Quantitative Laboratory Measurements of Biogeochemical Processes Controlling Biogenic Calcite Carbon Sequestration

Frank J. Zendejas, Pamela D. Lane and Todd W. Lane

Prepared by Sandia National Laboratories
Albuquerque, New Mexico 87185 and Livermore, California 94550

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy’s National Nuclear Security Administration under contract DE-AC04-94AL85000.

Approved for public release; further dissemination unlimited.
Quantitative Laboratory Measurements of Biogeochemical Processes Controlling Biogenic Calcite Carbon Sequestration

Frank J. Zendejas, Pamela D. Lane, and Todd W. Lane

Systems Biology Department and Biotechnology and Bioengineering Department
Sandia National Laboratories
PO Box 969
Livermore, CA 94550-0969
Contents

Executive Summary ................................................................................................................................. 6
1. Transcriptomic Analysis ...................................................................................................................... 7
   Introduction........................................................................................................................................... 7
   Materials and Methods ......................................................................................................................... 9
   Results .................................................................................................................................................. 10
   Discussion.......................................................................................................................................... 13
2. Calcite dissolution versus pressure ............................................................................................... 13
   Introduction.......................................................................................................................................... 13
   Materials and Methods ......................................................................................................................... 15
   Results ................................................................................................................................................. 17
   Discussion.......................................................................................................................................... 20
   Conclusions....................................................................................................................................... 22
   References.......................................................................................................................................... 22
   Distribution ........................................................................................................................................ 24

List of Figures
Figure 1: Growth curves for Emiliania huxleyi bubbled at various $p\text{CO}_2$................................. 11
Figure 2: Derived image of purified RNA separated on the Bioanalyzer. ........................................ 12
Figure 3: Average coverage of mRNA sequences .................................................................................. 13
Figure 4: A schematic representation of the calcium dissolution apparatus........................................ 14
Figure 5: six 50 mL conical tubes used to harvest a single 24 L culture of Emiliania huxleyi............. 17
Figure 6: Dissolution of calcium carbonate vs. pressure in calcium-free ESAW media..................... 18
Figure 7: Emission spectra of calcium standards in ESAW media....................................................... 18
Figure 8: Calcium carbonate dissolution vs. pressure ......................................................................... 19

List of Tables
Table 1: RNA for transcriptomic analysis............................................................................................ 12
Executive Summary

The purpose of this LDRD was to generate data that could be used to populate and thereby reduce the uncertainty in global carbon cycle models. These efforts were focused on developing a system for determining the dissolution rate of biogenic calcite under oceanic pressure and temperature conditions and on carrying out a digital transcriptomic analysis of gene expression in response to changes in $p$CO2, and the consequent acidification of the growth medium.
1. Transcriptomic Analysis

Introduction

The open ocean is the principal sink of atmospheric CO₂. Long-term sequestration of CO₂ requires dissolution into the surface layers, uptake by photosynthetic organisms, and eventually into ocean sediments. Significant uncertainties in computation models of climate change arise from a lack of fundamental understanding and accurate parameterization of biogeochemical processes involved in oceanic carbon sequestration. Elements of uncertainty arise from insufficient data on both 1) the behavior of the underlying biological systems that fix carbon at the ocean surface and properties of the resulting biogenic materials, and 2) the geochemical processing of biogenic carbon that occurs under relevant deep ocean conditions. Because of the magnitude of fluxes of oceanic carbon cycles, this lack of data leads to poorly constrained models of global carbon fluxes, and high levels of scientific uncertainty in the resulting predictions of CO₂ levels.

