Microbial biomass: A catalyst for CaCO$_3$ precipitation in advection-dominated transport regimes

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ABSTRACT

Microorganisms have long been thought to impact CaCO$_3$ precipitation, but determining the extent of their influence on sediment formation has been hampered by our inability to obtain direct experimental evidence about mineral formation processes in natural environments. We address this problem by conducting kinetic experiments within a modern terrestrial carbonate spring to determine aragonite precipitation rates and to quantify the relative influences of aragonite saturation state ($\Omega$), microbial biomass concentration and microbial viability on CaCO$_3$ mineralization in advection-dominated transport regimes. At an $\Omega$ value consistent with modern seawater (3.63 ± 0.09), our controlled in situ kinetic experiments show that: (1) the natural steady-state aragonite precipitation rate is more than twice that determined when microbial biomass on the aragonite mineral surface is depleted by 0.2 µm filtration; and (2) inhibiting microbial viability with ultraviolet (UV) irradiation has no significant effect on the mean precipitation rate. Furthermore, our modeling of the CaCO$_3$ precipitation process, which uses the empirical crystal growth rate expression and additional in situ kinetic measurements, reveals that reducing biomass concentrations by 45% can decrease the empirical rate constant by more than an order of magnitude. These findings strongly suggest that microorganisms catalyze CaCO$_3$ precipitation in advection-dominated systems and imply that changes in carbonate mineralization rates may have resulted from changes in local microbial biomass concentration throughout geologic history.

Keywords: biomineralization, carbonate, kinetics, crystal growth, nucleation, porosity, Yellowstone National Park

INTRODUCTION

One of the most controversial issues in the field of carbonate sedimentology and geochemistry is the degree to which microorganisms influence calcium carbonate precipitation (Ginsburg, 1991; Grotzinger and Rothman, 1996; Knorre and Krumbein, 2000). Microbial influences on CaCO$_3$ mineralization have been studied extensively for more than a century (Thompson and Ferris, 1990; Ehrlich, 1996), yet our understanding of the processes that produce carbonates composing the rock record remains limited. Three primary modes of mineral precipitation have been identified: (1) abiotic precipitation, which is independent of biological influences; (2) biologically induced precipitation, which is initiated by the physical presence and/or biogeochemical activity of an organism (e.g., biomineralization or organic matrix-mediated biomineralization); and (3) biologically controlled precipitation, which is directly determined by a living organism (e.g., skeletal carbonates; Lowenstam, 1981; Lowenstam and Weiner, 1982; Mann, 2001; and Weiner and Dove, 2003). However, diagnosing which of these modes was dominant in any given modern or ancient depositional environment has proven extremely difficult (Schlager, 2003).

A combination of morphological characterizations (Chafetz and Folk, 1984), geochemical reconstructions (Arp et al., 2001), microscopy and mineralogy (e.g., Neuweiler et al., 1999, among many others) has traditionally been used to differentiate abiotic carbonate precipitation from that attributed to biotic processes. However, despite the emergence of several important hypotheses from these investigations, none of these postulations have been quantitatively tested with controlled experimentation. Thus, the insights resulting from these types of studies lack the data necessary to support mechanistic interpretations of the CaCO$_3$ precipitation process.

To understand the processes responsible for the formation of CaCO$_3$ in natural environments, we must determine the factors that control the rate of CaCO$_3$ precipitation (Lasaga, 1998). The overall process of precipitation encompasses two stages, nucleation and crystal growth (Berner, 1980). Accordingly, any component which significantly lowers or raises the energy required for these closely linked processes to occur will respectively increase or decrease the overall precipitation rate. Numerous investigators have conducted in vitro kinetic experiments and have proposed phenomenological rate laws describing the rate of carbonate precipitation as a function of observable macroscopic quantities (Morse, 1983; Inskeep and Bloom, 1985; Burton and Walter, 1987; Zhong and Mucci, 1989; Ferris et al., 2004) such as:

$$R = k_{emp}A(\Omega - 1)^n$$

(Morse, 1983) (1)

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where $R$ is the precipitation rate, $k_{\text{win}}$ is the empirical rate constant, $A$ is reactive mineral surface area, $\Omega$ is the bulk solution CaCO$_3$ saturation state [$\text{Ca}^{2+}\cdot\text{CO}_3^{2-}\cdot\text{K}_{\text{sp, CaCO}_3}$] and $n$ is the reaction order with respect to the saturation state. Rate laws alone cannot directly reveal the mechanism by which microorganisms influence precipitation. However, by conducting controlled precipitation rate experiments under conditions where only the microbial component varies, it is possible to determine how microorganisms affect precipitation rates and whether they catalyze or inhibit the precipitation process.

