Microbial nucleation of calcium carbonate in the Precambrian

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ABSTRACT

Microbial sulfate reduction is thought to stimulate carbonate precipitation in modern stromatolites, yet whether this metabolism was important in shaping Precambrian stromatolites is unknown. Here we use geochemical modeling to suggest that the influence of sulfate reduction on the saturation index of calcite (SI) is negligible when seawater is in equilibrium with high pCO_2, as is thought for the Precambrian. Our laboratory experiments with heterotrophic bacteria in a medium mimicking Precambrian seawater chemistry show that even if sulfate reduction does not significantly change the SI, the presence of bacteria stimulates calcite precipitation over sterile controls by effectively increasing the SI over a pH range from 7.3 to 7.8. Under our experimental conditions, dead cells stimulate in situ carbonate precipitation equally, if not more, than active sulfate-reducing bacteria. Heterogeneous nucleation of calcite by microbial cell material appears to be the driving mechanism that explains this phenomenon.

Keywords: stromatolites, bacteria, nucleation, biogenic effects.

INTRODUCTION

Stromatolites are common features in the Precambrian rock record, but whether they can be used as evidence of widespread microbial life in ancient shallow water environments is debatable. While the contributions of various microorganisms and/or microbial communities to the deposition of rare modern stromatolites have been examined (Reid et al., 2000; Vasconcelos and McKenzie, 1997; Visscher et al., 2000), microbial contributions to the deposition of ancient stromatolites are often difficult to distinguish against a background of abiotic precipitation and to deconvolve mechanistically (Grotzinger, 1990; Grotzinger and Knoll, 1999; Grotzinger and Rothman, 1996).

By definition, stromatolites are laminated, lithified accretionary structures that grow by the addition of new lamina. Microbes can contribute to the formation of lamina by trapping and binding the sediment, or by influencing calcification. For example, the main lamina-forming process in modern stromatolites is trapping and binding of the carbonate sediment by cyanobacteria and diatoms, although micritic carbonates precipitate in the layers of sulfate reduction (Reid et al., 2000, Visscher et al., 2000). In contrast, in situ precipitation of carbonates dominated in Paleoproterozoic and Mesoproterozoic stromatolites, probably due to the higher saturation state of the Precambrian oceans with respect to calcium carbonate (Grotzinger and Kasting, 1993).

Sulfate reduction is a metabolism that has been associated with in situ carbonate precipitation in modern marine settings (Canfield and Raiswell, 1991; Krumbein, 1978; van Lith et al., 2002; Warthmann et al., 2000; Wright, 1999) and has been correlated with the formation of the micritic carbonate lamina in modern Bahamian stromatolites (Reid et al., 2000; Visscher et al., 2000). The mechanism by which sulfate reduction could induce precipitation of calcium carbonate by an increase in alkalinity is often described by the following equations:

$$SO_4^{2-} + 2CH_2O \rightarrow HS^- + 2HCO_3^- + H^+ \quad \text{and} \tag{1}$$

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3(s) + H^+. \tag{2}$$

However, it is unclear whether this mechanism would drive calcite precipitation in oceans that are well buffered by high concentrations of dissolved inorganic carbon (DIC), as inferred for the Early Proterozoic oceans. Here we consider how sulfate-reducing bacteria (SRB) stimulate in situ carbonate precipitation under environmental conditions relevant for the Precambrian, through modeling and laboratory studies of *Desulfovibrio desulfuricans* strain G20.

