American Mineralogist, Volume 83, pages 1503–1509, 1998

Production of carbonate sediments by a unicellular green alga

KIMBERLY K. YATES* AND LISA L. ROBBINS†

Geology Department, University of South Florida, 4202 E. Fowler Avenue, SCA 203, Tampa, Florida 33620, U.S.A.

ABSTRACT

This study investigates the ability of the unicellular green alga *Nannochloris atomus* to precipitate $CaCO_3$, quantifies mineral precipitation rates, estimates sediment production in a *N. atomus* bloom, and discusses the implications of microbial calcification for carbonate sediment deposition. A series of *N. atomus* cultures, isolated from Lake Reeve, Australia, were incubated at various pH and calcium concentrations to determine environmental parameters for calcification. Rates of calcification were calculated from initial and post-incubation alkalinity, pH, and calcium measurements. Replicate experiments and controls consisting of non-calcifying cultures, uninoculated media, and dead cell cultures were performed using environmental culture parameters determined in series cultures. Average calcification rates from replicate experiments were used to predict daily sediment production rates in a small bloom of *N. atomus. N. atomus* precipitates 0.138 g/L of calcite in approximately 4 h when incubated at pH 8.5, 14.24 m*M* calcium concentration, 33 °C, 100 $\mu E/m^2/s$ light intensity, and a cell population density of 10^7 cells/mL. Assuming continuous precipitation, this corresponds to a maximum estimated sediment production rate of 1.6×10^6 kg of $CaCO_3$ per 12 h day in a single bloom of 3.2×10^9 L. Our results suggest that microbial calcification contributes significantly to the carbonate sediment budget.

INTRODUCTION

The association of microbes with various types of calcium carbonate precipitation has long been noted in ancient and modern sedimentary environments. Microbial calcification has been suggested by the presence of fossil organisms resembling cyanobacteria and micro-algae in deposits ranging in age from Precambrian to Holocene (Horodyski and Mankiewicz 1990; Awramik 1991; Barattolo 1991; Flugel 1991; Mamet 1991; Riding 1991; Riding and Guo 1991; Roux 1991; Kazmierczak et al. 1994; Thompson et al. 1997). Several modern species of cyanobacteria and unicellular green algae are capable of producing calcium carbonate minerals in both natural and laboratory environments. Recently discovered calcifying microbes include such genera as the freshwater cyanobacteria *Synechococcus* (Thompson and Ferris 1991) and *Scytonema* (Merz 1992), unicellular green algae *Chlorella* (Stabel 1986) and *Chlorococcum* (Hartley et al. 1995), and marine cyanobacteria *Synechococcus* and *Synechocystis* (Yates and Robbins 1995). These species are ubiquitous in freshwater and marine ecosystems (Stockner 1988). The presence through geologic time and throughout the world of microbial species capable of precipitating calcium carbonate indicates that microbial calcification may have a significant impact on carbonate sedimentation and the CO_2 budget. However, only a few attempts have been made to quantify this process.

Species of the green alga *Nannochloris* are found in various freshwater (Naumann 1921; Sarokin and Carpenter 1982), marine (Butcher 1952; Ryther 1954; Jeffrey 1961; Thomas 1966), and brackish (Droop 1955) environments and are capable of rapidly adapting to salinity fluctuations (Brown 1985). The distribution of this organism is comparable to that of *Chlorella* and *Chlorococcum* sp. and is, thus, an ideal organism for investigating calcification potential in microalgal species.

This study examines the potential for precipitation of calcium carbonate by the unicellular green alga, *Nannochloris atomus,* collected from Lake Reeve in attempt to gain insight into the potential magnitude of microbial calcification. Environmental conditions for calcium carbonate precipitation in laboratory cell cultures were determined and used to calculate rates of calcification. These measurements were then used to predict the amount of $CaCO_3$ sediment that may be produced in a bloom of *N. atomus.*

METHODS

Collection and culture of microbes

Unfiltered water samples were collected from Lake Reeve, Australia and stored in 100 mL Nalgene bottles for transport back to the laboratory. These samples were initially examined via polarized light microscopy to determine general cell types associated with calcium car-

* Current address: U.S. Geological Survey, Center for Coastal Geology, 600 4th Street South, St. Petersburg, Florida 33701, U.S.A.

