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Carbon Dioxide Sequestration

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Abstract

Predictions of increasing levels of anthropogenic carbon dioxide (CO₂) 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 CO₂ from the environment through the growth of organisms such as coccolithophorids, which are capable of sequestering CO₂ 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 CO₂ 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 CO₂ emissions from power plants. Cultivation of coccolithophorids for calcium carbonate (CaCO₃) 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 CO₂ into the sea and ocean fertilization. Consequently, cultivation of coccolithophorids could be carried out immediately and the amount of carbon sequestered as CaCO₃ 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 CO₂ sequestration through coccolithophorid calcite production and to evaluate the costs/benefits of using coccolithophorid cultivation ponds or bioreactors to abate CO₂ emissions from power plants.

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Introduction

The major objective of this project is to determine the efficacy of using coccolithophorid CaCO_3 production in CO_2 removal technology. This project will determine the methods and the biological, chemical, and physical conditions needed to optimize the native ability of coccolithophorid algae to sequester CO_2 in the form of CaCO_3 . This project will identify the parameters necessary to produce coccolithophorid blooms and the factors required to maximize calcification rates. The information gained in this study can be incorporated into the design and construction of future algal ponds or bioreactors in follow-up research (not a part of this project) on CO_2 sequestration by coccolithophorids. The initial task of the research was 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 involves quantification of the amount of atmospheric CO_2 sequestered as CaCO_3 under conditions that maximize cell growth rates and calcification rates. During the first year of the project, we completed most of Task 1 and started work that is central to Task 2 and prerequisite for Tasks 3 and 4. In the second year of the project, we completed Task 1 and made substantial progress in completing Task 2. In addition, we did preliminary work regarding Task 4. In the third year of the project we focused on completing Task 2.0 and prepared for work in Task 4. Specifically, we completed nutrient analyses of samples from closed vessel experiments and completed our experimental work investigating coccolithophorid growth at different levels of dissolved CO_2 .

Here we review the progress during the last year with emphasis on the last quarter's work, from June 30, 2004 through September 30, 2004.

Experimental

During the last year, we focused our experimental work on the coccolithophorid *Emiliania huxleyi*, cell strain CCMP 371, which we previously identified as the best candidate among available coccolithophorid cell strains for CO_2 sequestration.

First, we investigated whether the growth rates, inorganic carbon uptake, and maximum yield of coccolithophorids could be increased by growing cells in two different growth media ? K media (Keller *et al.*, 1987) and F/50 (Guillard, 1975) ? which contain different sources and levels of nitrogen, phosphate, trace metals, and chelator.

Our experiments revealed no significant differences in growth rates or maximum yield of coccolithophorids when cells were grown in F/50 media versus K media. Therefore, we concluded that F/50 media, which is less costly to produce than K media, is a better choice for use in coccolithophorid CO_2 sequestration. We next investigated whether nitrate additions to F/50 media could enhance coccolithophorid growth and calcification. Another series of experiments addressed questions of coccolithophorid growth and calcification under initial conditions of elevated CO_2 in F/50 media made with artificial

seawater and in natural seawater enriched with nitrate and trace metals. These experiments are briefly described below.

F/50 media and nitrate utilization

We conducted closed vessel experiments in which 7 liters of F/50 media, containing 883 μM nitrate, was inoculated with a very small volume of *Emiliana huxleyi* cell strain CCMP 371 to give an initial cell density of 4×10^2 cells ml^{-1} . The culture was stirred continuously to keep cells uniformly suspended. This vessel was placed in a temperature and light-controlled environmental chamber. The temperature during the experiment was 18.5 $^{\circ}\text{C}$ and the light/dark cycle was 16 hours light/8hours dark. Subsamples were taken for cell densities and measurement of total alkalinity (TA) and dissolved inorganic carbon (DIC). Total alkalinity (TA) was determined using our own software. Total dissolved inorganic carbon in duplicate samples was measured using coulometry (Dickson and Goyet, 1994). Cell densities were determined daily by direct count. Additional samples were taken for nitrate analyses measured on a Lachat Quik Chem 8000 flow injection ion analyzer.