Here we report the results of in situ kinetic experiments we conducted within a terrestrial carbonate hot spring that contains a diverse community of thermophilic microorganisms and has an aqueous chemical composition similar to modern seawater. We determine steady-state CaCO$_3$ precipitation rates while inhibiting or promoting precipitation processes under conditions where only the microbial component varies, it is possible to determine how microorganisms affect precipitation rates and whether they catalyze or inhibit the precipitation process.

In this study, we conducted experiments to determine if inhibiting microbial activity affects CaCO$_3$ precipitation rates. In situ precipitation experiments (Fig. 1) are characterized by: an average cross sectional area of 975 cm$^2$; water depths ranging from 0.5 m (location 1) to 1 cm (location 5); and a spring water velocity of 32.5 ± 1.2 cm • s$^{-1}$. The flow path dimensions, water velocity, average spring water residence time (69.6 ± 2.9 s) and crystal growth rates (described below) indicate that material transport is dominated by advection and that any limitations to CaCO$_3$ precipitation are reaction controlled.

Emergent spring water is supersaturated with respect to both aragonite and its thermodynamically favored polymorph calcite, and is composed of Ca (7.2 mM), Na (5 mM), Sr (18.3 μM), Cl (5.1 μM), SO$_4^{2-}$ (6.0 mM), Mg (2.7 mM), K (1.3 mM), SiO$_2$ (0.85 mM) and dissolved inorganic carbon (21 mM). As spring water flows downstream along the 17 m flow path examined in this study (Fig. 1), its aqueous chemical composition is virtually invariant with the exception that water cools slightly and pH values increase as a result of the loss of gaseous carbon dioxide [CO$_2$ (g)] from the system. This process increases the tendency to precipitate CaCO$_3$ in accordance with the following reaction:

$$\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3(s) + \text{CO}_2(g) + \text{H}_2\text{O}$$

Precipitation rate experiments (this study) and X-ray diffraction measurements of travertine precipitates taken from the high temperature portions of springs at Mammoth (Friedman, 1970; Fouke et al., 2000) show that CaCO$_3$ deposition proceeds at a maximal rate of 0.0217 ± 0.0035 μmol • cm$^{-2}$ • min$^{-1}$ and that aragonite is the only mineral phase present in this part of the outflow system. Petrographic evidence (Fouke et al., 2000) also indicates that no sedimentation occurs. In addition, comparing observed precipitation rates with those predicted using a local equilibrium model (1.63 ± 0.21 μmol • cm$^{-2}$ • min$^{-1}$) demonstrates that equilibrium-based estimates grossly over predict precipitation in this spring; thus precipitation is kinetically controlled.

Diverse communities of microorganisms inhabit the spring system (Fouke et al., 2003) and exhibit visible effects on travertine crystal fabric in a number of locations along the spring outflow path. This ubiquitous microbial presence, coupled with the fact that precipitation is kinetically limited, leads us to hypothesize that microorganisms influence precipitation rates in this spring and may do so in other environments. In this report we use source water drawn from Spring AT-3 to conduct controlled kinetic experimentation that tests this hypothesis.
each experiment, water was removed from one meter upstream of location 2 in Spring AT-3 (Fig. 1) and delivered to the freestanding apparatus via a 25-m-long by 2.5-cm-diameter foam-insulated siphon connected to a peristaltic pump. In addition, the apparatus was prewashed with bleach and dissolved, deionized water before each run.

Two independent experimental treatments and a natural system control were conducted using the in situ kinetic apparatus. In order to determine whether microbial viability impacted precipitation rates, an ultraviolet (UV) irradiation unit (Aquafine Optima HX02ADS, Aquafine Corp., Valencia, CA) was installed upstream of the kinetic apparatus to expose natural microbial communities to UV light (254 nm at 30 mJ • cm^{-2} for 30 s), which damages DNA and inhibits microbial reproductive capacity. Subsequent plate count experiments, conducted on treated and untreated waters, relied on the infection of _Escherichia coli_ by MS-2 UV resistant phage as a measure of UV effectiveness. This verified that irradiation reduced the number of phage-infected _E. coli_ cells by a factor of 10^6. To determine whether changes in the concentration of microbial biomass existing on the surface of CaCO3 influence precipitation rates, a 293-mm-diameter filter holder (Millipore Corporation, Bedford, MA) loaded with a 0.2 μm polyether sulfone filter (Pall Europe Limited, Portsmouth, England) was installed upstream of the apparatus. Filters were replaced every 45 min over the course of the experiment by halting the pump for 30 s, installing a filter and subsequently reinitiating flow from the natural system. The natural control treatment utilized unaltered water pumped directly from Spring AT-3 to the in situ apparatus. Physical and chemical characteristics such as flow rate (19.5 ± 9.2 cm • s^{-1}), aragonite saturation state (Ω_a = 3.63 ± 0.09), temperature (62 ± 0.5 °C), pH (6.60 ± 0.01) and ionic strength (0.032 M) remained unchanged across all treatments and were nearly identical to those of the source water emanating from the natural spring system.