MATERIALS AND METHODS

Growth Medium and Conditions

We used *D. desulfuricans* strain G20 as a model organism. To test whether the effect of microbes on calcite precipitation was specific to SRB, we used the phylogenetically distant Gram-negative bacterium *Escherichia coli* HB101, a facultative anaerobe that colonizes the human gut and cannot reduce sulfate. G20 was grown in a bicarbonate-buffered medium on 0.5 m*M* Na-sulfate and 10 m*M* Na-lactate as the electron acceptor and donor, respectively (Rapp and Wall, 1987). The basal medium contained: 8 m*M* $MgCl_2$, 20 m*M* NH_4Cl, 0.5 m*M* KH_2PO_4, and 0.2 g/L yeast extract. The basal medium was boiled under N_2, autoclaved, and cooled under N_2/CO_2. A 1 mL/L SL 12-B trace element solution and 1 mL/L Pfennig vitamin solution, 10 m*M* Na-lactate, 0.5 m*M* Na_2SO_4, 10 mL/L 2.5% cysteine-HCl, 7.6 mL/L 8% Na_2CO_3, and 70 mL/L 1 *M* $NaHCO_3$ were added from sterile stock solutions, the pH was adjusted by 10 *N* NaOH to 7.4–7.5, and the medium was left to equilibrate with an atmosphere of 80% N_2, 15% CO_2, and 5% H_2 for at least one day. Finally, the medium was filter sterilized by a 0.2 μm filter. *Escherichia coli* strain HB 101 was grown in the same medium amended with 2 m*M* Na-fumarate as the electron acceptor. Both organisms were grown anaerobically under an atmosphere of 80% N_2, 15% CO_2, and 5% H_2.

Precipitation Experiments

We centrifuged 0.5 mL of early stationary phase bacteria (cell density 5×10^7 cells/mL) to remove the liquid phase. The bacteria were then inoculated into 0.4 mL of the fresh culture medium and transferred into 8-well LabTek (NalgeNunc International) culture dishes. After inoculation, $CaCl_2$ was added to sterile controls and cultures

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to 20 m*M* final concentration. Filter-sterilized nigericin was added to inhibit the cultures in two wells to the final concentration of 0.04 m*M*. These concentrations of nigericin completely inhibit the growth of G20 (data not shown). The ultraviolet (UV) treatment consisted of exposing bacteria to UV light on a Foto/Convertible transilluminator (Fotodyne Incorporated). Plate counts confirmed that the number of viable cells in UV-treated cultures was at least 10^{-6} times lower than in the original stock. The incubation time was 15–20 at 25 °C (relative comparisons were made only between cultures incubated for the same amount of time). The amount of precipitate[1] was determined by taking transmitted light micrographs of at least 10 different fields of view using a Zeiss Axiovert S100 microscope. The area covered by precipitates was measured by MetaMorph (Universal Imaging Corporation). When the crystal sizes varied (such as in the experiment with different initial pH values), their volumes were estimated by comparing them to a crystal with a defined unit volume. The relative volume for each experiment was then calculated by averaging the total volume in a given field of view for 10 independent fields.

Geochemical Modeling

Chemical parameters such as supersaturation and concentrations of chemical components in the medium were calculated using MINEQL+ (Environmental Research Software). We assumed a closed system (before and after the loss of sulfide). The model solution had basal ion concentrations equal to our freshwater culture medium, but with 10 m*M* $CaCl_2$ and 20 m*M* Na_2SO_4 (i.e., closer to their respective concentrations in present-day seawater).

RESULTS AND DISCUSSION

Modeled Effect of Sulfate Reduction on Calcite Saturation

To predict the effects of sulfate reduction on calcite precipitation in media with initial concentrations of DIC ranging from the present day (2 m*M*) to putative Precambrian conditions (72 m*M*), we modeled the saturation index (SI) of calcite before and after sulfate reduction (Fig. 1). SI is defined as $\log(IAP/K_s)$, where IAP is the ionic product of calcium and carbonate and K_s is the solubility constant of calcite. An increase in the SI after sulfate reduction reflects a higher potential for calcite precipitation. Conversely, if the SI decreases after sulfate reduction, calcite will be less likely to precipitate. The change in SI depends on the amount of sulfate reduced, the initial concentration of DIC, and the processes that remove sulfide and carbon dioxide from solution.