† E-mail: robbins@chuma.cas.usf.edu

0003–004X/98/1112–1503$05.00

bonate precipitation. Cultures containing primarily *Nannochloris atomus* cells were isolated from these water samples by inoculating 1% agar (wt./vol.) plates made with BG11 media (Rippka et al. 1981) prepared with a salinity of 58 by adding additional NaCl (the salinity of Lake Reeve during time of collection), using a standard culture loop, and incubating at 26–28 °C and 40 μE/m²/s light intensity. Cells from colonies on agar plates were subsequently transferred to 10 mL of liquid BG11 media and incubated until growth (observed as green coloration) of culture occurred. Cells from liquid cultures were then transferred back to 1% agar plates, and this procedure was repeated until isolated colonies were produced. Semi-continuous cultures were produced from 10 mL isolate cultures by raising culture volumes by addition of BG11 media to >3 L. These cultures were maintained at 27 °C and 40 to 100 μE/m²/s light intensity generated using alternating cool white and Grow Lux fluorescent lights (12 h light/dark cycles), and by periodically removing 10–33% culture volume and replacing this volume with fresh media. *Nannochloris atomus* was identified by Barry Rosen (Southwest Florida Water Management District) using criteria of Marshal (1986) and Griffith (1961). Samples were removed from larger volume semi-continuous cultures for use in experiments.

Calcium carbonate precipitation experiments

Calcium concentrations in Lake Reeve varied from approximately 10.0 to 32.5 m*M* (calculated from Davis et al. 1995), pH ranged from approximately 8 to 9, and temperature varied from approximately 24 °C to greater than 30 °C. Cultures of *Nannochloris atomus* were incubated in BG11 media with pH and calcium concentrations similar to those of natural Lake Reeve water, ranging in pH from 8.0 to 10.0 (increments of 0.5 pH unit), and ranging in calcium concentration from 8.24 to 14.24 m*M* at each pH increment. These culture parameters are also similar to those required for calcification by *Synechoccocus* and *Synechocystis* (Yates and Robbins 1995). This series of cultures was used to determine environmental parameters required for calcification in laboratory cultures within a reasonable and controlled time period. These culture parameters and incubation periods were then used in all subsequent calcification experiments. Cultures were prepared by removing aliquots (typically 100 to 500 mL) of cell culture from semi-continuous stock culture (pH 8.13) and adjusting to the desired pH by adding either HCl or NaOH. Appropriate amounts of 1 *M* $CaCl_2$ mixed in Na_2CO_3-free BG11 media (0.8 to 1.4 mL to yield final concentrations of 8.24–14.24 m*M*) were then added to 100 mL volumetric flasks, and the volumes were brought to 100 mL by adding pH adjusted *Nannochloris atomus* culture. Immediately after preparing cultures, 20 mL aliquots of each were removed and pressure filtered through 0.22 μm Gelman nylon acrodisc filters attached to syringes. Initial total alkalinity (T_{alk}), pH, and calcium concentration ([Ca]) were measured on the filtered aliquots. Total alkalinity and pH were measured (±0.02 meq/L and ±0.07 unit, respectively) using the Gran titration method (Dryssen and Sillen 1967) and a Brinkman 702 SM Titrino automated titrator calibrated with Tris base, Tris HCl seawater pH buffers at an ionic strength of 0.7. Samples were prepared for calcium analyses by diluting known volumes of filtered culture with 1 *M* HCl and storing in air-tight vials for analysis via acetylene flame atomic absorption spectrophotometry (AAS).

Cultures were then incubated at 33 °C and 100 μE/m²/s light intensity with low speed stirring, and examined periodically under a polarized light microscope for the onset of mineral precipitation. After precipitation had occurred, final alkalinity, pH, and calcium concentrations were measured as described above.

AAS calcium measurements were used to quantify precipitation of calcium carbonate assuming a 1:1 relationship between calcium and $CaCO_3$ such that a change in [Ca] corresponds stoichiometrically to the amount of $CaCO_3$ precipitated (decrease in [Ca] from initial to final measurements) or dissolved (increase in [Ca] from initial to final measurements). Carbonate alkalinity (C_{alk}) and dissolved inorganic carbon (DIC) were calculated in the following manner using equations modified from McConnaughey (1991):

$$K_1 = [HCO_3^-][H^+]/[H_2CO_3] = 4.68 \times 10^{-7}\ M \text{ at } 33\ ^\circ C \quad (1)$$

