**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<sub>2</sub> 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<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  $CO_2$  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 developing a method for separating calcareous coccoliths from cell organic matter such that the coccoliths may be recovered for biomedical and/or industrial uses.

# **Experimental**

Building on our previous work, we conducted experiments designed to fine tune our method of solubilization of the organic matter of coccolithophore cells while preserving the ultrastructue of the coccolith calcite. Our overarching objective was to eliminate as much organic matter as possible while preserving the surface microstructure of the calcareous coccoliths. These minute calcium carbonate structures may then be recovered and potentially used in biomedicine or industrial applications. Towards this end, we investigated whether we could reduce the duration of exposure to the detergent treatment such that the organic matter associated with cells would still be digested, but lessening the possibility of altering the surfaces of calcareous coccoliths.

We used a procedure modified from Paasche et al., 1996. First. we grew cultures in f/50 media (Guillard, 1975) under conditions described in previous reports. Subsequently, cell cultures were subsampled and centrifuged at 1000 rpm for 10 minutes. The resulting pellet was resuspended in 3 ml of supernatant. A 1% solution of Triton X-100 in 0.05 M NaHCO<sub>3</sub> was added to the resuspended pellet. Two drops of commercial NaOCl (ca. 8%) was added. The flask was then placed on rotating shaker at 100 excursions min<sup>-1</sup> for 15 minutes. The experimental control consisted of a subsample centrifuged and resuspended in the same manner. The control samples received the same detergent treatment, however, control samples were exposed to the detergent for twice as long (i.e., 30 minutes).

#### **Results and Discussion**

Examination by light microscopy revealed that the shortened Triton X-100 treatment solubilized fewer cells. Many coccospheres were still intact, with 1-3 layers of coccoliths covering the cell surface. Consequently, the reduced exposure time did not result in the

uniform suspensions of coccoliths as was observed in the control treatment which had twice the exposure time. Thus, at the cell concentrations we used ( $6 \times 10^7$  cell ml<sup>-1</sup>), separation of the calcareous coccoliths from organic matter requires more than 15 minutes of exposure to the Triton-X solution combined with the NaOCl to digest the organic cytosol and any possible organic coverings of the individual coccoliths. In contrast, the control treatment which exposed cells to the detergent treatment for 30 minutes appeared to digest nearly all of the organic matter associated with cells and result in a uniform, suspension of coccoliths, as determined by light microscopy.

# **Conclusion**

We investigated whether we could reduce the exposure time to solubilizing agents during the method we previously used to detach coccoliths from the cell surface of coccolithophores. Examination with light microscopy revealed that the reduced exposure time to the detergent did not effectively digest the organic matter. Results suggest that the modified method we had previously developed was superior and resulted in a uniform suspension of calcareous coccoliths. The last remaining task for this project is to draft and submit the final project report.

# **References**

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