In order to quantify precipitation as a function of time in each treatment and within Spring AT-3, rectangular Na2CO3 precipitation substrates (5.1 cm × 7.6 cm × 0.2 cm) were placed along the sampling runway of the kinetic apparatus and within the natural spring system at locations 1–5. Triplicate measurements of temperature and pH were taken multiple times throughout the duration of each experiment, and water samples were collected for subsequent chemical analysis. Aragonite precipitation rates, aragonite saturation states, reactive mineral surface area and all other quantities were determined for each experiment after field experimentation was completed. In addition, average microbial biomass concentrations were quantified for the natural control and filtered treatments as well as for samples taken from the natural spring system. Detailed explanations of all materials and methods are presented in the following sections.

Flow Rate Quantification

Flow velocities were determined for each experiment on the kinetic apparatus by dividing the average volumetric flow rate (obtained from triplicate measurements of the rate at which water discharging from the apparatus filled a 2000 mL graduated cylinder) by the average cross-sectional area of water flowing across the platform (2.34 ± 0.62 cm²) in each of the experimental runways. Natural system flow velocities were also obtained volumetrically in triplicate. Accordingly, a reinforced 1.9-cm-diameter polyvinyl hose was placed in the system (location 3; Fig. 1) at a 2 cm depth parallel to the water surface, and discharge was collected in a graduated 5 L bucket near location 4 over three separate one-minute intervals. The resultant average flow rate was assumed to be characteristic of the full channel cross section; thus average velocity (32.5 ± 1.2 cm • s^{-1}) was calculated by normalizing the volumetric flow rate by the average channel cross section area (975 cm²) computed using theodolite measurements. Independent system velocity measurements obtained using Pitot tubes (28.9 ± 5.4 cm • s^{-1}) were within uncertainty of this value.

Precipitation Experiments

Sodium carbonate slides (38.71 cm²; heat resistant up to 600 °C; Erie Corporation, Portsmouth, NH) were used as precipitation substrates in each experiment. Prior to any
experimentation, all slides were: washed in 95% ethanol; rinsed with MilliQ water; dried at 100 °C for 2 h in a drying oven; weighed on an analytical balance; wrapped in aluminum foil and then transported to the field. Just prior to conducting each of the three kinetic experiments on the apparatus, triplicate sets of slides were assigned a removal time of 120, 240, 360, 480 or 600 min. Individual slides within these sets were then randomly placed in 1 of 42 grooved slots on the experimental surface. Minutes later, water was pumped to the kinetic apparatus via the various treatments and CaCO₃ precipitation ensued. As slides were removed over the course of the experiment, they were rewrapped in aluminum foil and placed in a storage carrier until completion of the experimental run. Subsequently, slides were taken back to the laboratory and dried at 100 °C for 2 h before CaCO₃ precipitation (mol • cm⁻²) was determined gravimetrically. Each slide was then carefully placed back into aluminum foil and stored in the carrier until further analyses were completed.

Additional precipitation experiments were conducted over a period of 73 h in the natural system at locations 1–5 (Fig. 1) to determine steady-state precipitation rates across a broad range of saturation states. All slides and preparatory/storage techniques were the same for this experiment; however, 12 substrates were placed at each location by hand or with forceps directly on top of the preexisting travertine. Six slides were removed from the system after 32 h and the remaining substrates were collected at the end of the experimental period. Each sample was handled as previously described to determine aragonite precipitation.

**Chemical Sampling and Analysis**

Aqueous samples for chemical analyses including—cations (Ca, Sr, B, Ba, Mg, Na, K, Cu, Fe, Mn, Zn, P, Al, As, Si); anions (SO₄²⁻, Cl⁻, S, O₂⁻, F⁻, and NO₃⁻); calcium; and dissolved inorganic carbon (DIC)—and triplicate measurements of temperature and pH were collected twice during each experiment conducted on the kinetic apparatus. Water directly above each location within the natural system was also sampled for cations and anions at the beginning and end of the 73 h experimental run. In addition, individual calcium and DIC samples were similarly collected for each location along with triplicate measurements of pH and temperature at four intervals (0, 12, 32 and 73 h) during this period.