At relatively low rates of sulfate reduction similar to those measured in modern Bahamian stromatolites (Visscher et al., 1998), we estimate that ~0.5 m*M* sulfate could be reduced in 24 h in the layer of maximum sulfate reduction. If all metabolic products were to stay in solution over this time period, our model predicts that both the pH and the SI will decrease regardless of the initial concentrations of DIC (Fig. 1A). If we assume that 10 times more sulfate is reduced over the same time period, we expect SI to increase only when DIC is low, as is the case for present-day seawater (Fig. 1A).

For any concentration of DIC, it is also possible for sulfate reduction to increase the SI if we assume that CO_2 and H_2S leave the solution by outgassing, or if sulfide reacts with Fe(III) minerals (Canfield and Raiswell, 1991). If sulfide reacts with Fe(II), however, the pH will decrease and reduce the SI: $Fe^{2+} + H_2S \rightarrow FeS(s) + 2H^+$. Because reactions with iron will rapidly remove sulfide from solution, for the purpose of our model we have assumed that all sulfide is removed instantaneously through such reactions, enabling us to neglect the slower dynamics of outgassing.

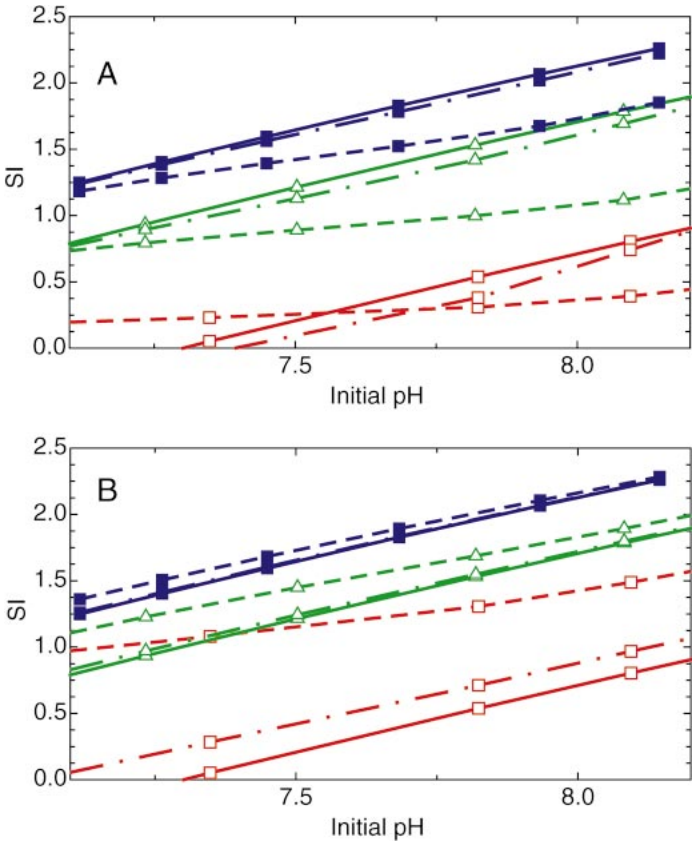

Figure 1. A: Saturation index (SI) of calcite calculated before (solid line), after reduction of 0.5 m*M* SO_4^{2-} with lactate (dot-dashed line), and after reduction of 5 m*M* SO_4^{2-} with lactate (dashed line), assuming that all sulfide stays in medium. SI is calculated for initial dissolved inorganic carbon concentrations of: 2 m*M* (red), 22 m*M* (green), and 72 m*M* (blue). B: Same as above, but assuming that all sulfide produced by sulfate reduction is removed from system.

