1}$ . When stationary phase of cell growth was

reached a second time, we suspected coccolithophorid growth was limited by carbon availability and, therefore, allowed limited exchange of the headspace of the vessel with the ambient atmosphere. Upon exposure to the atmosphere, the pH decreased as expected.

During the period of exponential cell growth, pH increased dramatically. Values of pH oscillated on a diel basis. As shown in Figure 3, pH increased during light hours and decreased during the dark. Over the course of the experiment thus far, the pH ranged from 8.02 to 8.67, indicating that this cell strain of *Emiliana huxleyi* is very tolerant to pH changes.

In the immediate future, we will complete the analyses of samples from this experiment. We will use our recently finished alkalinity titration program to measure the total alkalinity of samples taken over the course of the experiment. This will be followed by total dissolved CO<sub>2</sub> measurements determined by coulometry (Dickson and Goyet, 1994). The results of this initial experiment will allow us to trouble-shoot and perform any adjustments to experimental protocols that may be needed for the suite of experiments described in Task 2.0.

## **Conclusion**

We have begun Task 2 and produced preliminary results from a closed-vessel experiment with *E. huxleyi* that ran for 26 days. The data acquisition programs that we developed for this project worked well and enabled us to continuously monitor temperature and electromotive force (EMF in mV). In addition, subsamples were taken for total alkalinity and total dissolved inorganic carbon analyses which we will complete in the upcoming weeks. This first run of our equipment, software, and methods was successful and forms the foundation of further experiments described in Task 2.

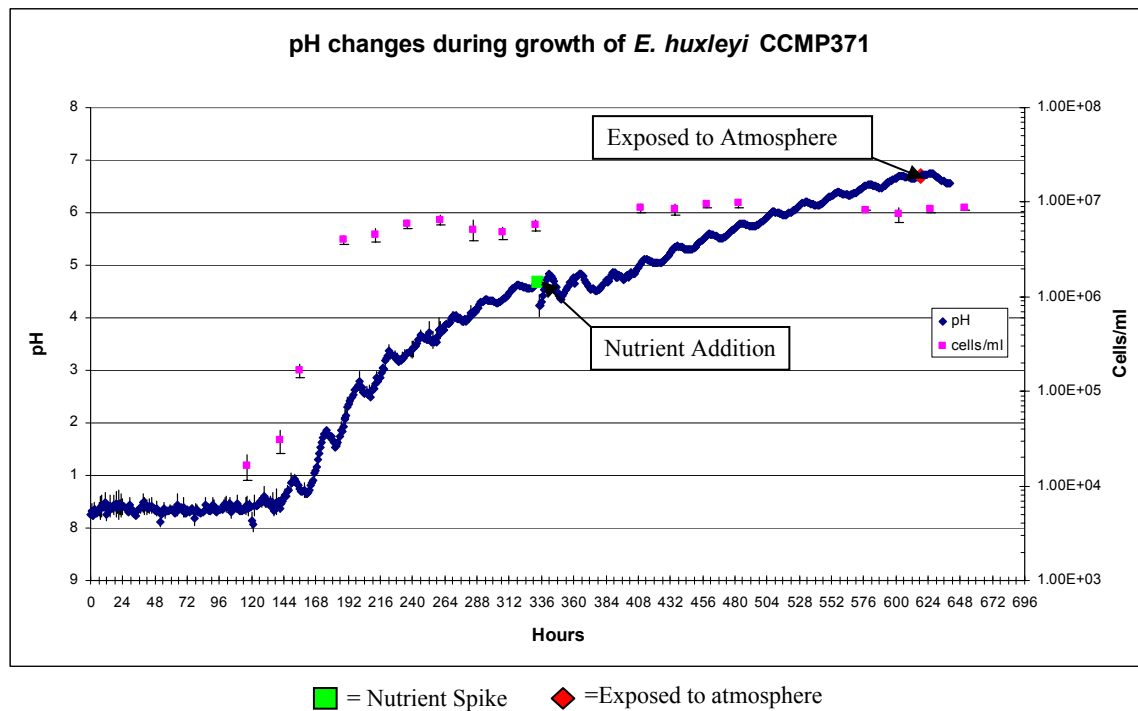
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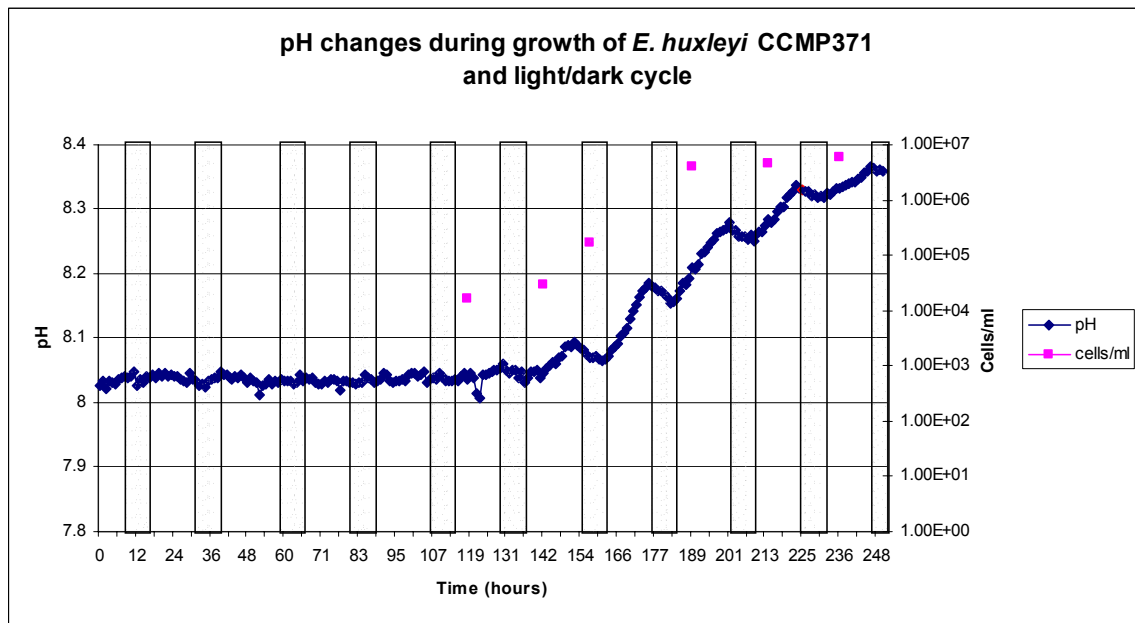


**Fig.1.** Experimental vessel equipped with temperature and pH probes, sampling ports (with 3-way stopcock) and mylar argon reservoir. Note the turbidity of the media due to a high cell density.





**Fig. 2.** Changes in pH (left Y-axis; blue line) and cell density (right Y-axis; pink squares) over 26 days from initial inoculation of coccolithophorids in a closed vessel experiment. On day 13, nutrients were added to the media. On day 26, the vessel was partially exposed to the atmosphere.



**Fig. 3.** First 10 days of closed vessel experiment illustrating the relationships among pH (left Y-axis; blue line), cell density (right Y-axis; pink squares) and light/dark cycle (16 hours light/8 hours dark). Dark hours denoted by shaded bars. Note the pH decrease dark hours.