

Stromatolites below the photic zone in the northern Arabian Sea formed by calcifying chemotrophic microbial mats

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ABSTRACT

Chemosynthesis increases alkalinity and facilitates stromatolite growth at methane seeps in 731 m water depth within the oxygen minimum zone (OMZ) in the northern Arabian Sea. Microbial fabrics, including mineralized filament bundles resembling the sulfide-oxidizing bacterium *Thioploca*, mineralized extracellular polymeric substances, and fossilized rod-shaped and filamentous cells, all preserved in ¹³C-depleted authigenic carbonate, suggest that biofilm calcification resulted from nitrate-driven sulfide oxidation (ND-SO) and sulfate-driven anaerobic oxidation of methane (SD-AOM). Geochemical batch modeling reveals that the collective effects of ND-SO and SD-AOM increase alkalinity more than SD-AOM alone, explaining the preservation of sulfide-oxidizing bacteria in authigenic carbonate. These findings demonstrate the biogenicity of a conical stromatolite associated with OMZ methane seeps and confirm the fact that, apart from photosynthesis-based metabolisms, chemosynthesis-based metabolisms can also account for stromatolite formation.

INTRODUCTION

Since the early 1900s, phototrophic and associated chemoheterotrophic microorganisms have been the presumed chief builders of calcareous stromatolites throughout Earth history (Riding, 2000; Dupraz et al., 2009). However, the view that only light-dependent microbes form stromatolites was challenged by Bailey et al. (2009), who pointed out that chemotrophic Archaea and Bacteria associated with marine methane seeps can form stromatolites as well. Indeed, stromatolitic fabrics have been recognized in ancient (Peckmann et al., 2001) and modern methane-seep carbonates from deep-water settings (Greiner et al., 2002).

At methane seeps, microbial consortia of methanotrophic archaea and sulfate-reducing bacteria mediate the sulfate-driven anaerobic oxidation of methane (SD-AOM: $\text{CH}_4 + \text{SO}_4^{2-} \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$), consuming methane and sulfate while producing bicarbonate and hydrogen sulfide (e.g., Reeburgh, 2007). SD-AOM-produced bicarbonate increases carbonate alkalinity and induces the precipitation of ¹³C-depleted authigenic carbonates (e.g., Ritger et al., 1987), while the hydrogen sulfide produced by SD-AOM nourishes benthic sulfide-oxidation-dependent organisms (e.g., Levin, 2005). Whereas sulfide oxidation with oxygen increases acidity and, thus, induces carbonate dissolution rather than precipitation, Bailey et al. (2009) put forward the idea that sulfide-oxidizing bacteria can stimulate calcification when coupling sulfide oxidation to nitrate

reduction: $\text{HS}^- + \text{NO}_3^- + \text{H}^+ + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + \text{NH}_4^+$ (cf. Fossing et al., 1995; Otte et al., 1999). The effect of this process, referred to as nitrate-driven sulfide oxidation (ND-SO), is amplified when it takes place near hotspots of SD-AOM (Siegert et al., 2013). Therefore, it has been hypothesized that fossilization of sulfide-oxidizing bacteria may occur during seep carbonate formation (Bailey et al., 2009). Yet, seep carbonates resulting from the putative interaction of sulfide-oxidizing bacteria with the SD-AOM consortium have, to the best of our knowledge, only been recognized in ancient seep deposits (Peckmann et al., 2004).

The oxygen minimum zone (OMZ) in the northern Arabian Sea (Fig. 1) is most pronounced at 760 ± 340 m water depth, showing oxygen and nitrate concentrations as low as 2 μM and as high as 20 μM , respectively (Paulmier and Ruiz-Pino, 2009). The hypoxic water conditions are unfavorable for benthic metazoans, but they are ideal for chemotrophic microbial mats of sulfide-oxidizing bacteria due to the presence of locally produced electron donors (hydrogen sulfide) at methane seeps. Methane seeps within the OMZ are colonized by filamentous sulfide-oxidizing bacteria of the genus *Thioploca* (Schmaljohann et al., 2001; Fischer et al., 2012), now reclassified as *Candidatus Marithioploca* (Salman et al., 2013), which store nitrate within cell vacuoles, using it as an electron acceptor