Based on these assumptions, our model predicts that the reduction of 0.5 m*M* sulfate will increase SI at most by 0.3 log units in a poorly buffered aqueous environment (2 m*M* total DIC) when all sulfide is removed. Even if sulfate reduction were to occur at lower concentrations of organic acids, as is likely the case in modern stromatolites (Visscher et al., 2000), this would not affect the difference in SI before and after sulfate reduction (data not shown). The greater the amount of reduced sulfate, the greater the increase in SI and the potential for calcite precipitation in modern seawater (Fig. 1B). In well-buffered systems that are supersaturated with calcium carbonate, even if all sulfide is lost from solution, the increase in SI will be negligible (Fig. 1B). Because sulfate concentrations in the Archean and Early Proterozoic are thought to be <0.5 m*M* (Anbar and Knoll, 2002; Canfield et al., 2000; Habicht et al., 2002), and pCO_2 is considered to have been much higher than today (Kasting, 1987), we believe that the modeling results shown in Figure 1B rule out a significant role for sulfate reduction in stimulating calcite precipitation at that time.

Effects of Bacteria on Calcite Precipitation

Because our geochemical modeling does not predict a significant effect of sulfate reduction on SI, we hypothesized that sulfate-reducing bacteria could stimulate calcite precipitation by a mechanism other than sulfate reduction. To test this, we used *D. desulfuricans* G20 as a representative SRB to determine how its presence affected calcite precip-

[1]GSA Data Repository item 2003083, Figure DRI, flow chart of the precipitation experiments, is available on request from Documents Secretary, GSA, P.O. Box 9140, Boulder, CO 80301-9140, USA, editing@geosociety.org, or at www.geosociety.org/pubs/ft2003.htm.

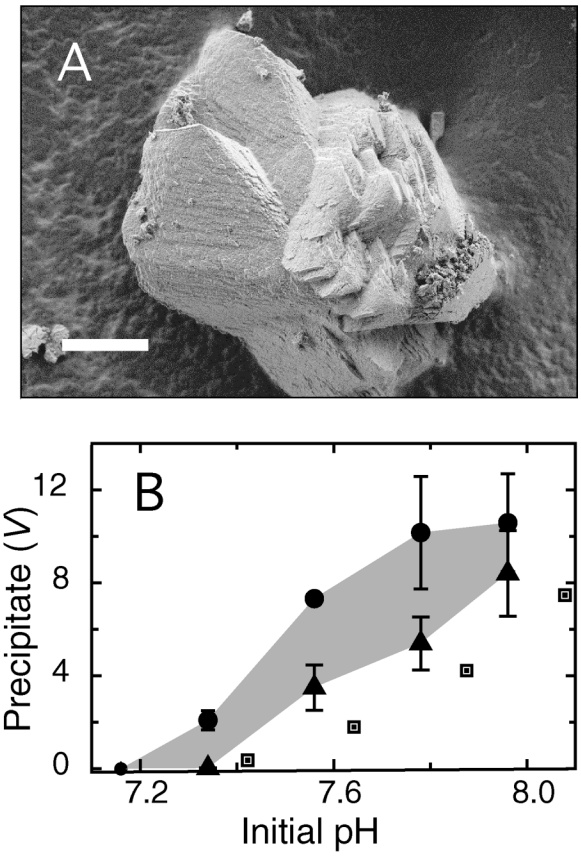

Figure 2. A: Scanning electron micrograph of representative calcite crystal precipitated in G20 cultures at pH 7.5. Image was obtained on LEO1550 VP field emission scanning electron microscope operating at 10 kV. Scale bar: 20 μm. B: Relative volume (*V*) of calcite in nigericin-inhibited G20 cultures (circles) and sterile controls (triangles). Squares: predicted volume, assuming it is proportional to homogeneous nucleation rate that is proportional to saturation index of calcite (calculated by MINEQL+). Shaded area shows range of measurable differences between biologically influenced precipitation and sterile controls. Results shown are averages of two independent experiments (error bars are larger than standard error).

itation under conditions that mimicked Archean and Early Proterozoic seawater chemistry. At the beginning of our precipitation experiments, the culture medium was almost 100 times supersaturated with calcite. Calcite crystals (identified by X-ray diffraction) precipitated both in the uninoculated (sterile) controls and G20 cultures. A characteristic crystal is shown in Figure 2A. These experiments consistently showed that ~82% more calcite formed in the presence of G20 than in the sterile controls at initial pH 7.4 (Table 1).