Recently it has been recognized that, aside from potential contributions to global warming, there is a "second problem" arising from increased atmospheric CO₂: that of acidification of the ocean surface layers. It is well established that total atmospheric CO2 levels are rising. Human activities presently release about 7.1 Petagrams of carbon per year (PgC/yr) to the atmosphere, by fossil fuel burning and land use change: this CO₂ has to go somewhere, and much of it dissolves directly into the ocean. Of the 7.1 PgC/yr, 3.3 PgC/yr remain in the atmosphere, while the open ocean behaves as a sink estimated to be 1.9 PgC/yr (the terrestrial biosphere is often assumed to trap the remaining 1.9 PgC/yr). The increased flux of dissolved CO₂ resulting from elevated total atmospheric CO2 leads, through elementary chemical processes considerably less complex than those involved in atmospheric greenhouse effects, to lower ocean surface pH, unless it is buffered or fixed and transported to the deep ocean. However, the buffering capacity of the ocean surface waters is well measured and not large, and acidification of ocean surface layers is now well documented. Increase in $pCO_2$ has
been shown to result in a decrease in biogenic calcium carbonate formation in a variety of experiments involving single species and natural assemblages (for review see Doney et al 2009.) However, the net effects on long-term carbon sequestration by the ocean are depend on carbon fixing capacity of the organisms dominant under increasingly acid ocean surface conditions, and the geochemical fate of biogenic carbon that is fixed by them.

This LDRD is designed to address shortcomings in our understanding of biogenic calcite deposition and dissolution, and provide to climate modelers improvements in the accuracy and uncertainty of key parameters. We have focus on producing data from organisms responsible for major biogenic carbon fluxes. A specific group of marine algae referred to as the Coccolithophores are considered to be the most productive calcifying organism on earth. As part of the carbon acquisition and fixation process, coccolithophores produce calcite plates, called coccoliths, which eventually cover the cell surface. The most abundant coccolithophore, *Emiliania huxelyi* often forms massive blooms in temperate and sub-polar oceans, and in particular at continental margins and shelf seas. These blooms are often visible in satellite imagery. Coccolithophores produce low magnesium calcite -- the form that is most resistant to dissolution and likely to be buried in sediment.

A focus of the LDRD was to measure, under thermodynamic conditions found in the deep ocean, the rates of chemical reactions involved in the geochemical processing of coccoliths produced by *E. huxleyi*, using material grown in temperature and pH ranges relevant to modern ocean surface conditions. The understanding of the regulation of calcite deposition and the effects of increased $pCO_2$ and pH on cellular physiology is rudimentary at best (Gangsto et al 2008). There have been several studies measuring calcite dissolution under varying conditions of temperature and pressure (for review see Morse et al 2007), but most of these were carried out with reagent grade calcite. It is clear however that biogenic calcite displays significantly different solubility characteristics than the abiotically produced form (Morse et al 2006). Although it has been observed that increased $pCO_2$ causes a decrease in calcification in a number of relevant species it is not clear what effect that increase will have on productivity and the basic biochemistry of carbon acquistion. We hypothesize that the
underlying physiology of the cell will also impact the structure of the biogenic calcite and that will directly affect its dissolution behavior.

We will culture *E. huxelyi* under different conditions of pCO$_2$ and seawater chemistry. We measure the resulting calcification rates and characterize the morphology of the coccoliths by scanning electron microscopy. We will harvest coccoliths from culture grown under various conditions and determine their rate of dissociation under different conditions of pressure and seawater chemistry. We will use and artificial seawater formulation so that we can rigorously control the concentration of the relevant constituents. At the same time we will carry out a digital transcriptomic analysis of culture to correlate specific growth conditions to physiological state and regulation of the calcification process. Digital transcriptomics will be carried out by ultra high throughput sequencing of mRNA.

The digital transcriptomics analysis will be correlated with physiological data and will allow us to identify biomarkers indicative of specific physiological conditions. These biomarkers can then be used to rapidly characterize the physiological state of laboratory cultures and, more importantly, field populations. The development of biomarkers that can rapidly attest to the physiological state of field population is of significant importance when trying to extend laboratory results to ecosystem studies. It allow the field researcher to replace time consuming physiological studies and shipboard incubations with assays that can be used directly on the field samples allowing larger numbers of assays to be carried out and a reduction in experimental error due to “bottle effects”. Because *E. huxelyi* is the most ubiquitous calcifying microalgal species, biomarkers identified for this species can play a significant role biological oceanography.