All pH and temperature measurements were made using an automatic temperature correcting meter (Orion 290A equipped with 9109 WL electrode). Temperature measurements were verified using an alcohol thermometer, and the pH meter was calibrated with three standard pH buffer solutions (4.0, 7.0, 10.0) prior to each triplicate measurement in order to minimize measurement drift. Water samples were drawn into sterile syringes, sterilized with 0.22 μm cellulose membrane syringe filters (Fisher, USA), acidified to a pH of 2 with 1M ultrapure nitric acid (Sigma Aldrich, St. Louis, MO) and stored in polyethylene bottles at 4 °C prior to cation and calcium analyses. Cations were determined in triplicate via inductively coupled plasma-emission spectrometry at the Montana State University Soil Analytical Laboratory. Calcium concentrations were also analyzed in triplicate for each sample using flame atomic absorption spectrometry. Water samples collected for anion determination were filter sterilized prior to analysis at the field site by ion chromatography in accordance with previously described equipment and methods (Inskeep et al., 2004).

For each sample collected for DIC analysis, a 6 mL aliquot of spring water was drawn into a sterile syringe and immediately equipped with a cellulose membrane filter and a sterile 22 gauge needle. Samples were then transferred into pre-weighed and evacuated (50 milliTorr) 60 mL serum bottles that were crimp-capped with butyl rubber stoppers and contained 2 g of 100% Crystalline Phosphoric Acid (Fluka). Post-sampling weights of all bottles were measured and samples were stored at room temperature for two weeks before laboratory extractions were conducted. Sample extraction proceeded via three steps: (1) water removal, where gas was drawn through a methanol–dry ice cold trap (~70 °C); (2) carbon dioxide isolation via a ~197 °C liquid nitrogen trap; and (3) total CO₂ (g) measurement using a mercury manometer. Total DIC concentrations (mol C • kg⁻¹) were determined by normalizing each total CO₂ (g) measurement by the sample mass.

**Microbial Biomass Determinations**

Duplicate slides from the 120, 360 and 600 min time intervals of the natural control and filtration treatments were dried at 100 °C along with duplicate slides from locations 3, 4 and 5 that were collected at the end of the 73 h experiment. Masses were reanalyzed using a microbalance accurate to ± 5 μg. In addition, three laboratory-grown Iceland spar samples were dried and analyzed similarly. All samples were placed in a perforated aluminum foil boat, transported to a muffle furnace in an airtight glass chamber and heated to 500 °C for 5 h, via a method consistent with Dean (1974), in order to combust microbial organic matter while preventing inorganic carbon evolution from CaCO₃. Each sample was returned to the airtight chamber immediately after being removed from the furnace and was allowed to cool before its mass was analyzed on the microbalance. Precipitated mass was determined for each sample by subtracting slide mass from the dried sample mass. The difference between this value and the mass obtained after heating at 500 °C was assumed to represent the total microbial biomass in each sample, given that dissolved organic matter incorporation into the solid phase is not expected due to its negligible concentration in spring water (Nordstrom et al., 2005). In addition, consistency between pre- and post-heating masses of Iceland spar calcite verified that carbon was not lost from CaCO₃ during the heating process.

Steady-state microbial biomass concentrations (mg biomass • g precipitate⁻¹) for the filtration and control treatments were calculated using the duplicate 360 and 600 min samples from each experiment. Duplicate slides from all three locations were used to characterize steady-state biomass in the natural system. Individual biomass concentrations were calculated by normalizing biomass values (mg biomass) by sample dry mass (g precipitate). Average concentrations and 1σ uncertainties, which were determined as the mean and standard deviation of the slide set from each experiment, were assumed to represent the best estimate of microbial biomass in this system due to the fact that the accuracy of cell counts is hampered by the presence of numerous filamentous microorganisms. Therefore, all experimentally determined precipitation rates were adjusted using the appropriate steady-state microbial biomass concentrations to account for the microbial contribution to precipitated mass.

