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_2) 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_2 from the environment through the growth of organisms such as coccolithophorids, which are capable of sequestering CO_2 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_2 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_2 emissions from power plants. Cultivation of coccolithophorids for calcium carbonate ($CaCO_3$) 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_2 into the sea and ocean fertilization. Consequently, cultivation of coccolithophorids could be carried out immediately and the amount of carbon sequestered as $CaCO_3$ 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_2 sequestration through coccolithophorid calcite production and to evaluate the costs/benefits of using coccolithophorid cultivation ponds to abate CO_2 emissions from power plants.

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Introduction

The 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 biological and chemical 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 obtain maximum 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. This report describes progress made towards Task 4.0 which investigates coccolithophorid calcification using low-salinity water and waste concrete that is artificially weathered by the addition of CO_2 gas. Here we report our preliminary results on the growth and calcification of coccolithophores in seawater prepared by artificially weathering concrete by bubbling CO_2 into an aqueous solution.

Experimental

Building on our results described in the last quarterly report, we prepared seawater media enriched with alkalinity resulting from the artificial weathering of waste concrete. We used seawater of two different salinities (20‰ and 33‰) in 2-L jars. To each replicate jar (n=3) we added porous bags containing waste concrete broken into medium – fine size pieces ($\leq$ 1mm) and then bubbled bottles with concentrated CO_2 gas for 48 hours. Three replicate bottles for each salinity treatment were also prepared with similar porous bags of waste concrete and bubbled with compressed air as a control. After the bubbling of CO_2 gas and compressed air, subsamples were taken for total alkalinity measurements determined by potentiometric titration (Dickson and Goyet, 1994).

After the total alkalinity measurements confirmed that the artificial weathering of waste concrete by CO_2 gas resulted in seawater with substantially higher alkalinity, we then sterile filtered the different media types and inoculated replicate flasks with *Emiliania huxleyi* CCMP 371 that had been previously acclimated to the appropriate salinity (i.e., 20‰ or 33‰). Flasks were kept in a growth chamber at $17^{?}$ C in a light: dark cycle of 16 hours light: 8 hours dark. After 10 days, cell counts were conducted on replicate samples and the percentage of calcified cells in each treatment was recorded.

In a second thrust of our work during this period, we acquired three new coccolithophorid cultures: two of the bloom-forming coccolithophore *Gephyrocapsa oceanica* and one of coccolithophore *Coccolithus pelagicus*, which contains up to 50 times more calcite than *Emiliania huxleyi*. We are growing these cultures in different media in order to find the media which best promotes growth and calcification. If we can successfully maintain these new cultures, we will investigate their rates of calcification and cell growth and compare them to those we have previously measured in *Emiliania huxleyi* CCMP371.

Results and Discussion

The total alkalinity in the water samples differed significantly (Table 1). In the normal salinity (33‰) seawater, the artificial weathering of waste concrete with CO_2 gas resulted in a total alkalinity of 7083 µmol kg^{-1}, an increase of 320%. In contrast, the treatment of bubbling in compressed air into 33‰ seawater containing waste concrete resulted in a total alkalinity value of 2777 µmol kg^{-1}, an increase of 560 µmol kg^{-1} over the initial total alkalinity. In the low salinity seawater (20‰), the CO_2 weathering of waste concrete resulted in a total alkalinity of 7285 µmol kg^{-1} and the compressed air treatment resulted in a total alkalinity value of 2107 µmol kg^{-1}.

Table 1. Mean values of total alkalinity (µmol kg^{-1}) produced by bubbling CO_2 gas or compressed air into low and normal salinity seawater containing waste concrete.

	33‰ Salinity	20‰ Salinity
Bubbled with CO_2 gas for 48 h	7083	7285
Bubbled with compressed air for 48 h	2777	2107

Growth of *Emiliania huxleyi* CCMP 371 in each seawater treatment occurred over the 10-day incubation period. Cell growth rates were highest in the high alkalinity, 33‰ seawater, reaching more than 2 x 10^5 cells ml^{-1}. In each treatment, the percentage of calcified cells was high (e.g., see Figure 1), ranging from 75 to 97%. No significant differences in calcified cells were observed between treatments, however, this preliminary result requires additional testing.