Cell growth in artificial seawater enriched with F/50 at high pH and low pH

In another experiment, we made F/50 media using laboratory-made seawater and filled each closed vessel with 7 L. In one closed vessel, we added additional CaCO_3 to increase the initial pH to 8.2; we refer to this experimental chamber as the “high CO_3 vessel or high pH and low CO_2 vessel.” In the second closed vessel, we injected CO_2 gas into the media which lowered the initial pH to 7.6; we refer to this experimental chamber as the “low pH or high CO_2 vessel.” We inoculated each vessel with a small volume of *Emiliana huxleyi* cell strain CCMP 371 to give an initial cell density of 2.5×10^4 cells ml^{-1} in the high CO_3 vessel and 5.6×10^3 cells ml^{-1} in the high CO_2 vessel. Each experimental vessel was stirred continuously to keep cells uniformly suspended. Vessels were placed in a temperature and light-controlled environmental chamber at 18.5 $^{\circ}\text{C}$ and a light/dark cycle of 16 hours light/8 hours dark. At periodic time intervals, subsamples were taken for cell densities, total alkalinity (TA), and total dissolved inorganic carbon (DIC). Total alkalinity (TA) was determined using our own software in a computer-driven, potentiometric titration. Total dissolved inorganic carbon was measured using coulometry (Dickson and Goyet, 1994). Cell densities were determined by direct count. Additional subsamples were taken for phosphate and nitrate measurements with a Lachat Quik Chem 8000 flow injection ion analyzer and particulate calcium concentrations measured with a Varian 220 FS atomic absorption spectrometer. Using the appropriate dissociation constants, we calculated the CO_2 fugacity ($f\text{CO}_2$) and dissolved CO_2 in each closed vessel over the course of the experiment.

Cell growth in natural seawater enriched with nitrate and trace metals at elevated $p\text{CO}_2$

Emiliana huxleyi (strain CCMP371) cells were grown in two closed, 7-L cylindrical polycarbonate vessels. Media consisted of 0.2 μm filtered seawater enriched with ~ 400 μM nitrate and F/2 levels of vitamins and trace metals (Guillard, 1975). No phosphate was added to the seawater. Phosphate concentration was ~ 0.5 μM . Incubation occurred

at 17°C and a light/dark cycle of 18 hour light/6 hours dark. Cultures were continuously mixed using a magnetic stirrer. In one closed vessel, CO₂ gas was bubbled into the seawater for an initial $p\text{CO}_2$ of 735 μatm and a dissolved CO₂ concentration of 26.5 $\mu\text{mol kg}^{-1}$. In the second closed vessel, mixed gas with a known CO₂ concentration was bubbled into the seawater for an initial $p\text{CO}_2$ of 390 μatm and a dissolved CO₂ concentration of 13.9 $\mu\text{mol kg}^{-1}$. After inoculating each closed vessel with a small volume of *E. huxleyi* cells, vessels were periodically subsampled for total cell counts, relative numbers of calcified cells versus noncalcified cells, and measurements of DIC, TA, total particulate calcium, organic carbon, nitrate, phosphate, and silicate. Additional samples of cells were taken for examination with scanning electron microscopy (SEM). Subsamples for these measurements were taken without introducing additional atmospheric CO₂ into the experimental vessel. Each subsample volume removed for analyses was replaced with argon from a mylar reservoir.

Results and Discussion

F/50 media and nitrate utilization

Population cell growth showed the expected exponential growth curve. Maximum cell density of $> 10^7$ cells ml^{-1} was reached in 11 days. The decrease in DIC from day 7 to day 12 is marked and indicates that cells were probably carbon-limited by day 12. The changes in nitrate concentration and cell density are shown in Figure 1B of Quarterly Report #11. At day 12, the nitrate concentration was still high, having declined by only about 100 μM or 10% of the initial concentration. These results indicate that cell growth was not limited by nitrate, and thus enhanced nitrate concentrations would not increase cell growth. These data suggest that cell growth was limited by DIC levels and support our previous hypothesis that introducing additional dissolved CO₂ at day 12 could possibly lead to greater cell growth and CO₂ sequestration.

Cell growth in artificial seawater enriched with F/50 at high pH and low pH

In the high CO₃/high pH closed vessel experiment, cell densities reached stationary phase by day 4 and the experiment was terminated at day 8. As expected, as cell density increased, DIC steadily decreased (see Fig. 2A of Quarterly Report #11). By day 8, the DIC concentration approached zero and limited further population growth of the coccolithophorids. In contrast, the high CO₂/low pH closed vessel experiment was conducted over 26 days (see Fig. 2B of Quarterly Report #11). In response to the initial low pH of 7.58, the cell density decreased for the first four days, after which cell numbers steadily increased and reached approximately the same maximum cell density as the high pH vessel by day 20. These results demonstrate that coccolithophorid cell strain CCMP 371 shows a marked delay in population growth in response to low pH. Interestingly, however, enough cells survived the low pH conditions to grow and eventually reach a population density similar to the high pH vessel. By the end of the experiment at day 26, the DIC concentration approached zero, again indicating that DIC was limiting further population cell growth.