**Reactive Surface Area Determination**

An independent kinetic experiment was conducted in the natural system to determine a characteristic reactive mass of aragonite. Accordingly, triplicate substrates were placed in the system (location 3; Fig. 1) as before and removed at intervals of 120, 240, 360, 600, 760 and 1130 min. Calcium carbonate precipitation was then determined for each interval and plotted as a function of time (Fig. 3). Linear regression analyses [precipitation (μg CaCO₃ • cm⁻²) = a • time (min) + b; R²] revealed that precipitation dependence on time was markedly different for data obtained from the first three time intervals (a = 3.27 μg • min⁻¹ • cm⁻²; b = −283 μg • cm⁻²; R² = 0.986) relative to data measured for time points beyond 360 min (a = 4.78 μg • min⁻¹ • cm⁻²; b = −982 μg • cm⁻²; R² = 0.999). This indicated that steady-state precipitation occurred after 360 min and that surface and substrate effects on precipitation rates were
Precipitation rates were calculated by normalizing reactive mass experiments with that of the BET natural spring in order to compute the average mass of reactive aragonite for all experiments.

Total mineral surface area was determined via N\(_2\)g-BET measurements for three samples drawn from the 1130 min experimental interval. Resultant aragonite surface areas (3.37 ± 0.12 m\(^2\) • g\(^{-1}\)) were then multiplied by the average reactive mass of aragonite and the geometric surface area of either an experimental slide or the natural spring in order to compute the average reactive surface area (m\(^2\)). The relative uncertainty of reactive surface area was determined by summing the absolute uncertainty from the reactive mass experiments with that of the BET measurements in quadrature.

Geochemical Modeling and Data Analysis

Equilibrium speciation and local equilibrium modeling was conducted using React version 5.0.6 from the Geochemist’s Workbench suite (Bethke, 2004). Average local equilibrium precipitation rates were calculated by normalizing the product of aragonite output (3954 \(\mu\)mol • kg H\(_2\)O\(^{-1}\)) and the mass of water (1794 kg) contained within the 17.0 m \(\times\) 0.75 m natural system transect (Fig. 1) by the average residence time (69.6 ± 2.9 s) and the average travertine reactive surface area of this region of the spring (376 ± 36 m\(^2\)). Relative local equilibrium precipitation rate uncertainty was derived by summing the 1\(\sigma\) uncertainties of area and residence time in quadrature.

Steady-state precipitation rates were calculated for the natural system experiments by normalizing the precipitation (mol CaCO\(_3\)) on all slides from an experimental interval by the amount of time a sample was in the system. Area normalized rates were determined by averaging these individual rates and then dividing this result by the empirically determined reactive surface area (964 ± 52 cm\(^2\)). Steady-state rates obtained from the kinetic apparatus were calculated similarly; however, solely the slides collected for the 360, 480 and 600 min time intervals of each experiment were used. Relative uncertainties were computed as before in quadrature using the standard deviation of precipitation rates from each slide set and reactive surface area uncertainty.

Mean aragonite saturation states (\(\Omega\)) and associated uncertainties were determined via a Monte Carlo (MC) approach to the equilibrium speciation results obtained for each location and treatment. Accordingly, we assumed that each spring water component concentration was drawn from a random normal distribution that exhibited a mean given by the average analytical concentration of each component and a standard deviation given by the appropriate analytical or experimental uncertainty. We constructed 15,000 synthetic data sets using random values taken from the individual distributions of each chemical component. Each of these data sets was then analyzed via React, and resultant HCO\(_3\)\(^{-}\) and Ca\(^{2+}\) species activities were recorded and binned into two synthetic distributions for each of the eight experiments considered. \(\Omega\) values were computed by multiplying the median values of the HCO\(_3\)\(^{-}\) and the Ca\(^{2+}\) activity distributions together, normalizing this result by the average hydrogen ion activities (H\(^{+}\)) determined via in situ pH measurements and then dividing this quantity by the ionic strength adjusted aragonite solubility product (K\(_{\text{HCO}_3} \cdot \text{Ca}^{2+}\)). Relative \(\Omega\) uncertainties were then determined by adding the MC derived standard deviations of the HCO\(_3\)\(^{-}\) and Ca\(^{2+}\) activity distributions to the experimental uncertainty of the (H\(^{+}\)) measurements in quadrature.

The relationship between area normalized precipitation rates and (\(\Omega\) – 1) was also determined via an MC best-fit approach. However, in this case we assumed that the average rate and (\(\Omega\) – 1) values represented the means of two random normal distributions for each of the five locations and that the relative uncertainties of these quantities were the standard deviations of these distributions. One million synthetic data sets were generated from the 10 distributions. Each data set was then fit via nonlinear regression to Equation 1, and k\(_{\text{HCO}_3}\) and n values were recorded and binned into separate distributions. The MC best-fit equation was then derived using the median n value and its associated k\(_{\text{HCO}_3}\). Analysis of resultant histograms revealed that n was lognormally distributed and that 68% of the variation was found to lie within the interval from 1.05 to 2.13.