The newly acquired coccolithophore cell lines are being kept in F/2 and F/50 media (Guillard, 1975). To date, these cell lines appear to be growing satisfactorily. Figure 2 illustrates the differences in cell size in *Emiliania huxleyi* CCMP 371, the two strains of *Gephyrocapsa oceanic*, and the one strain of *Coccolithus pelagicus*.

Conclusion

Preliminary experiments were conducted investigating the growth and calcification of the coccolithophore *Emiliania huxleyi* CCMP 371 in low salinity and normal salinity seawater which had been treated by bubbling CO_2 or compressed air over waste concrete. Cells grew in all seawater treatments and had high percentages of calcified cells. Cell growth rates were greatest in normal salinity seawater in which the alkalinity had been increased more than 3 times by the artificial weathering of waste concrete. These preliminary results should be confirmed by additional experiments.

The next phase of experimental work will extend the results presented here on the growth and calcification of *E. huxleyi* CCMP 371 on media prepared by artificially weathering waste concrete. In addition, we will conduct experiments to determine which media composition results in maximal growth and calcification of *Coccolithus pelagicus*, a coccolithophore species which contains up to 50 times more $CaCO_3$ than *E. huxleyi*.

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, R.R.I. (1975) In: Smith, WH & Chanley, MH (eds.) *Culture of Marine Invertebrate Animals*. Plenum. New York, 726 pp.

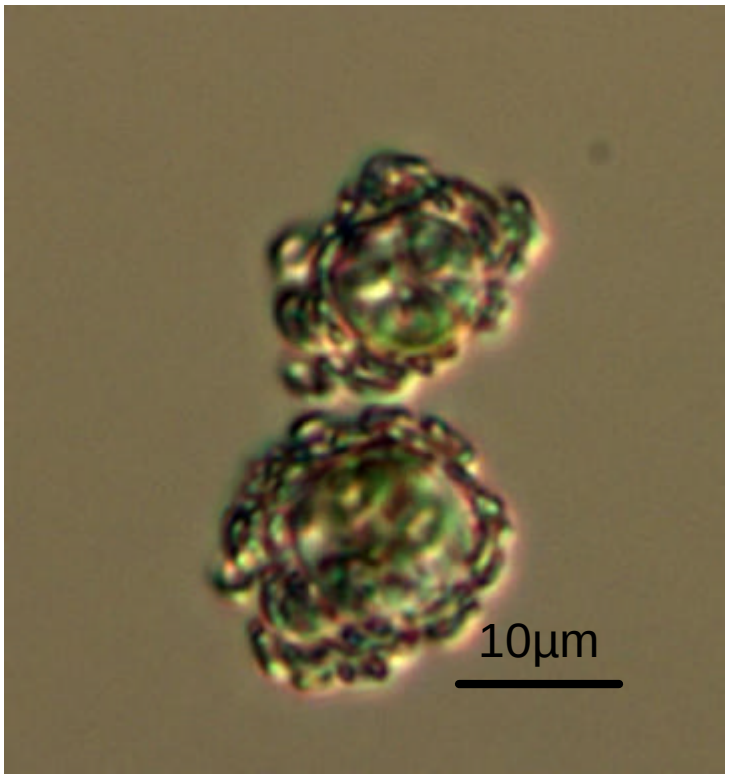

Figure 1. Photomicrograph of heavily calcified cells of *E. huxleyi* CCMP 371 grown in seawater with 33‰ salinity and high alkalinity.