$$K_2 = [CO_3^{2-}][H^+]/[HCO_3^-] = 5.46 \times 10^{-11}\ M \text{ at } 33\ ^\circ C \quad (2)$$

$$[C_{alk}] = [T_{alk}] - ([OH^-] - [H^+]) \quad (3)$$

$$[DIC] = [C_{alk}]([H^+]/K_1 + 1 + K_2/[H^+]) \div (1 + 2K_2/[H^+]) \quad (4)$$

where C = change in [Ca, mols/L], initial-final measurement; $[H^+] = -\log$ pH; and $[OH^-] = Kw/[H^+]$. Dissociation equilibrium constants K1 and K2 for carbonic acid were calculated using free energy of formation values (G_f^0) from Faure (1991) via $\ln K = \Delta G^0/-RT$ where ΔG^0 = standard free energy of reaction, R = 1.9865 cal/mol·K, and T = 298.15 K. K1, K2, and Kw were corrected for experimental temperature of 33 °C using the equations $\ln K_2/\ln K_1 = -\Delta H^0/R(1/T_2 - 1/T_1)$ where K_1 = known constant, K_2 = unknown constant, T1 = temperature of known constant, and T2 = temperature of unknown constant, and $\log Kw = -4470.99/T + 6.0875 - 0.01706\ T$ where T = temperature in degrees Kelvin.

Replicate experiments were performed on cultures prepared at the same combination of pH and calcium concentration that exhibited precipitation of $CaCO_3$ in series cultures as observed by polarized light microscopy and calcification calculations. Control experiments consisted of cell cultures incubated at the same pH, temperature, and light intensity as replicate cultures without addition of $CaCl_2$, uninoculated (cell-free) BG11 media and dead cell cultures (prepared by adding 0.1% sodium azide) in-

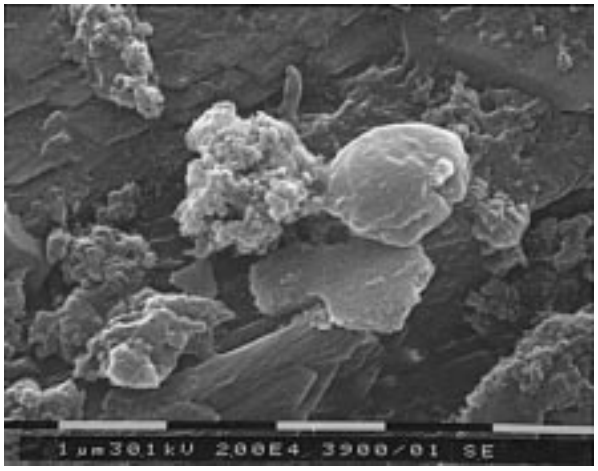

FIGURE 1. SEM photomicrograph of a single *Nannochloris atomus* cell showing a small seed crystal and a larger clump of crytals attached to different locations on the cell membrane. Note larger crystals in the background, which are characteristic of later stages of crystallization. Scale = 1 μm.

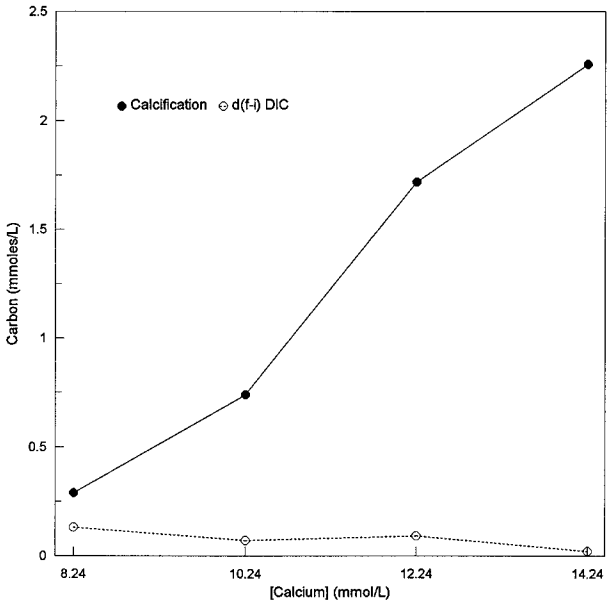

FIGURE 2. Cultures of cell population density 1.19×10^7 cells/mL were incubated at pH 8.5, 33 °C, and 100 μE/m²/s light intensity with calcium concentrations of ranging from 8.24 to 14.24 m*M*. Optimum calcification occured at 14.24 m*M* [Ca]. DIC is presented as ΔDIC = post- incubation DIC values − pre-incubation DIC values. Note lack of DIC shift commensurate with calcification. Calcification calculated as the change in [Ca] from pre- to post-incubation is represented on the *y* axis as millimoles per liter of carbon (see text).

cubated with chemical and physical parameters identical to those of calcifying replicates. All replicates and controls were incubated for 4 h, the amount of time required for $CaCO_3$ precipitation in initial series cultures.