**Materials and Methods**

**Strains and Culture Conditions.**

An axenic culture of *Emiliania huxelyi* (CCMP1516) was obtained from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton at Bigelow Laboratory for Ocean Sciences (West Boothbay Harbor, MA). Cultures were maintained in ESAW medium (Harrison *et al* 1980) a 16/8 hr light/dark cycle, at 23°C and 1000 µmol photons
m$^{-2}$ s$^{-1}$. Cell numbers were determined by direct counting using a Coulter Z2 particle counter (Beckman Coulter Inc, Fullerton, CA). Experimental cultures were grown in ESAW in 1 L polycarbonate bottles and bubbled with 100ppm CO$_2$, room air or 750ppm CO$_2$ (Figure 1). Experimental cultures were inoculated from mid logarithmic phase cultures that had been maintained for at least 3 passages under the same nutrient and CO$_2$ conditions as the experimental cultures. Cultures were grown to early logarithmic and harvested 75 minutes after the start of light phase by gentle filtration (5 mm Hg vacuum) onto three micron pore size nucleopore filters (Whatman, USA) which were immediately frozen in Liquid nitrogen and at -80 °C until RNA extraction.

**RNA extraction.**

Cell pellets were resuspended in Tri Reagent (Sigma St Louis MO) at a concentration of 2.5 X 10$^7$ cells•mL$^{-1}$. Total RNA was extracted from each sample following the manufacturers instructions. Quality of the purified total RNA was characterized by electrophoretic analysis using an Agilent bioanalyzer and both purity and concentration of the RNA was determined by UV spectrometry (Figure 2). 150-350 micrograms of purified total RNA (Table 1) was delivered to the Eureka Genomics for each sample.

**Nucleic acid sequencing and analysis**

Transcriptomic analysis by next generation nucleic acid sequencing was carried out by Eureka Genomics using 51 base-pair, paired-end sequencing on an Illumina platform. The samples were indexed and run three per lane. Sequencing generated approximately 15 million reads per sample or about 750 Mbases of sequence data per sample. These read were mapped back to the predicted transcriptome of *E. huxelyi* CCMP 1516 produced by the Joint Genome Institute (Walnut Creek, CA) and the transcript abundances were determined.

**Results**
Preliminary results indicated that transcripts from the genes encoding the flagellar structure were not present. This result is not inconsistent with the diploid, calcifying form of the organism present in the culture. Surprisingly little variation was observed in the transcript levels across \( p\text{CO}_2 \) treatment. Specific transcripts, such as those encoding carbonic anhydrase, which seemed likely to vary in abundance with \( p\text{CO}_2 \), showed little variation.

Figure 1: Growth curves for Emiliania huxleyi bubbled at various \( p\text{CO}_2 \)
Figure 2 Derived image of purified RNA separated on the Bioanalyzer.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>ng/ul</th>
<th>Vol (ul)</th>
<th>ug RNA</th>
<th>260/280</th>
</tr>
</thead>
<tbody>
<tr>
<td>020810, 100 ppm RNA</td>
<td>88.76</td>
<td>200</td>
<td>17.8</td>
<td>1.82</td>
</tr>
<tr>
<td>020810, Air RNA</td>
<td>82.34</td>
<td>200</td>
<td>16.5</td>
<td>1.81</td>
</tr>
<tr>
<td>020810, 750 ppm RNA</td>
<td>82.3</td>
<td>200</td>
<td>16.5</td>
<td>1.77</td>
</tr>
<tr>
<td>021210, 100 ppm RNA</td>
<td>72.33</td>
<td>200</td>
<td>14.5</td>
<td>1.77</td>
</tr>
<tr>
<td>021210, Air RNA</td>
<td>133.65</td>
<td>133</td>
<td>17.8</td>
<td>1.86</td>
</tr>
<tr>
<td>021210, 750 ppm RNA</td>
<td>89.05</td>
<td>200</td>
<td>17.8</td>
<td>1.84</td>
</tr>
<tr>
<td>022710, 100 ppm RNA</td>
<td>67.58</td>
<td>200</td>
<td>13.5</td>
<td>1.76</td>
</tr>
<tr>
<td>022710, Air RNA</td>
<td>129.83</td>
<td>200</td>
<td>26.0</td>
<td>1.82</td>
</tr>
<tr>
<td>022710, 750 ppm RNA</td>
<td>155.57</td>
<td>200</td>
<td>31.1</td>
<td>1.84</td>
</tr>
</tbody>
</table>