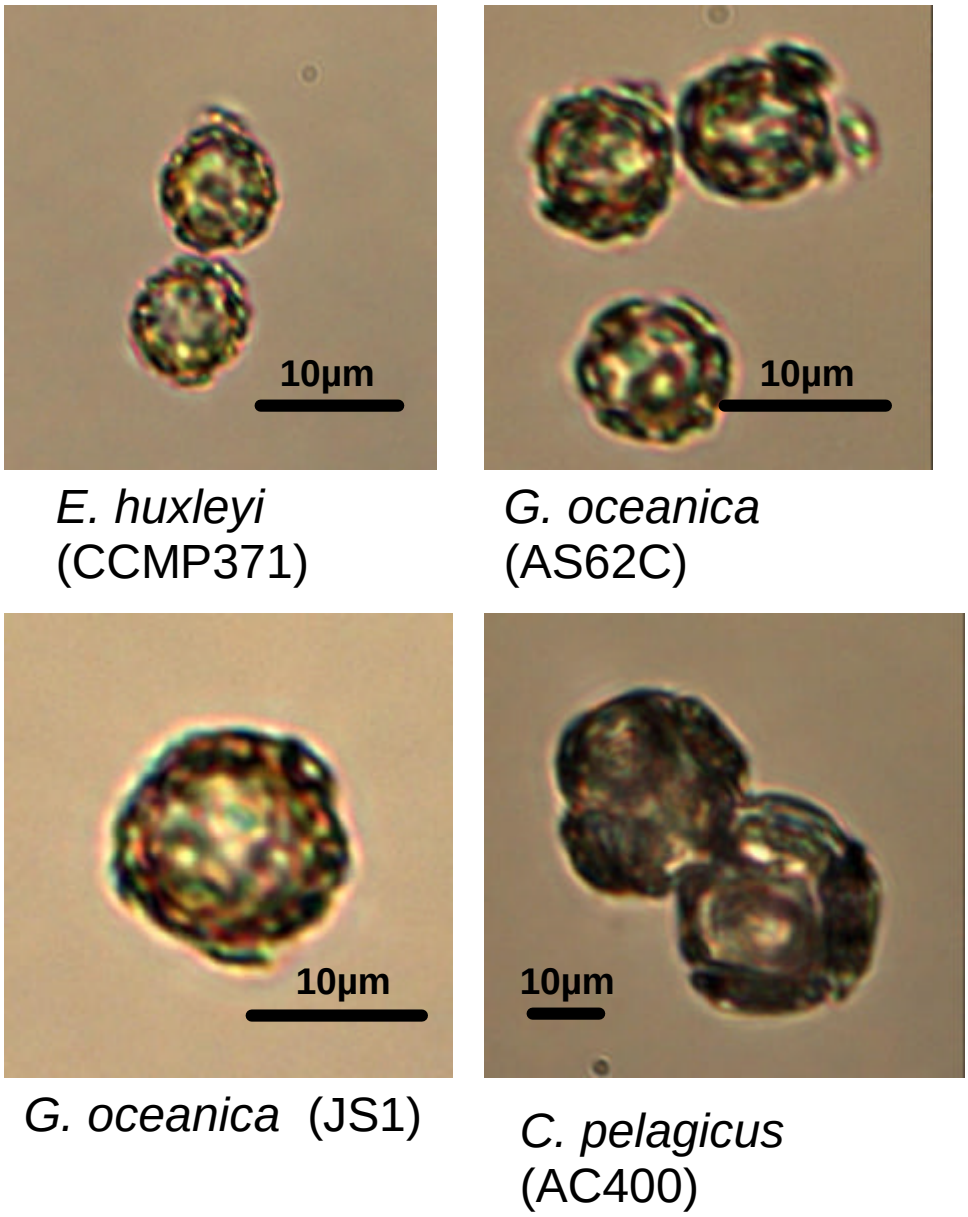

Figure 2. Photomicrographs of four cell lines representing three species of coccolithophores currently maintained in our lab. Note difference in size between *Coccolithus pelagicus* and *Emiliania huxleyi*.