Autotrophic cell counts were performed on cell cultures after initial pH adjustment using an Olympus epifluorescent microscope fitted with a blue-violet cube, Whippel grid, and ocular micrometer following the procedures of Boehme et al. (1993). Cells per grid were counted for each of 10 frames from each of three slides per culture, and cells per milliliter were calculated as cells/mL = (cells/grid)(filter area/grid area)(1/volume filtered). Cells per milliliter values reported represent the average cell count from three slides counted per culture.

X-ray diffraction analysis

Calcium carbonate precipitation was confirmed using X-ray diffraction (XRD) analysis. Cells and calcium carbonate were collected by centrifugation at 7000 g for 15 min. After discarding the supernatant, pellets were resuspended in ethanol to remove water and remaining soluble salts, then centrifuged again to collect cells and $CaCO_3$. Pellets were allowed to air dry overnight, then ground in a mortar and pestle, and analyzed on quartz glass slides via Scintag X-ray diffractometer.

RESULTS

Calcification was observed in *N. atomus* series cultures incubated at pH 8.5 with calcium concentrations of 8.24, 10.24, 12.24, and 14.24 m*M* within 4 h of incubation. No calcification was observed in cultures incubated at higher and lower pH with the same range of calcium concentrations. Examination of cell cultures via polarized light microscopy (magnification of 400 to 1000×) and scanning electron microscopy (SEM) revealed individual cells with morphologically indistinct seed crystals (Fig. 1) adjacent to outer cell membranes and, occasionally, clumps of cells with needle-shaped crystals. Maximum $CaCO_3$ precipitation of 2.26 mmoles/L in 4 h occurred in *N. atomus* cultures containing 1.19×10^7 cells/mL at pH 8.5 and 14.24 m*M* [Ca] (Fig. 2). Calcification increased with increasing calcium concentration at pH 8.5 while DIC showed no significant change. Alkalinity and pH measurements indicate only a slight decrease of 0.15 pH unit and 0.09 mmoles/L in 4 h, respectively, corresponding to the maximum calcification rate at [Ca] of 14.24 m*M*. (Table 1).

Replicate experiments performed at pH 8.5, [Ca] of 14.24 m*M*, and average cell density of 3.20×10^7 cells/mL resulted in average calcification and DIC shifts of 1.46 mmoles/L in 4 h and 8.4×10^{-5} mmoles/L in 4 h, respectively, with calcification ranging from 0.61 to 2.31 mmoles/L in 4 h (Fig. 3). Again, only slight decreases in alkalinity and pH were observed in these experiments (Table 1).

Control experiments consisting of *N. atomus* cultures (average cell density 3.4×10^7 cells/mL) incubated at pH 8.5 with no additional $CaCl_2$ added (i.e., not induced to calcify) showed only a slight decrease in average [Ca] of 0.085 mmoles/L in 4 h corresponding to an average decrease in DIC of 4.4×10^{-5} mmoles/L in 4 h (Fig. 3). Alkalinity and pH showed no change from initial mea-

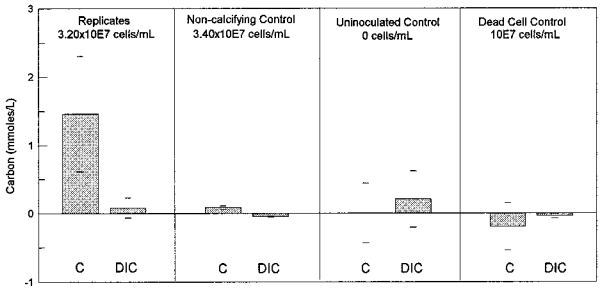

FIGURE 3. Calcification and DIC trends for replicate cultures of calcifying *Nannochloris atomus* at [Ca] of 14.24 m*M*, non-calcifying control cultures, and uninoculated BG11 media at 14.24 m*M* [Ca]. Each set of calcification and DIC peaks represents averages from triplicate experiments. Horizontal bars represent calculated standard deviations. Calcification calculated as the change in [Ca] from pre- to post-incubation is represented on the *y* axis as millimoles per liter of carbon (see text), assuming a stoichiometic 1:1 relation between Ca and C in calcium carbonate.