To determine whether metabolically inactive G20 could enhance carbonate precipitation, we inhibited its metabolism chemically (by adding nigericin) and physically (by exposing it to UV light). Nigericin has been shown to collapse the membrane proton gradient in sulfate-reducing bacteria (Cypionka, 1989; Kroder et al., 1991), whereas UV light kills cells by damaging DNA and preventing cell division. We observed significantly more precipitate at pH 7.4 relative to sterile controls in metabolically inactive cultures (Table 1). In contrast to our results, Chafetz and Busczynski (1992) observed lithification in modern microbial mats only when living bacteria (presumably heterotrophs) were present. In keeping with our modeling results (Fig. 1), we suggest

TABLE 1. DIFFERENCE IN THE VOLUME OF PRECIPITATE BETWEEN BACTERIAL CULTURES AND STERILE CONTROLS

Condition	Difference (%)
Sulfate reducer G20 in "freshwater" medium*	
G20 uninhibited	82 ± 17
G20 inhibited by UV†	138 ± 8
G20 inhibited by nigericin§	137 ± 18
Sulfate reducer G20 in "saltwater" medium#**	
G20 uninhibited	41 ± 1
G20 inhibited by UV†	77 ± 18
Anaerobically grown *E. coli* HB101#	
HB101 uninhibited	18 ± 11
HB101 inhibited by UV†	54 ± 19

Note: Differences shown are averages of at least two independent experiments, with volumes determined from at least 10 different fields of view. Uninhibited and inhibited cultures were incubated at the same initial cell density. E. is *Escherichia.*
*Determined at initial pH 7.4.
†UV is ultraviolet light.
§Nigericin did not stimulate precipitation in the absence of bacteria.
#Determined at initial pH 7.5.
**Saltwater medium contained an additional 20 g/L NaCl and 1.4 g/L $MgCl_2$.

that these differences are due to metabolically induced changes in SI, given the lower buffering capacity of modern seawater.

Although we used G20 as our model species, stimulation of calcite precipitation is not specific to SRB. *Escherichia coli* HB101, a bacterium that colonizes the human gut, also stimulates precipitation of calcite when growing on fumarate instead of sulfate and even more so when inhibited by UV (Table 1). An increase in heterogeneous nucleation in G20 and HB101 cultures is probably due to the binding and accumulation of metal ions by negatively charged carboxylate and phosphoryl groups on bacterial surfaces (Ferris et al., 1988; Fortin et al., 1997). As shown by Mera et al. (1992), cell walls of the Gram-positive bacterium *Bacillus subtilis* bind more metal when the proton gradient across the cell membrane is artificially collapsed. The release of charged compounds through compromised cell membranes of the inhibited organisms into the extracellular medium could additionally stimulate mineral precipitation.

Having experimentally confirmed that bacteria stimulate calcite precipitation by mechanisms other than metabolic activity, we sought to determine the range of pH conditions over which this stimulation could be measured. Assuming a constant radius of calcite nuclei, the free energy of homogeneous nucleation, ΔG, increases linearly with SI:

$$\Delta G \propto \mathrm{SI}. \quad (3)$$

The nucleation rate, J, increases exponentially with the free energy of nucleation:

$$J \propto \exp(\Delta G). \quad (4)$$

SI (as defined herein) increases linearly with pH. The value of J will therefore increase exponentially with SI and pH (Stumm and Morgan, 1996). The relative difference in the amount of precipitate between inhibited cultures of G20 and sterile controls depends on the initial pH of the medium and is measurable in the pH range 7.3–7.8 (Fig. 2B). This range is consistent with the estimated pH range of the Precambrian oceans (Grotzinger and Kasting, 1993). When the initial pH of the medium was below 7.2, we did not observe any mineral grains in either G20 cultures or controls, even though the medium was more than 30 times supersaturated with calcite. The absence of precipitation

at lower pH values is probably due to kinetic inhibition of nucleation in our medium. The critical value of SI needed to start mineral precipitation in our experiments is consistent with critical SI values of ~1 (10-fold supersaturation) that have been observed in modern soda lakes (Arp et al., 1999). When the initial pH was 8.0, the amount of precipitate in the control medium and G20 cultures was the same within the experimental error (Fig. 2B).