The high CO₃/high pH experimental vessel showed consistently greater particulate calcium levels as cell population increased over 8 days (see Fig. 3 of Quarterly Report # 11). In the high CO₂/low pH closed vessel, however, the particulate calcium concentration was low through day 10. By day 20, the particulate calcium increased by 100 times the particulate calcium on day 1. However, at day 26 the particulate calcium was only 50% that of the high CO₃/high pH closed vessel on day 8.

The fugacity of CO₂ (fCO₂) at 1-atm pressure is very similar to the partial pressure of CO₂ in water. At the beginning of the experiment, the fCO₂ in the low CO₂ vessel was about 400 μ atm, whereas in the high CO₂ vessel, the initial fCO₂ was about 3 times higher, at 1200 μ atm (see Fig. 2 in Quarterly Report #12). It is particularly interesting that the high CO₂ level did not kill all the cells; although their population decreased initially, the final population density was as high as in the low CO₂ vessel and the final fCO₂ was also similar.

Cell growth in natural seawater enriched with nitrate and trace metals at elevated pCO₂

The two closed vessels started the experiment with very different CO₂/carbonate chemistries. After bubbling the “High CO₂” vessel with CO₂ gas, the initial pCO₂ of the seawater was 735 μ atm and contained a dissolved CO₂ concentration of 26.5 μ mol kg⁻¹. After bubbling a mixed gas with a lower CO₂ concentration in the second closed vessel, this “Normal CO₂” vessel began the experiment with an initial pCO₂ of 390 μ atm and a dissolved CO₂ concentration of 13.9 μ mol kg⁻¹. We then inoculated each vessel with a small volume of *E. huxleyi* coccolithophorid cells and ran the experiment for 18 days. The growth of the cells clearly changed the CO₂ chemistry of the ambient seawater in both closed vessels. Although the “High CO₂” vessel began the experiment with a pCO₂ value almost two times that of the “Normal CO₂” vessel, the high CO₂ conditions were transient and did not persist after the cell population reached mid-late exponential growth phase. With cell growth and calcification, the levels of DIC and total alkalinity (TA) declined in both vessels (Fig. 1). Cells in both vessels were likely carbon-limited by the termination of the experiment.

As shown in Fig. 2, the total number of cells ml⁻¹ in the two vessels was similar throughout the experiment. Cell populations in both CO₂ treatments reached stationary phase on about day 10. The maximum number of cells produced was slightly greater in the “High CO₂” vessel.

Although the total number of cells in the “High CO₂” vessel was equal to or greater than cell densities in the “Normal CO₂” vessel, the percentage of calcified cells vs. non-calcified cells was significantly lower in the “High CO₂” vessel (Fig. 3). Even after the CO₂ chemistry of the seawater in the initially “High CO₂” vessel reached the conditions of the “Normal CO₂” vessel, the lower percentage of calcified cells relative to non-calcified cells persisted.

The particulate calcium measurements were used to calculate the particulate inorganic carbon (PIC) production in each vessel. As Fig. 4A reveals, the particulate calcium

concentration in the “High CO₂” vessel was initially lower than the particulate calcium in the “Normal CO₂” vessel. The “High CO₂” vessel had a lower particulate calcium concentration than the “Normal CO₂” vessel until about day 13. After day 13 and until the end of the experiment, the particulate calcium concentration in both vessels was equivalent. As shown in Fig. 4B, the decrease in total alkalinity mirrored the increase in particulate calcium concentration in both vessels over the course of the experiment.

Examination of the cells with light microscopy and scanning electron microscopy (SEM) revealed that in response to high CO₂ conditions, cells in the “High CO₂” vessel were either fully calcified or were naked (i.e., without coccolith plates). Figure 5A illustrates a heavily-calcified cell next to a naked cell from a subsample of the “High CO₂” vessel. Figure 5B is a photomicrograph of cells from the “Normal CO₂” vessel. In the “Normal CO₂” vessel, more than 95% of the total number of cells were calcified by the end of the experiment. In contrast, about 60% of the total number of cells in the “High CO₂” vessel were calcified at the termination of the experiment. Although the percentage of calcified cells was lower in the “High CO₂” vessel, preliminary SEM work revealed no discernible differences in the morphology of coccoliths produced under high CO₂ conditions. Figure 6 depicts a SEM photograph of a cell taken from the “High CO₂” vessel on day 3 of the experiment.