RESULTS

The mean steady-state biomass concentration of the natural control treatment is a factor of 1.8 greater than that of the filtered treatment (Fig. 4A), although the mass and rate of aragonite precipitation increase as a function of time during both of these experiments. Precipitation at early time intervals (t ≤ 240 min) is consistent between the two treatments because Na\(_2\)CO\(_3\) substrates control mineralization in this interval and arming of the experimental slides (i.e., covering of the entire substrate surface with CaCO\(_3\) crystals) proceeds similarly in both treatments. Significant deviations between the natural control and filtration experiments occur beyond 360 min as precipitation rates reach their steady-state values. Correspondingly, for the period 360 min ≤ t ≤ 600 min, the control treatment average rate (15.0 ± 2.2 mol CaCO\(_3\) • 10\(^{10}\) • min\(^{-1}\) • cm\(^{-2}\)) is greater than twice that of the filtration treatment (7.23 ± 0.98 mol CaCO\(_3\) • 10\(^{10}\) • min\(^{-1}\) • cm\(^{-2}\)).
Mean calcium concentrations were slightly lower (≤ 2%) in the filtration experiment relative to the natural control owing to the fact that small amounts of CaCO₃ precipitate at the aqueous-filter interface during in situ experimentation. However, mass balance calculations reveal that CaCO₃ precipitation on filters accounts for only 0.2% of the mean separation between the natural control and filtration treatments at t = 600 min (Fig. 4A). Thus, filtration serves to significantly reduce microbial biomass concentrations while negligibly impacting other aspects of the precipitation environment (i.e., aragonite saturation state, pH, temperature, flow rate and ionic strength) during our experiments.

The mass and rate of CaCO₃ precipitation also increase as a function of time in the UV irradiation experiment (Fig. 4B). However, mean precipitation in this treatment is statistically indistinguishable from the natural control results. Accordingly, steady-state aragonite precipitation rates are correlative between the two treatments, and mean precipitation is within experimental uncertainty throughout the duration of the experiment. Furthermore, the fact that no significant changes in bulk aqueous chemistry occurred across all experiments shows that microbial metabolism has little effect on the saturation state when transport is advective.

Precipitation rates from the natural spring system, normalized by reactive surface area, exhibit a strong dependence on the aragonite saturation state, Ω₋₁ (Fig. 5). In addition, Monte Carlo (MC) results for the two-parameter data fit show that the median k₋₁ (0.907 mol CaCO₃ • 10⁰⁰ • min⁻¹ • cm⁻²) and n (1.33) values represent the best model of the data given the calculated uncertainties for rate and Ω. The good correspondence between our experimental data and the MC best-fit model demonstrates that the empirical rate law (Equation 1) accurately describes aragonite precipitation in the natural depositional system.

**DISCUSSION**

**Modeling the Microbial Biomass Effect**

In our natural control and filtration kinetic experiments, we held the physical and chemical conditions constant and varied microbial biomass concentrations. Thus, the steady-state rate difference we observed between these treatments suggests that microbial biomass: (1) catalyzes aragonite precipitation by altering the reaction rate constants in our experiments; (2) enhances precipitation by increasing the amount of aragonite surface area that is reactive; or (3) alters the dependence that precipitation rate has on saturation state. Given that we completed controlled experimentation at only one Ω value and obtained information regarding rate dependence on saturation state solely at conditions similar to the natural control treatment, we need to establish a model to determine whether the rate increase exhibited at an elevated biomass concentration is attributable to the effects of increased surface area, a larger rate constant or a higher order dependence on saturation state, (Ω₋₁)ⁿ.

Determining reactive mineral surface area has historically been a difficult task (e.g., White and Peterson, 1990), but our independent experimentation (Fig. 3; Reactive Surface Area Determination section) gives us an excellent quantitative estimate of the mass of aragonite that is reactive under natural system conditions. When this result is considered along with the fact that our natural system N₂(g)-BET aragonite surface area determinations (3.37 ± 0.12 m² • g⁻¹) are within the uncertainty of surface areas measured for laboratory synthesized aragonite (Zhong and Mucci, 1989), it is apparent that biomass has a negligible effect on the surface area term. Therefore, if we assume that reactive surface area is equivalent for both treatments at steady-state, modeling the sensitivity of our experimental results to changes in n enables us to test whether: (1) microbial biomass catalyzes precipitation in the control treatment; or (2) a disparate rate dependence on saturation state is responsible for the observed rate differences.