A comparison of the theoretical rate of homogeneous calcite nucleation with our experimental data suggests that bacteria affect calcite formation mainly kinetically, by increasing the rate of heterogeneous nucleation. In other words, metabolically inactive G20 effectively increases the SI by at least 0.3 SI units over sterile controls. We estimate the effective difference in SI by assuming that the measured volume of precipitate is linearly proportional to the nucleation rate: $V \propto$ rate (Fig. 2B). Because the nucleation rate depends exponentially on the SI (equation 4), the logarithm of the ratio of precipitate volumes in G20 cultures and sterile controls defines the effective difference in SI, i.e.,

$$\log\frac{\text{rate}_{G20}}{\text{rate}_{control}} = \text{SI}_{G20} - \text{SI}_{control}. \quad (5)$$

Although this is only a first-order approximation, the effective increase in SI appears to be at least as great as the maximum modeled increase in SI due to sulfate reduction under conditions relevant for the Precambrian (Fig. 1).

SUMMARY

In contrast to field studies of lithification in modern stromatolites, our results demonstrate that sulfate-reducing bacteria can influence the rates of calcium carbonate precipitation by mechanisms that do not require metabolic activity. Given the proposed composition of Archean and Early Proterozoic seawater, heterotrophic sulfate reduction is not likely to have had a significant effect on calcification. Rather, microbial viability may have been a more important parameter in determining the rates of heterogeneous calcite nucleation. Dying cells remaining in the lower portions of upwardly moving microbial communities may have been the sites of most rapid carbonate nucleation in ancient stromatolites. In modern environments, microbial cell death may also significantly contribute to lithification, although metabolic effects are more likely to dominate.

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REFERENCES CITED

Anbar, A.D., and Knoll, A.H., 2002, Proterozoic ocean chemistry and evolution: A bioinorganic bridge?: Science, v. 297, p. 1137–1142.

Arp, G., Thiel, V., Reimer, A., Michaelis, W., and Reitner, J., 1999, Biofilm exopolymers control microbialite formation at thermal springs discharging into the alkaline Pyramid Lake, Nevada, USA: Sedimentary Geology, v. 126, p. 159–176.

Canfield, D.E., and Raiswell, R., 1991, Carbonate precipitation and dissolution: Its relevance to fossil preservation, *in* Allison, P.A., and Briggs, D.E.G., eds., Taphonomy: Releasing the data locked in the fossil record: London, Plenum, p. 411–453.

Canfield, D.E., Habicht, K.S., and Thamdrup, B., 2000, The Archean sulfur cycle and the early history of atmospheric oxygen: Science, v. 288, p. 658–661.

Chafetz, H.S., and Busczynski, C., 1992, Bacterially induced lithification of microbial mats: Palaios, v. 7, p. 277–293.

Cypionka, H., 1989, Characterization of sulfate transport in *Desulfovibrio desulfuricans*: Archives of Microbiology, v. 152, p. 237–243.

Ferris, F.G., Fyfe, W.S., and Beveridge, T.J., 1988, Metallic ion binding by *Bacillus subtilis*—Implications for the fossilization of microorganisms: Geology, v. 16, p. 149–152.

Fortin, D., Ferris, F.G., and Beveridge, T.J., 1997, Surface-mediated mineral development by bacteria, *in* Geomicrobiology: Interactions between microbes and minerals: Reviews in Mineralogy, v. 35, p. 161–180.

Grotzinger, J.P., 1990, Geochemical model for Proterozoic stromatolite decline: American Journal of Science, v. 290A, p. 80–103.

Grotzinger, J.P., and Kasting, J.F., 1993, New constraints on Precambrian ocean composition: Journal of Geology, v. 101, p. 235–243.