Table 1
Figure 3: Average coverage of mRNA sequences by reads from 100ppm (y axis) and 750ppm (x axis) samples. Each point corresponds to a single mRNA sequence from database. The Linear Regression line is shown in magenta. The red lines correspond to upper and lower limit of the three (3) standard deviations Confidence Band (under Gaussian assumptions underlying regression model this should include 99.7% of observations). Shown in green are the scaffolds exhibiting statistically significant differences in average coverage between samples.

Discussion

2. Calcite dissolution versus pressure

Introduction

To measure the rates of biogenic calcite dissolution, we will design and build a variation of the reactor used by Wolf et al (1989) capable of operating under higher pressures (Figure 4). The reactor used by Wolfe et al was designed to operate under low pressure. We anticipate operating at pressures up to those that would be encountered at or about the depth of the lysocline. Currently the lysocline is at an average depth of 3700 to 4500 meters which corresponds to a pressure of
approximately 5300 to 6400 psi. We will design our reactor to operate under the appropriate pressure and temperature regime. To prevent precipitation of the calcite upon pressure relief, it would be optimal if the determination of dissolve calcite was carried out in situ. We will investigate a variety of gravimetric and spectrographic methods for determining either residual solid or dissolved calcite. We will compare the rate of dissolution of biogenic calcite in the form of coccoliths to that of reagent grade calcite. By using an artificial seawater formulation we will be able to control the concentrations of the relevant chemical species and correlate cell physiology, and coccolith production conditions and resulting structure with dissolution behavior of coccoliths.

Figure 4. A schematic representation of the calcium dissolution apparatus consisting of a high pressure syringe pump with controller, recirculation bath, reactor, and UV-plate reader. The inset is a close up of our packed reactor.
Materials and Methods

The same methods and procedures developed to culture the *Emiliania huxelyi* for the digital transcriptomics work were also used to cultivate the coccolithophorid cells for the dissolution studies. For the dissolution studies the main objective was to yield the maximum amount of cells; therefore, three 24L cultures were harvested between days 8 and 10. The cells were harvested and pooled together using a Sharples continuous centrifuge (Sharples Specialty Company, Philadelphia, PA) and subsequently stored at -80°C until coccolith purification.

The calcium dissolution apparatus consisted of a Teledyne ISCO Model 260D high pressure syringe pump capable of handling flow rates from tens of microliters to hundreds of milliliters per minute with a resolution of 1.0 µL min⁻¹ at pressures of (10 – 7500 psi). The syringe pump was controlled using a Teledyne ISCO series D pump controller (Teledyne ISCO, Lincoln, NE). This setup provided flow and pressure control throughout our operating range. Fused silica capillary tubing was used to plumb the syringe pump to the inlet of the packed column and from the outlet of the column to the low dead volume flow restrictor valve (High Pressure Equipment Company, Erie, PA). The temperature of the column was maintained by submersion in a LAUDA-Brinkmann Ecoline RE106 (LAUDA-Brinkmann, Delran, NJ) refrigerating circulator. The syringe was filled with calcium-free ESAW media.

A CapTite™ microfluidic refillable cartridge/column (LabSmith, Livermore, CA), was vacuum packed with a slurry of reagent grade calcium carbonate (98%, Sigma Aldrich, St Louis, MO). All of the initial calcium dissolution experiments focused on calcium carbonate as the model compound and later experiments would focus on packed columns containing the purified biogenic coccoliths for comparison.

Before running the dissolution experiments, the column was conditioned. First, we allowed the column to acclimate to the desired experimental temperature for 4 hrs. Secondly, since the column was originally vacuum packed, the column was packed a second time using the high pressure syringe pump. To do this the flow restrictor valve was closed and the column was pressurized to 7000 psi. The restrictor valve was then
slightly opened to allow a flow rate of 1.0 mL min\(^{-1}\) at the outlet for 5 minutes. After five minutes the column pressure was decreased to 500 psi and the valve closed. This process was repeated for a total of five times, at which point the column was considered properly packed.