surements (Table 1). No crystals were observed via polarized light microscopy in these cultures. Therefore, the decrease in [Ca] from initial to final measurements most likely represents the portion of calcium bound or metabolized by cells. To arrive at true calcification values, the average portion of bound calcium from non-calcifying controls (0.085 mmoles/L in 4 h) was subtracted from the average calcification observed in replicate experiments of 1.46 mmoles/L in 4 h resulting in an actual calcification value of 1.38 mmoles/L in 4 h or 0.138 g/L of calcium carbonate precipitated in 4 h. XRD results from replicate experiments allowed to incubate for seven days confirm the precipitation of calcite with a primary peak occurring at *d*-spacing 3.03 Å (Fig. 4).

Control experiments consisting of uninoculated (algae-free) BG11 media incubated under the same chemical and physical parameters as calcifying cultures showed no crystals after 4 h when examined via polarized light microscopy and no significant change in [Ca] or alkalinity (Fig. 3, Table 1). However, average pH showed a greater decrease (0.31 pH unit) in uninoculated controls than in any other experimental cultures presumably due to re-equilibration of controls with atmospheric CO_2 during the incubation period (Table 1). Control experiments consisting of sodium azide killed cells incubated in BG11 media at pH 8.5, [Ca] of 14.24 m*M*, 33 °C, and 100 E/m²/s also showed no crystals after 4 h when examined by polarized light microscopy and no significant change in [Ca] or DIC (Fig. 3, Table 1).

TABLE 1. Total alkalinity and pH measurements

Culture description	Pre-incubation measurements				Post-incubation measurements			
	pH*	std.	T_{alk}*	std.	pH*	std.	T_{alk}*	std.
8.24 m*M* [Ca]	8.19	0.05	1.59	0.13	7.98	0.08	1.50	0.04
10.24 m*M* [Ca]	8.12	0.01	1.49	0.01	8.01	0.02	1.45	0.03
12.24 m*M* [Ca]	8.07	0.03	1.49	0.01	7.91	0.01	1.44	0.01
14.24 m*M* [Ca]	7.97	0.00	1.47	0.01	7.82	0.01	1.38	0.01
Reps. 14.24 m*M*								
A	8.33	0.00	1.59	0.00	8.18	0.00	1.51	0.00
B	8.19	0.01	1.65	0.01	8.18	0.01	1.60	0.01
C	7.97	0.00	1.47	0.01	7.82	0.01	1.38	0.01
Non-calcifying								
A	8.38	0.01	2.06	0.01	8.41	0.01	2.07	0.00
B	8.37	0.00	2.06	0.01	8.39	0.00	2.06	0.00
C	8.35	0.00	2.06	0.01	8.36	0.01	2.05	0.00
BG11 controls								
A	8.03	0.02	0.69	0.01	7.69	0.06	0.69	0.01
B	7.97	0.03	0.68	0.00	7.69	0.02	0.68	0.00
C	7.96	0.02	0.68	0.00	7.64	0.04	0.68	0.00
Dead cultures								
A	8.22	0.01	4.08	0.03	8.17	0.01	3.99	0.01
B	8.19	0.01	4.05	0.02	8.14	0.00	3.94	0.01
C	8.17	0.01	4.06	0.01	8.14	0.01	3.92	0.01

Notes: pH = 8.5. Cultures labeled A, B, and C represent replicates within an experimental group. std. = standard deviation.

* Total alkalinity (meq/L) and pH measurements represent averages of two measurements per filtered sample. These average values were used to calculate photosynthesis and calcification values for each culture.