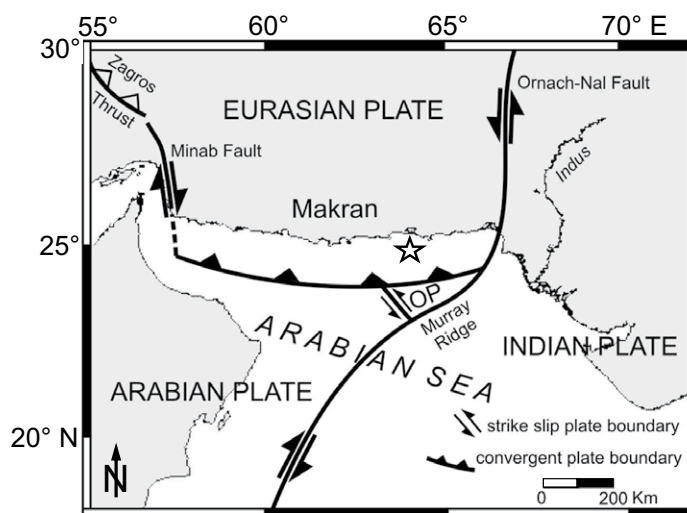


Figure 1. Tectonic sketch map of Makran region (northern Arabian Sea), modified from Kukowski et al. (2001); OP—Ormara plate. Star indicates sample location “Flare site 15” located at 24°48.46'N, 63°59.65'E, in ~731 m water depth on upper slope of Makran convergent margin.

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for sulfide oxidation when oxygen is not available (Fossing et al., 1995; Otte et al., 1999). In contrast to other genera of sulfide-oxidizing bacteria, *Thioploca*, whose filaments live inside a common sheath, is sensitive to high concentrations of molecular oxygen (Jørgensen and Gallardo, 1999).

Previous studies have shown that SD-AOM and cyclic sedimentation facilitate growth of stromatolitic bioherms at the Makran OMZ seeps (Himmler et al., 2015, 2016). In addition to the observation that microbial mats cover domal bioherms, we here report microbial fabrics indicative of the involvement of *Thioploca* in stromatolite growth. We reconcile carbonate precipitation through ND-SO by using a PHREEQC batch model (https://www.brr.cr.usgs.gov/projects/GWC_coupled/phreeqc/), providing further insight into the interactions between biogeochemical processes that govern stromatolite formation.

SEAFLOOR OBSERVATIONS

Abundant white and orange bacterial mats were observed scattered across an area of $\sim 15 \text{ m} \times 15 \text{ m}$ in 731 m water depth (for detailed methods, see the GSA Data Repository¹). The mats were separated by 0.5–1 m distance from each other (Fig. 2A), and their distribution reflects sites of active methane seepage (Römer et al., 2012). No benthic metazoans were observed. The mats were circular or oval-shaped, with distinct domal morphologies up to $\sim 30 \text{ cm}$ high (Figs. 2A–2C). The central parts of the domes were covered by either orange or white mats. Their domal morphology suggests that these mats cover stromatolitic bioherms made of microbial carbonate like other, similar structures sampled at the study site (Fig. 2D; Himmler et al., 2015, 2016).

MICROBIAL FABRICS

Methane-derived, clotted microcrystalline and fibrous aragonite cements comprise the framework of the stromatolitic bioherms, confirming that microbially mediated SD-AOM induced aragonite precipitation ($\delta^{13}\text{C}_{\text{aragonite}}$ as low as -55.1% , Vienna Peedee belemnite; Himmler et al., 2015, 2016). Abundant microbial fabrics were observed directly associated with clotted and fibrous aragonite of the topmost layer of one stromatolite (Fig. 2D), resembling lithified *Thioploca* filament bundles in size and geometry (Figs. 3A–3C; Fig. DR1). Living *Thioploca* from other Makran OMZ seeps exhibit segmented filaments 3–75 μm in diameter that are combined into bundles of 10–20 filaments within sheaths of up to 400 μm

in diameter (Schmaljohann et al., 2001). Fossilized microorganisms preserved within mineralized extracellular polymeric substances (EPS) were also observed on laminae surfaces, including rod-shaped cells ($\sim 2 \mu\text{m}$ long and $\sim 0.5 \mu\text{m}$ in diameter) and filamentous microorganisms (Figs. 3D–3F).