In order to determine whether microbial biomass catalysis is the dominant effect, we use Ω and the experimentally determined precipitation rates along with the empirical rate law (Equation 1) to identify the n-phase space where the k₋₁ value of the filtration treatment is less than that of the control. To accomplish this we rearrange the rate law equation and solve for the empirical rate constants of the filtered (k₋₁) and control treatments (k₋₁). We then normalize the rate equation for the filtered case by that of the control to produce the expression:

\[
\frac{k₋₁}{k₋₁} = \frac{R₋₁(Ω₋₁)⁻¹}{R₋₁(Ω₋₁)⁻¹} \tag{3}
\]

where R₋₁ and R₋₁ are the experimentally determined rates for the filtration and natural control treatments.
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Figure 5. Mean steady-state aragonite precipitation rates plotted versus $\Omega_a - 1$ for each of the five natural system experimental locations. The MC Best Fit line represents the predicted rate dependence on $\Omega_a - 1$ when median $k_{\text{emp}}$ and $n$ values determined from Monte Carlo simulations of the data are used along with the empirical rate law (Equation 1) to calculate aragonite precipitation rates across a range of $\Omega_a - 1$. Error bars represent 1σ uncertainties.

Figure 6. Phase diagram of $n$ for the control ($n_f$) and filtration ($n_c$) treatments. Results depict regions where the inequality $(k_c \times k_f^{-1} < 1)$ is satisfied (dark), is within 1σ uncertainty of unity (gray), and is not satisfied (white) when worst case scenario 1σ precipitation rate uncertainties from the two treatments are used to compute the value of $k_c \times k_f^{-1}$. The median $n$ value determined for the natural system via Monte Carlo simulations is also displayed (Best Fit $n_c$) and shows that $k_c$ is always significantly less than $k_f$ for the natural system.

treatments respectively, and $n_f$ and $n_c$ are the corresponding exponents that describe how precipitation rate depends on saturation state in these experiments.

For all $k_f < k_c$, Equation 3 yields a result that is less than unity. Therefore, when we input the known values of $R_f$, $R_c$, and $\Omega_a$ while varying $n_f$ and $n_c$, we determine the phase space where the inequality $(k_f \times k_c^{-1} < 1)$ is satisfied. The dark area of the resultant phase diagram (Fig. 6) depicts the region where this relationship is true across a range of $n$ typically found for in vitro mechanistic experiments (Lasaga, 1998). However, our results are not constrained to these $n$ values as the value of $k_c$ is less than that of $k_f$ for all $(n_f - n_c \leq 0.75)$. This indicates that microbial biomass catalysis is the dominant influence on rate difference when treatment $n$ values are within this modeled interval.

Thus, if we use our best fit results from the natural system to characterize $n_f$ (Fig. 6) and assume that $n_c$ is equivalent to this value, the resultant average $k_f k_c$ ratio of 0.483 is equal to the quotient obtained by normalizing the average steady-state filtration rate by that of the control treatment. Alternatively, if we use the experimentally derived $n_f$ while assuming that $n_c$ is 3.7, which was recently determined for aragonite precipitation via experiments using filtered seawater (sensu Morse et al., 2003), model predictions yield a natural control rate constant value that is more than twenty times $k_c$ when microbial biomass concentrations are correlated with those measured in the natural control treatment. These results demonstrate that microbial biomass acts as a catalyst in Spring AT-3 and may do so in other advection-dominated carbonate depositional systems. It is important to note, however, that we would need to conduct further controlled experimentation at a broad range of $\Omega_a$, temperature, pH and ionic strength to determine if the catalysts identified by this study can be extrapolated to other carbonate depositional environments.

Microbial Influence on Aragonite Precipitation

Numerous investigators have conducted mineralization experiments (e.g., van Lith et al., 2003; Cherkoun et al., 2004) or analyzed precipitates from the geological record (e.g., Chafetz and Folk, 1984) to propose mechanisms of microbially influenced CaCO$_3$ precipitation. The general applicability of these results is limited, however, given that no direct measurements of precipitation rates were made under controlled conditions. Mechanistic examinations have been much less common; although, Bosak and Newman (2003) recently determined the volume of CaCO$_3$ precipitated in the presence of Desulfovibrio desulfuricans, a sulfate-reducing bacterium, via in vitro experimentation. Their results show that when microbial viability and metabolism are inhibited by UV irradiation across a range of ionic strengths, CaCO$_3$ volumes increase by more than an order of magnitude relative to sterile controls. However, the applicability of these results to natural systems where diverse communities of microorganisms exist is unknown.