Conclusions and Overview of Future Work

The results of these experiments suggest that when the coccolithophorid *Emiliani huxleyi* cell strain CCMP 371 is grown in F/50 media nitrate is still in excess when stationary growth phase is reached. We conclude that cell growth in our experiment was limited by insufficient dissolved inorganic carbon, not nitrate. Experiments comparing cell growth and calcification in F/50 media made with high CO₃/high pH seawater versus high CO₂/low pH seawater substantially delayed the population growth in the low pH vessel relative to the high pH vessel. Although similar maximum cell densities were achieved over very different timeframes, the particulate calcium content of the low pH vessel was only half that of the high pH vessel at the end of the experiment. The population growth in the low pH vessel was initially delayed compared to that of the high pH vessel. Although the fCO₂ was about 3 times higher in the high CO₂ vessel than the low CO₂ vessel, cells survived to eventually reach logarithmic growth and a final density of nearly $1 \times 10^7 \text{ ml}^{-1}$. The decrease in dissolved CO₂ was particularly striking in the high CO₂ vessel, as cells took up the excess dissolved CO₂ and may have been carbon limited at the end of the experiment.

The results using natural seawater and two different values of pCO₂ at the start of the experiment indicate that initially high pCO₂ promotes the production of particulate organic carbon and increases the proportion of non-calcified cells relative to calcified cells. However, cells exposed to initially high CO₂ conditions displayed a similar population growth rate and maximum density at stationary phase compared to cells in

“Normal CO₂” seawater. Finally, preliminary SEM work revealed no discernible differences in coccolith morphology when cells were exposed to “High CO₂” conditions.

Our workplan for the next year focuses on three areas:

- ? Completion of the development of methods to artificially weather concrete by bubbling CO₂ into an aqueous solution and determination of the amount of nutrients must be added to that media for good coccolithophorid growth. The final solution must be economical to prepare and able to support healthy growth of coccolithophorid algae.
- ? Completion of preliminary experiments investigating coccolithophorid growth and calcification using low salinity water and waste concrete. The use of waste concrete to supply the calcium and carbonate needed for CaCO₃ formation and deposition in coccolithophorid algae would expand the use of these organisms in CO₂ abatement in power plants located in inland areas.
- ? Preparation and submittal of the final report for this project. The final report will summarize the research efforts for this project, describes its results, and provide recommendations for application of coccolithophorid algae in reducing anthropogenic CO₂.

References

- Dickson, A. G. and Goyet, C. (1994). Handbook of Methods for the Analysis of the Various Parameters of the Carbon Dioxide System in Sea Water. U.S. Department of Energy.
- Guillard, RRI (1975) In: Smith, WH & Chanley, MH (eds.) *Culture of Marine Invertebrate Animals*. Plenum. New York, 726 pp.
- Keller, MD, Selvin, RC, Claus, W & Guillard, RRI (1987) *Journal of Phycology*. 23: 633-638.

A

Cell Growth, Alkalinity, and DIC in Normal CO₂ Vessel

B

Cell Growth, Alkalinity, and DIC in High CO₂ Vessel

Fig. 1. Changes in cell density (black circles), total dissolved inorganic carbon (DIC; open triangles), and total alkalinity (black triangles) in closed vessel experiments with *Emiliania huxleyi* cell strain CCMP 371 grown at different initial conditions. (A) The “Normal CO₂” vessel had an initial $p\text{CO}_2$ value of 390 μatm . (B) The “High CO₂” vessel had an initial $p\text{CO}_2$ value of 735 μatm .

Fig. 2. Changes in the total number of cells in the “High CO₂” vessel (black circles) and the “Normal CO₂” vessel (open circles) during an 18-day experiment in which *E. huxleyi* cells were grown in closed vessels with different initial *p*CO₂ values.

Fig. 3. Changes in the percentage of cells that were calcified during the experiment. Results from the closed vessel which started with an initially high $p\text{CO}_2$ are denoted with black circles and data from the closed vessel which started with a “Normal CO₂” value are shown with open circles.

Fig. 4. Changes in (A) the particulate calcium concentration versus time and (B) total alkalinity versus time in the “High CO₂” vessel (black circles) and the “Normal CO₂” vessel (open circles).

Fig. 5. Photomicrographs of *Emiliani huxleyi* cells grown in closed vessels with different initial $p\text{CO}_2$. (A) “High CO_2 ” at start of experiment produced both calcified and non-calcified cells; arrows point to naked or non-calcified cells. (B) “Normal CO_2 ” treatment at start of experiment produced primarily calcified cells, as evidenced with polarizing optics. Size bars in both photographs are 10 μm .

Fig. 6. SEM photograph of an *Emiliana huxleyi* cell grown in “High CO₂” in a closed vessel experiment. This cell was sampled on day 3 of the experiment. Preliminary work revealed no clear differences in coccolith morphology in cells exposed to transient high CO₂ conditions.