CHEMOSYNTHESIS-INDUCED BIOFILM CALCIFICATION

It was previously shown that stromatolite growth at the Makran OMZ seeps is controlled by cyclic sedimentation and SD-AOM (Himmler et al., 2015, 2016). Regardless, the preservation of fossils resembling the sulfide-oxidizing bacterium *Thioploca* exclusively in the topmost layer of one stromatolite is remarkable: Whereas sulfide-oxidizing bacteria are unlikely to be preserved in carbonate precipitates if molecular oxygen is used as the electron acceptor for sulfide oxidation (Peckmann et al., 2004), our finding suggests that sulfide oxidation was coupled to nitrate reduction (cf. Bailey et al., 2009). Fossilization of bacterial cells by in situ–formed carbonate minerals is likely to require a very local source of alkalinity, which is ideally represented by the metabolism of the fossilized bacterium itself (Dupraz et al., 2009). Although a contribution of ND-SO to the alkalinity increase induced by SD-AOM is not required to explain the precipitation of ^{13}C -depleted aragonite, such a contribution could have favored the fossilization of minute details of *Thioploca* filaments, including their typical arrangement in bundles and segmentations (Figs. 3A–3C). Both biogeochemical processes favor carbonate precipitation. By consuming protons, ND-SO increases the local pH, and SD-AOM produces bicarbonate (HCO_3^-), shifting the carbonate equilibrium toward supersaturation. Given the virtually unlimited availability of divalent cations (Mg^{2+} , Ca^{2+} , and Sr^{2+}) from seawater, the EPS cation-complexation capacity is likely to be surpassed easily, resulting in biofilm calcification (e.g., Dupraz et al., 2009). The apparent ease of biofilm calcification and fossilization is also indicated by remnants of fossilized EPS partly covering aragonite crystals (Figs. 3D–3F).

In a geochemical batch model, ND-SO favors calcite precipitation. ND-SO produces 0.37 g of calcite $\text{L}^{-1} \text{ yr}^{-1}$, SD-AOM produces 1.26 g $\text{L}^{-1} \text{ yr}^{-1}$, and both reactions together yield 1.52 g $\text{L}^{-1} \text{ yr}^{-1}$ (Fig. 4). It is apparent that ND-SO supports calcite precipitation through consumption of protons (Table DR1; see also Visscher and Stolz, 2005). A similar interaction between ND-SO and SD-AOM was suggested for microbial mats in the euxinic Black Sea (Siebert et al., 2013). Since both processes

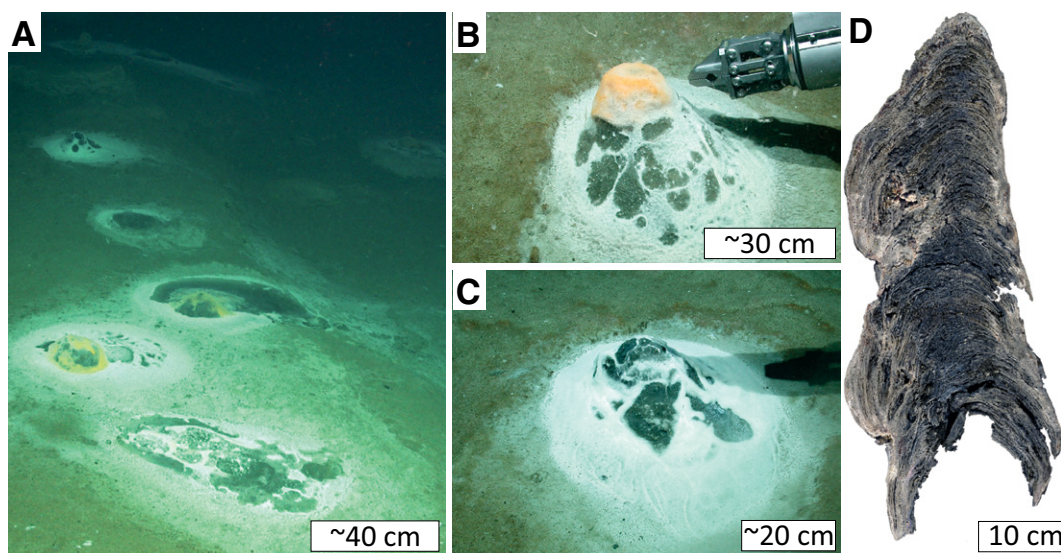


Figure 2. Area photographs of dome-shaped mats on seafloor and conical stromatolite sampled at Flare site 15 ($24^{\circ}48.46'N$, $63^{\circ}59.65'E$). **A:** Neighboring chemotrophic microbial mats; note black sediment associated with microbial mats. **B:** Domal bioherm covered by orange and colorless mats. **C:** Domal bioherm covered by colorless mat. **D:** Conical stromatolite (sample GeoB12353–11; adapted from Himmler et al., 2016).