Here we demonstrate that field-based kinetic experimentation can bridge the gap between in vitro mechanistic studies and interpretations of the precipitation process via the rock record. Accordingly, because Bosak and Newman (2003) show that UV irradiation inhibits microbial metabolic activity and viability, the statistically indistinguishable precipitation rates we observe in our natural control and microbial viability experiments indicate that specific microbial metabolisms do not influence the CaCO$_3$ precipitation process in systems where advective mass transport dominates. Conversely, results presented in Figure 4A show that increased microbial biomass concentrations on the surface of CaCO$_3$ substantially enhance aragonite precipitation rates. Therefore, our experimental and modeling results indicate that microbial surfaces derived from the natural microbial communities of Spring AT-3 passively catalyze mineralization in a manner consistent with biologically induced precipitation.

The exact pathway by which microorganisms catalyze precipitation is beyond the scope of this study. However, many investigations focusing
more specifically on CaCO$_3$ nucleation and crystal growth in the presence of microbial cell surface constituents have verified that heterogeneous nucleation is facilitated when carboxyl (Levi et al., 1997; Teng et al., 1998), hydroxyl (Dickinson and McGrath, 2004) and phosphate groups (Fortin and Beveridge, 2000) bind divalent cations such as Ca$^{2+}$. Mann (2001) also provides numerous examples of how proteins, polysaccharides and lipids increase CaCO$_3$ nucleation rates by lowering the activation energy for precipitation to occur. Figure 7 shows that aragonite crystal density is enhanced in the region surrounding a bacterial filament. This suggests that microbial biomass induced CaCO$_3$ nucleation and subsequent crystal growth may be responsible for the rate effects we observe. However, because organic molecules may influence preferential crystal growth on mineral faces and may inhibit carbonate precipitation (Berner et al., 1978), future kinetic experiments are required to distinguish microbial biomass impacts on nucleation from those attributed to crystal growth.

**IMPLICATIONS**

Our modeling and experimental data demonstrate that aragonite precipitation is catalyzed by microbial biomass (microbial cell surfaces and/or EPS) present on the surface of CaCO$_3$ crystals in Spring AT-3. Thus, fluctuations in biomass alone can cause precipitation rate changes in advection-dominated carbonate depositional environments while saturation state remains constant. In addition, because microbial viability has no impact on precipitation rate, these results strongly suggest that microbial surfaces catalyze CaCO$_3$ precipitation in many natural environments regardless of microbial metabolic activity.

Given that rates of authigenic marine carbonate precipitation can be determined from measurements of cement thickness, porosity, compaction and absolute age, we may be able to use reconstructions of seawater $\Omega_a$ (e.g., Locklair and Lerman, 2005) together with aragonite precipitation rates to identify biologically induced precipitation. If rates of CaCO$_3$ cement accumulation are observed to change at constant $\Omega_a$ in marine systems where microorganisms are ubiquitous and material transport is dominated by advection (Hawke and Hunter, 1992), it would suggest that microbial biomass could be involved in the precipitation process (Fig. 8). This implies that changes in calcium carbonate

![Figure 7. Scanning electron micrograph showing aragonite precipitation on an experimental slide from the natural control treatment (time = 600 min). Larger aragonite crystals surround densely packed smaller crystals that are coating a bacterial filament (black arrows).](image)

![Figure 8. Predicted CaCO$_3$ accumulation rates as a function of sediment porosity ($\phi$). Spring system catalyzed and depleted curves are the result of expressing the natural control and filtration treatment mean steady-state precipitation rates, respectively, in cm$^3$ CaCO$_3$ • yr$^{-1}$ • cm$^{-2}$ geometric while accounting for the volume attributed to pore space within a sediment and relying upon several simplifying assumptions (see GSA Data Repository$^1$ for details). Marine system aragonite accumulation rates are expressed similarly but are derived from either an abiotic precipitation rate — which is calculated by incorporating $\Omega_a$ as well as the $k_{emp}$ and $n$ values determined at 25 °C by Burton and Walter (1987) into Equation 1 — or a biomass-catalyzed precipitation rate that results when the abiotic rate is multiplied by a factor of 20.5 to represent the maximum level of microbial biomass catalysis we identify in this report.](image)

$^1$GSA Data Repository Item 2007256, Modeling Details, is available at www.geosociety.org/pubs/ft2007.htm. Requests may also be sent to editing@geosociety.org.
mineralization rates may have been intimately linked with changes in local microbial biomass concentration throughout geologic history.

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