To understand the calcium carbonate dissolution rate at the various experimental operating conditions we began by setting the column temperature and pressure to 3.5°C and 1000 psi respectively. The flow restrictor valve was then opened to allow a constant flow rate of 1.5 mL min\(^{-1}\). Samples were collected every 2 minutes for the first 10 minutes, then every 5 minutes for the next 20 minutes and finally every 15 minutes for the next 60 minutes. The experiment was repeated at 3.5°C and 5000 psi. From these experiments, it was determined that the concentration of calcium in solution increased within the first 5 minutes, but quickly reached steady state after 10 minutes. As a result, samples were collected 20 minutes after changing the operating pressure.

To assay the calcium in solution, we used a 500 mM stock solution of Fluo-4FF calcium (Ca\(^{2+}\)) binding dye from Invitrogen (Invitrogen, Carlsbad, CA). As the sample was collected it was immediately diluted (five fold for 3.5°C and 13.5°C experiments), and (fifteen fold for 23.5°C experiments) with calcium-free ESAW media to ensure that the calcium did not precipitate out of solution. Then 200 µL of the diluted sample was transferred to a 96 well plate and the calcium binding dye was added (100 µM final concentration). Using a Sectra Max Gemini XS plate reader (Molecular Devices, Sunnyvale, CA) the excitation wavelength was set to 488 nm, while the emission wavelength was scanned between (400 – 600 nm). The emission peak was measured at 520 nm. Using calcium calibration standards we determined the concentration of calcium in each sample taking into account the background.

To purify the coccoliths from the -80°C stored *Emiliania huxelyi* cells, we used the process described by De Jong et al. (1976). The first step of the process was to suspended the pellet in calcium-free ESAW media and ultrasonicate the solution for 1 minute. The solution was centrifuged at 10k G’s for 10 minutes. The supernatant was removed and the pellet was suspended by ultrasonication in a 50 mM solution of ammonium bicarbonate (98%, Sigma Aldrich, St Louis, MO). A sucrose gradient centrifuge step was done to isolate the coccoliths from the cells. The sucrose gradient
consisted of 20 mL of a (1:1) Ludox:DI-water solution adjusted to a pH of 8 using 4 M HCl, followed by 5 mL of 20% sucrose, followed by 4 mL of the ammonium bicarbonate solution containing the *Emiliania huxelyi* cells. The centrifuge tube was spun at 23k G’s for 20 minutes. The pellet was washed with 50 mM ammonium bicarbonate and centrifuged. The washing step was repeated a total of five times.

**Results**

The growth rates for our *Emiliania huxelyi* cultures reached a final cell density of \((6.8 \pm 0.2) \times 10^6\) cells mL\(^{-1}\) in approximately 8 – 10 days. The harvested *Emiliania huxelyi* yielded \((0.286 \pm 0.032\) grams L\(^{-1}\)\) of wet biomass is shown in Figure 5.

![Figure 5](image)

Figure 5. A digital photograph depicting six 50 mL conical tubes used to harvest a single 24 L culture of *Emiliania huxelyi* at day 8. The conical tube at the far right displays the cells prior to being centrifuged and stored at -80ºC.

We measured the dissolution rate of calcium carbonate at a temperature of 3.5ºC and pressures of (1000 and 5000 psi) over a 90 minute period. The results of the experiments are shown in Figure 6. The flow restrictor valve was opened to allow a constant flow rate of 1.5 mL min\(^{-1}\). When the pressure was set to 1000 psi, the concentration of calcium measured between times (0 – 8 min) was (30 – 32 µM). After 10 minutes the concentration of calcium measured in solution had stabilized at 36 µM for the duration of the experiment. The experiment was repeated with the pressure increased to 5000 psi. The concentration of calcium measured between times (0 – 8 min) was (145 – 156 µM). After 10 minutes we observed a similar trend to that of the 1000 psi experiment. At 5000 psi the concentration calcium measured in solution had
stabilized at 182 µM. The concentrations for each sample collected were calculated using calcium standards curves measured at 520 nm using Fluo-4FF dye at a final working concentration of 100 µM. The standards curves are shown in Figure 7.