DISCUSSION

Mineralogy and sediment production

The solution chemistry, carbonate speciation, and mineral saturation with respect to calcite was calculated for BG11 at standard and experimental calcium concentrations as described by Yates (1996). While experimental cultures and controls were supersaturated (SI = 91.4) with respect to calcite, controls showed no mineral precipitation within the incubation period of 4 h. Periodic microscopic examination of semi-continuous stock cultures of *N. atomus* in undersaturated conditions frequently showed mineral precipition indicating that cells are capable of inducing precipitation in undersaturated media, and that cellular metabolism likely plays a role in generating a microenvironment near the cell that is conducive to calcification. Results of calcium carbonate precipitation experiments indicate that the presence of live *Nannochloris atomus* cells are required for precipitation of calcite in BG11 at the experimental parameters described above. The absence of calcite precipitation at pH values above 8.5 may have been due to a shift in response of cellular metabolism to changing hydrogen ion concentration. Cells must maintain intracellular pH and, therefore, shift the ratio of $[H^+]_{in}/[H^+]_{out}$ in response to changes in pH in the media. While we have some insight into the types of ion fluxes that may play a role in generating microenvironments of calcification (McConnaughey and Falk 1991; Yates 1996), the effects of cellular stress due to changing environmental conditions on calcification remain to be examined. However, the absence of calcification at pH values above 8.5 again emphasizes the role of cellular metabolism in precipitation. In cultures with pH greater than 8.5, mineral precipitation should have been enhanced if calcite was precipitating inorganically because of the increased calcite saturation state.

The calcite mineralogy of precipitates by *N. atomus* is consistent with that of natural sediments associated with

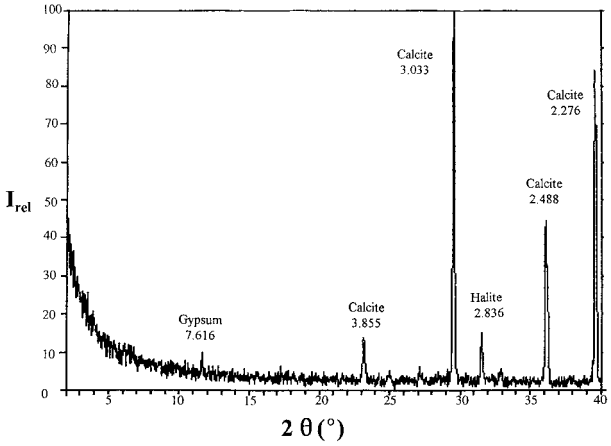

FIGURE 4. X-ray diffraction pattern from replicate calcium carbonate precipitation experiments. Cultures of *Nannochloris atomus* showed precipitation of calcite after 7 days of incubation. *y* axis is in relative intensity. Peaks are labeled with *d* spacings in angstroms.

microbes from Lake Reeve Australia (Davis et al. 1995). The absence of precipitation in controls containing dead cells indicates that organic material is not simply acting as a seed for nucleation of mineral precipitation, but that the metabolism of these cells plays a role in inducing mineral precipitation. Based on average calcification rates of 0.138 g/L per 4 h by *N. atomus* in replicate experiments (average cell population density 3.20×10^7 cells/mL), microbial calcification may have a significant impact on deposition of carbonate sediments and cycling of inorganic carbon. As a first esitmate, if a constant rate of precipitation throughout a 12 h light cycle was assumed, these organisms could produce approximately 0.5 g/L of calcium carbonate in a 12 h day. In a single bloom of *N. atomus* of 0.64 km^2 and 5 m depth (i.e., 3.20×10^9 L) this production rate results in precipitation of 1.60×10^6 kg of $CaCO_3$ per day. The effect of *N. atomus* cell population density, daily variations in the rates of precipitation, and cell growth cycle on $CaCO_3$ precipitation remains to be determined. However, for a conservative estimate of sediment production rates in natural cell populations, it was assumed that $CaCO_3$ precipitation decreases proportionally with cell count. Adjusting the precipitation rate of 0.138 g/L per 4 h for a lower cell population density of 1.0×10^5 cells/mL [consistent with photosynthetic picoplankton cell counts in Bahamian whitings (Robbins et al. 1996)] yields a calcification value of 1.55 mg/L in 12hr. Considering a *N. atomus* bloom in a water mass of 3.20×10^9 L, this production rate results in 5.0×10^3 kg of $CaCO_3$ per day. These values are comparable to $CaCO_3$ sediment yield from Bahamian whitings, a potential product of microbial calcification (Robbins and Blackwelder 1992; Robbins et al. 1997).

