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

**Type of Report:** Quarterly Progress Report #6  
**Reporting Period Start Date:** October 1, 2002  
**Reporting Period End Date:** December 31, 2002

**Principal Author:** V. J. Fabry, Ph.D.

**Date Report Was Issued:** December 15, 2002

**DOE Award Number:** DE-FC26-01NT41132

**Name and Address of Submitting Organization:**  
Dr. V. J. Fabry  
Department of Biological Sciences  
California State University San Marcos  
San Marcos, CA  92096-0001
Disclaimer:

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.
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.
Table of Contents

| Title Page | 1 |
| Disclaimer | 2 |
| Abstract | 3 |
| Table of Contents | 4 |
| List of Figures | 5 |
| Introduction | 6 |
| Experimental | 6 |
| Results and Discussion | 6 |
| Conclusion | 7 |
| References | 7 |
# List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.</td>
<td>Cell growth in different cell lines of <em>E. huxleyi</em> in low salinity media, with and without selenium.</td>
<td>8</td>
</tr>
<tr>
<td>Figure 2.</td>
<td>Nomarski and polarized light photomicrographs of <em>E. huxleyi</em> grown in low salinity medium.</td>
<td>9</td>
</tr>
</tbody>
</table>
**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 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.

**Experimental**

We continued experiments investigating coccolithophorid cell growth as a function of selenium enrichment. Previously, we focused primarily on K media (Keller et al., 1987); in the experiments described here, we used F/50 media (Guillard, 1975). K media contains nitrogen in the form of nitrate and ammonium, phosphorus in the form of organic phosphate ($\beta$-glycerophosphate), EDTA as a chelator, and selenium. In contrast, F/50 media contains nitrogen only as nitrate, inorganic phosphate, about 20 times less EDTA than K media, and no selenium.

In other experiments, we grew *E. huxleyi* cell strains CCMP 371 and 374 in low-salinity (20 ppt), F/50 media and investigated cell growth as a function of selenium enrichment.

**Results and Discussion**

Our results indicated that the addition of selenium to F/50 media did not significantly increase the population growth rate or the maximum cell density reached at stationary phase in calcifying *Emiliania huxleyi* cell strains CCMP 371, 374, or 1516. Moreover, when compared to growth in K media, these strains exhibited similar growth rates, but lower maximum cell densities (approximately 0.5 order of magnitude lower).

Consistent with our previous results in F/50 media which typically has a salinity of 31 ppt, the addition of selenium did not increase the growth rate or the maximum cell density achieved (Fig 1).

An unexpected result of the low salinity experiments was the appearance of inordinately heavily calcified cells. While only 25 to 50% of the total number of cells were calcified, those cells which were calcified had multiple layers of coccoliths. In many cases, the extra layers of coccoliths resulted in a coccosphere (cell plus attached coccoliths) diameter that was twice the diameter of the cell itself (Fig. 2). In future work, we plan to attempt to produce a monoculture of these extraordinarily heavily calcified cells.
Conclusion

We continued to investigate the hypothesis that selenium is a requirement for coccolithophorid growth. Our data using three calcifying cell lines of *E. huxleyi* indicated that selenium enrichment alone does not increase the rate of cell growth. In other experiments, we grew coccolithophorids in low salinity water. Although not all cells calcified in low salinity water, those that did showed extraordinarily high calcification, with multiple layers of calcified coccoliths readily visible with light microscopy. We will investigate this unexpected result in the future. This part of the project has particular relevance to Task 4, which will investigate the efficacy of using low salinity water supplied with alternative calcium sources, such as waste concrete, to grow coccolithophorids for CO$_2$ sequestration.

References


Fig 1. Cell growth in different cell lines of *Emiliania huxleyi* at a salinity of 20ppt with or without selenium enrichment in F/50 media. (A) *E. huxleyi* strain CCMP371 with and without selenium. (B) E. huxleyi strain CCMP 374 with and without selenium.
Fig. 2. Nomarski and polarized light photomicrographs of *Emiliania huxleyi* strain CCMP 371 grown in F/50 at a low salinity of 20 ppt. Cell diameter = 5-7 micrometers (without coccoliths). (A) Upper cell non-calcified and lower cell heavily calcified. (B) Single cell with multiple layers of coccoliths. (C) Two cells surrounded by multiple layers of coccoliths. (D) Aggregate of 4-5 cells with multiple layers of coccoliths.