Figure 6. Dissolution of calcium carbonate vs. pressure in calcium-free ESAW media. The temperature was set at 3.5°C and pressures of (1000 and 5000 psi) were investigated. In both instances the dissolution rate reached a steady state after 10 minutes. The data was collected in triplicates.
Figure 7. Emission spectra of calcium standards in ESAW media. Excitation wavelength was 488 nm, while the emission wavelength was scanned between (480 – 600 nm). The emission peak was measured at 520 nm.

As a result of the calcium carbonate dissolution rate study it was determined that it was necessary to wait 20 minutes after changing the pressure before collecting a sample. Although we found that the dissolution rate stabilized after 10 minutes we decided to be conservative in our sample collection approach.

Using the high pressure syringe pump along with the temperature controlled recirculation bath we demonstrated the dissolution of calcium carbonate with varying pressures and temperatures. Figure 8 shows the concentration of calcium in solution while maintaining a constant temperature of (3.5º, 13.5º, and 23.5ºC) at varying pressures (1, 2, 3, 4, and 5×10³ psi). For temperature settings of (3.5º and 13.5ºC) the concentration of calcium in solution gradually increased from (30 to 124 µM) and from (300 – 363 µM) respectively, as the pressure was increased. When the temperature was set to 23.5ºC the concentration ranged between (423 and 479 µM).

Figure 8. Calcium carbonate dissolution vs. pressure. Three different temperatures were investigated (3.5º, 13.5º, and 23.5ºC). The pressure was varied between (1000 – 5000 psi). The concentration of calcium carbonate measured in solution increased with increasing temperature and pressure. Data is based on triplicates.
Discussion

In order to measure the rates of calcium carbonate and biogenic calcite dissolution we built a reactor capable of operating under high pressures. Two critical components to our system were the high precision syringe pump which was capable of generating up to 7500 psi and the pump controller. Using conventional HPLC capillary tubing we connected the pump to the packed column. Our operating pressures ranged between (1000 and 5000 psi) which are similar to those that would be encountered at or about the depth of the ocean where the rate of calcite dissolution increases significantly. We also invested three operating temperatures (3.5º, 13.5º and 23.5ºC). At pressures greater than 5000 psi the temperature of the ocean would most likely correspond to 3.5ºC. Our primary objective was to compare the rate of dissolution of biogenic calcite from purified *Emiliania huxleyi* coccoliths to that of reagent grade calcite (calcium carbonate).

We observed that our *Emiliania huxleyi* cultures began to reach a peak cell density after 8 days, therefore we harvested between days 8 – 10. We produced 20.6 grams of wet biomass from three 24 L cultures, whereas De Jong et al. (1976) generated 30 grams of biomass from a 300 L culture. Since the reported biomass values are based on wet mass and not lyophilized mass it is difficult to directly compare our results with their values.

To compare the rate of dissolution of biogenic calcite to that of reagent grade calcite we opted to use calcium carbonate. We vacuum packed our column using a modified technique described by Lynen *et al* (2005) and Chirica *et al* (1999). Simply, the column is opened at both ends and a membrane is placed at one end of the column and sealed with a capillary fitting. A vacuum is placed at the capillary fitting end while the column is packed with calcium carbonate slurry from the opposite end. Once the column is completely filled, a membrane is carefully placed at the filling end. Lastly, a capillary fitting seals the opening.

We intended to measure the dissolved calcite *in situ* to prevent precipitation of the calcite upon pressure relief, but we determined that filling a 250 mL syringe pump with
enough dye to ensure a final working concentration at the detector of 100 µM was not realistic. In order to run in situ we would need to use a micro syringe pump capable of producing the pressures necessary for this research. We therefore prevented precipitation by minimizing the time the sample was not under pressure as well as quickly diluting the sample into calcium-free media. By using a 96 well plate reader we minimized the amount of sample and dye required for our measurements.