Robbins et al. (1997) measured small whitings of 0.64 km^2 in water of approximately 5 m depth (volume 3.20×10^9 L). Assuming an average $CaCO_3$ density in whitings of 10.6 mg/L from data of Shinn et al. (1989), a small whiting of this size contains 4.0×10^4 kg of calcium carbonate in a given day. This value falls well within the range of values calculated for calcium carbonate production by *N. atomus* in blooms of equal size consisting of 3.20×10^7 and 1.0×10^5 cells/mL. Although the effects of dark cycle respiration on calcification and preservation potential of carbonates produced by unicellular green algae remain to be examined, clearly, given the extensive distribution of *Nannochloris* sp. alone, preservation and deposition of only a small portion of $CaCO_3$ potentially precipitated by this organism can contribute significantly to the carbonate sediment budget. Considering the wide distribution of other known calcifying microbes and others that may yet be discovered, microbial production of carbonate sediments may represent a phenomenon of tremendous magnitude.

DIC shifts and estimated photosynthesis

The initial series of calcium carbonate precipitation experiments examining calcification and DIC from [Ca] 8.24 to 14.24 m*M* showed no significant change in DIC. Replicate experiments at [Ca] of 14.24 m*M* showed a similar result. This is confirmed by the fact that only small shifts in both pH and alkalinity were observed from pre- to post-incubation in calcifying cultures. The lack of DIC shift commensurate with calcification or photosynthesis suggests that DIC utilization for calcification and photosynthesis was compensated for in some manner during this process. One possible explanation for this result has been addressed in great detail by McConnaughey (1994). He suggests that biological precipitation of calcium carbonate by a trans-calcification mechanism whereby Ci uptake and calcification occur at distinctly separate surfaces in a 1:1 calcification to photosynthesis ratio is characterized by only minor shifts in alkalinity and pH. Trans-calcification typically involves a transmembrane $Ca^{2+}/2H^+$ exchange creating a Ca^{2+}-rich, alkaline, extracellular microenvironment that absorbs CO_2 from cells, hydrates it to HCO_3^-, and precipitates $CaCO_3$ (McConnaughey 1991; McConnaughey and Falk 1991; McConnaughey 1994). In this mechanism, McConnaughey (1994) suggests that calcification generates CO_2 which is then utilized in photosynthesis resulting in consumption of HCO_3^- without affecting aquatic CO_2 concentrations. In addition, photosynthesis utilizes protons generated by calcification resulting in consumption of HCO_3^- without affecting pH. Furthermore, C/P ratios increase with increasing pH as more protons are required to convert Ci to CO_2 for photosynthetic uptake. The minor pH, alkalinity, and, thus, DIC shifts observed in calcifying cultures of *N. atomus* may result from the presence of a trans-calcification type mechanism in these organisms.

Another possible explanation for lack of DIC shifts during calcification results from effects of photosynthesis on ambient water chemistry. It is well known that microbes are capable of increasing the pH of media during

photosynthetic uptake of inorganic carbon (Ci). It is not unreasonable to assume that, in an open system, CO_2 is drawn into the medium at a rate near that of photosynthetic uptake resulting in only minor pH, alkalinity, and DIC shifts. It is unlikely that photosynthesis was inhibited at experimental pH, [Ca], and salinity because these values are typical for the natural environment of *N. atomus* in Lake Reeve. Pilot experiments (Robbins and Yates, unpublished manuscript) in which atmospheric pCO_2 fluctuations were monitored in closed-system environments containing cultures of photosynthesizing *N. atomus* cells (10^7 cells/mL) indicate that atmospheric pCO_2 decreases by approximately 0.358 mmoles/L in 4 h when incubated at 33 °C and 100 $\mu E/m^2/s$ light intensity. This result is consistent with net photosynthesis values calculated for *N. atomus* of 0.719 mmoles O_2/L in 4 h (Geider and Osborne 1986) and for *Chlorella vulgaris* of 0.227 to 2.50 mmoles O_2/L in 4 h in cultures ranging in NaCl concentration from 0 to 300 m*M* (Ahmed et al. 1989). Assuming this represents CO_2 ''draw down'' from atmosphere to media due to net photosynthesis, this value can be used to correct DIC fluctuations and estimate net photosynthesis (*P*) in cultures of *N. atomus.* Subtracting 0.358 mmols C/L from post-incubation DIC calculations such that

$$P = [\text{pre-incubation DIC} - (\text{post-incubation DIC} - 0.358\ \text{m}M)] - \text{C} \quad (5)$$

provides an estimate of CO_2 fixation, which is unobscured by the flux of CO_2 from atmosphere to media. Calculation of photosynthesis in non-calcifying cultures using Equation 5 results in an average net photosynthesis value of 0.316 mmoles/L in 4 h. However, this exercise yields a photosynthesis value of −1.18 mmoles/L in 4 h indicating that more CO_2 is drawn from the atmosphere to media in calcifying cultures than can be accounted for by net photosynthesis alone.