Grotzinger, J.P., and Knoll, A.H., 1999, Stromatolites in Precambrian carbonates: Evolutionary mileposts or environmental dipsticks?: Annual Review of Earth and Planetory Sciences, v. 27, p. 313–358.

Grotzinger, J.P., and Rothman, D.H., 1996, An abiotic model for stromatolite morphogenesis: Nature, v. 383, p. 423–425.

Habicht, K.S., Gade, M., Thamdrup, B., Berg, P., and Canfield, D.E., 2002, Calibration of sulfate levels in the Archean Ocean: Science, v. 298, p. 2372–2374.

Kasting, J.F., 1987, Theoretical constraints on oxygen and carbon-dioxide concentrations in the Precambrian atmosphere: Precambrian Research, v. 34, p. 205–229.

Kroder, M., Kroneck, P.M.H., and Cypionka, H., 1991, Determination of the transmembrane proton gradient in the anaerobic bacterium *Desulfovibrio desulfuricans* by P-31 nuclear-magnetic-resonance: Archives of Microbiology, v. 156, p. 145–147.

Krumbein, W.E., 1978, Photolithotropic and chemoorganotrophic activity of bacteria and algae as related to beachrock formation and degradation (Gulf of Aqaba, Sinai): Geomicrobiology Journal, v. 1, p. 139–203.

Mera, M.U., Kemper, M., Doyle, R., and Beveridge, T.J., 1992, The membrane-induced proton motive force influences the metal-binding ability of *Bacillus subtilis* cell-walls: Applied and Environmental Microbiology, v. 58, p. 3837–3844.

Rapp, B.J., and Wall, J.D., 1987, Genetic transfer in *Desulfovibrio desulfuricans*: National Academy of Sciences Proceedings, v. 84, p. 9128–9130.

Reid, R.P., Visscher, P.T., Decho, A.W., Stolz, J.F., Bebout, B.M., Dupraz, C., Macintyre, L.G., Paerl, H.W., Pinckney, J.L., Prufert-Bebout, L., Steppe, T.F., and DesMarais, D.J., 2000, The role of microbes in accretion, lamination and early lithification of modern marine stromatolites: Nature, v. 406, p. 989–992.

Stumm, W., and Morgan, J.J., 1996, Aquatic chemistry: Chemical equilibria and rates in natural waters: New York, John Wiley & Sons, Inc., 1022 p.

van Lith, Y., Vasconcelos, C., Warthmann, R., Martins, J.C.F., and McKenzie, J.A., 2002, Bacterial sulfate reduction and salinity: Two controls on dolomite precipitation in Lagoa Vermelha and Brejo do Espinho (Brazil): Hydrobiologia, v. 485, p. 35–49.

Vasconcelos, C., and McKenzie, J.A., 1997, Microbial mediation of modern dolomite precipitation and diagenesis under anoxic conditions (Lagoa Vermelha, Rio de Janeiro, Brazil): Journal of Sedimentary Research, v. 67, p. 378–390.

Visscher, P.T., Reid, R.P., Bebout, B.M., Hoeft, S.E., Macintyre, I.G., and Thompson, J.A., 1998, Formation of lithified micritic laminae in modern marine stromatolites (Bahamas): The role of sulfur cycling: American Mineralogist, v. 83, p. 1482–1493.

Visscher, P.T., Reid, R.P., and Bebout, B.M., 2000, Microscale observations of sulfate reduction: Correlation of microbial activity with lithified micritic laminae in modern marine stromatolites: Geology, v. 28, p. 919–922.

Warthmann, R., van Lith, Y., Vasconcelos, C., McKenzie, J.A., and Karpoff, A.M., 2000, Bacterially induced dolomite precipitation in anoxic culture experiments: Geology, v. 28, p. 1091–1094.

Wright, D.T., 1999, The role of sulphate-reducing bacteria and cyanobacteria in dolomite formation in distal ephemeral lakes of the Coorong region, South Australia: Sedimentary Geology, v. 126, p. 147–157.

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Notes