As described earlier, the dissolution rate of calcium carbonate reached a steady state after 10 minutes regardless of which pressure we investigated at 3.5°C. Although we did not repeat this experiment for the two other experimental temperatures, we expect a similar trend. Additionally, we sampled after 20 minutes to make certain that a steady state dissolution rate was reached. The measured dissolution of calcium carbonate not only increased as a function of increased pressure, but also as a function of increased temperature which was expected. Although we observed a four fold decrease of calcium carbonate in solution from 23.5°C to 3.5°C we nonetheless achieved a significant dissolution of calcium carbonate at the lowest temperature setting. An advantage of using a high pressure dissolution apparatus is the ability to provide calcium dissolution rate data within minutes whereas other previous low pressure studies required days (Wolf et al 1989).

We attempted to purify the coccoliths using the technique described by De Jong et al (1976) with little success. We found it difficult to recover any significant amount of coccoliths using their sucrose gradient. Either the coccoliths ended up trapped in the sucrose gradient, or we lost the coccoliths during the washing steps. A few possible approaches to increase the likelihood of success are to centrifuge more than 4 mL of Emiliania huxelyi at a time, or adjust the sucrose gradient concentration. Another approach would be to use a different coccolith purification technique altogether like those described by Takano et al (1993) and Marsh et al (1992) Using these new purification approaches we could potentially collect enough coccolith material to pack a column in order to perform a direct comparison between the rates of dissolution of biogenic calcite and reagent grade calcite.
Conclusions
As mentioned earlier the main focus of this research was to address shortcomings in our understanding of biogenic calcite deposition and dissolution, and provide to climate modelers improvements in the accuracy and uncertainty of key parameters. To deliver on our objectives we successfully built a reactor capable of operating under highly controlled pressures and temperatures analogous to those of the deep ocean. We grew cultures of *Emiliania huxelyi* with biomass yields similar to those found in the literature. To measure the concentration of calcium in solution we developed a method capable of assaying the dissolution rate of calcium. Although we did not carry out any dissolution experiments with biogenic calcite, our current setup and assaying method provides the groundwork to test a column packed with purified coccoliths. In addition, other variables such as the pH of the media can also be easily adapted with our current setup to investigate the role that pH undertakes in the dissolution of calcium.

References


Gangsto, R.; Gehlen, M.; Schneider, B.; Bopp, L.; Aumont, O. Joos, F., Modeling the marine aragonite cycle: changes under rising carbon dioxide and its role in shallow water CaCO₃ dissolution *Biogeoosci* 2008, 5:1057-1072


Wolf, M.; Breitkopf, O.; Puk, R., Solubility of calcite in different electrolytes at temperatures between 10° and 60°C and at CO2 partial pressures of about 1 kPa. Chemical Geology 1989, 76 (3-4), 291-301.
## Distribution

<table>
<thead>
<tr>
<th></th>
<th>MS</th>
<th>Name</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>MS9292</td>
<td>Todd Lane,</td>
<td>8623 (Electronic Copy)</td>
</tr>
<tr>
<td>1</td>
<td>MS9292</td>
<td>Joseph Schoeniger,</td>
<td>8623 (Electronic Copy)</td>
</tr>
<tr>
<td>1</td>
<td>MS</td>
<td>Pamela Lane</td>
<td>8623 (Electronic Copy)</td>
</tr>
<tr>
<td>1</td>
<td>MS</td>
<td></td>
<td>8621 (Electronic Copy)</td>
</tr>
<tr>
<td>1</td>
<td>MS9291</td>
<td>Catherine Branda,</td>
<td>8623 (Electronic Copy)</td>
</tr>
<tr>
<td>1</td>
<td>MS0899</td>
<td>Technical Library,</td>
<td>4536 (Electronic Copy)</td>
</tr>
<tr>
<td>2</td>
<td>MS9018</td>
<td>Central Technical Files,</td>
<td>8944</td>
</tr>
<tr>
<td>2</td>
<td>MS0899</td>
<td>Technical Library,</td>
<td>4536</td>
</tr>
<tr>
<td>1</td>
<td>MS0123</td>
<td>D. Chavez, LDRD Office,</td>
<td>1011</td>
</tr>
</tbody>
</table>