A comparison of calcification in calcifying cultures (1.38 mmoles/L in 4 h) to photosynthesis in non-calcifying controls (0.316 mmoles/L in 3.33 h) yields a calcification: photosynthesis ratio of 4.37. If a trans-calcification mechanism is present, then this result indicates that net photosynthesis increased by 4.37 times in calcifying cultures to produce a 1:1 C/P ratio, or that calcification exceeded net photosynthesis by some value up to 4.37 times while maintaining pH and alkalinity levels. The latter scenario requires maintenance of high pH in the calcifying environment to facilitate conversion of excess CO_2 (not utilized for photosynthesis) to HCO_3^- /CO_3^{2-} for incorporation into $CaCO_3$. It has been demonstrated that *Chara* cells are capable of maintaining high extracellular pH (10.4) in their calcifying microenvironment under conditions in which photosynthesis was limited, indicating that alkalinization of these zones does not result from CO_2 uptake alone (McConnaughey 1994). McConnaughey (1994) also suggests that locally raising CO_2 concentrations through calcification can potentially double photosynthesis. Net photosynthesis measurements that are independent of alkalinity and pH data are required to accurately determine the extent that photosynthesis was affected by calcification in *N. atomus.* Research by Fabry and Robbins (1994) indicates that microbial calcification may decrease atmospheric CO_2. Regardless of the C/P ratio for calcifying *N. atomus*, removal of HCO_3^- while maintaining alkalinity, pH, and DIC requires conversion of CO_2 to HCO_3^- and, in lab cultures where the only additional source of DIC is from atmospheric CO_2, uptake of CO_2 from atmosphere to media to maintain aqueous pCO_2 and, thus, DIC levels. However, the fate of CO_2 taken up by this process depends on the preservation potential of $CaCO_3$ and organic material produced. Assuming that all $CaCO_3$ produced under 1:1 C/P ratio conditions was preserved, upon death and degradation of cellular material, an amount of CO_2 at least equivalent to half that taken up by the process will be released back to the medium. Thus, the likelihood for long-term storage of Ci sequestered increases as calcification exceeds net photosynthesis due to the fact that the preservation potential of $CaCO_3$ is greater than that of organic material. Results of this research indicate that DIC calculations based on alkalinity and pH measurements may not accurately reflect calcification or photosynthesis and that other methods of measuring these parameters must be employed. However, maintenance of pH and alkalinity levels in calcifying cultures and a potentially high C:P ratio of 4.37 based on photosynthesis in non-calcifying cultures indicates that the potential exists for sequestration of CO_2 through this process. As calcium carbonate experiments indicate that these organisms are capable of generating significant amounts of carbonate sediments, perhaps several kilograms per day per individual plankton bloom, further examination of the effects of calcification by unicellular green algae on photosynthesis and atmospheric CO_2 are warranted. *Nannochloris atomus* represents only one of many species of calcifying microbes. While the calcification potential of other microbes, perhaps utilizing various mechanisms for calcification, remains to be examined, it is likely that other species are capable of producing comparable amounts of $CaCO_3$ sediments as well. The contribution of microbial carbonates to carbonate sediment budgets has generally been overlooked. However, this research indicates that the magnitude of this process is such that these budgets may require reevalulation as new data on microbial calcification potential is acquired.

Acknowledgments

This research was supported primarily by the Electric Power Research Institute (EPRI) and in part by a research assistantship from the University of South Florida Institute for Biomolecular Science. We thank Bob Benson for assistance with atomic absorption spectrophotometry analyses and John Compton for assistance with XRD analyses. Assistance from Lara Nicosia and Sharon Hawkins with experiments is greatly appreciated. We gratefully acknowledge J. McKenzie and an anonymous reviewer for suggestions that improved this manuscript.

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MANUSCRIPT RECEIVED FEBRUARY 24, 1998
MANUSCRIPT ACCEPTED AUGUST 14, 1998
PAPER HANDLED BY JILLIAN F. BANFIELD