¹GSA Data Repository item 2018102, methods, model results (Table DR1), and mosaics of thin section micrographs (Fig. DR1) showing alternation of laminated and clotted fabrics comprising the framework of stromatolite sample GeoB12353–11, is available online at <http://www.geosociety.org/datarepository/2018/> or on request from editing@geosociety.org.

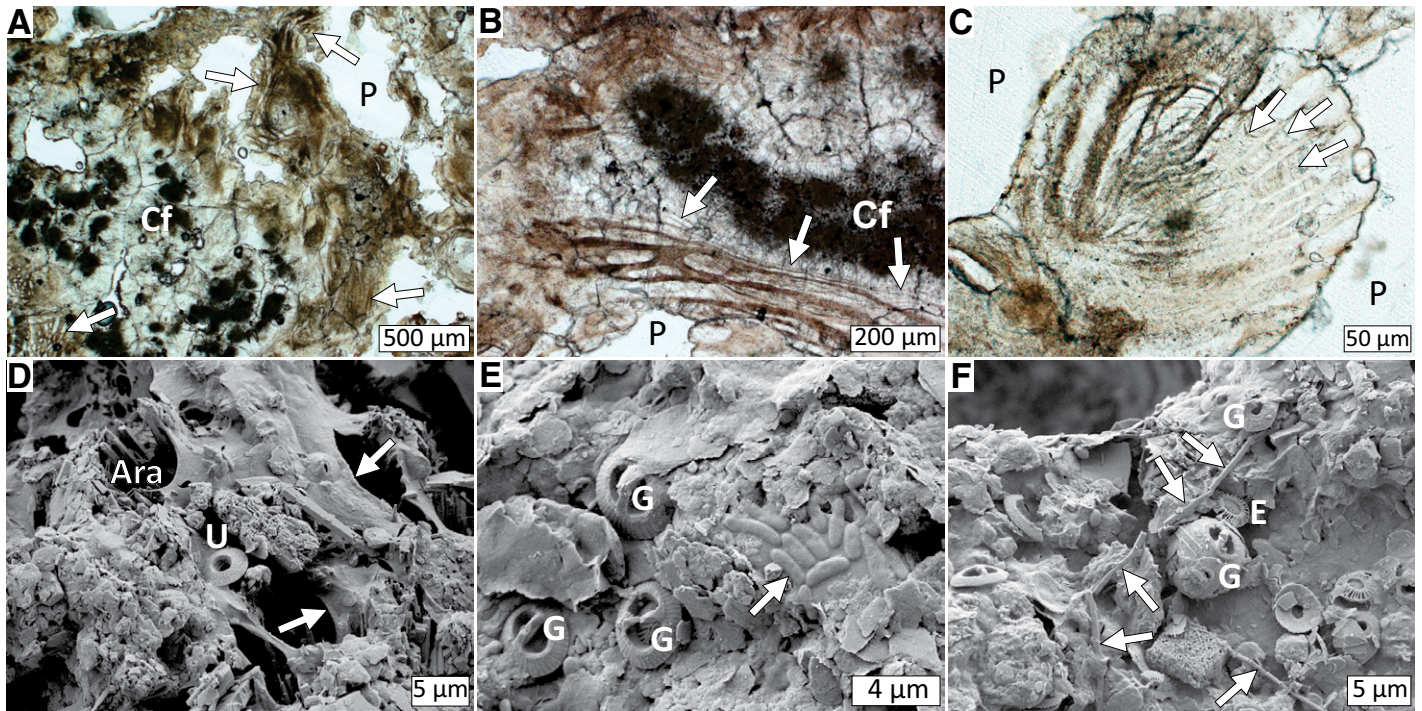


Figure 3. Thin section micrographs (plane-polarized light) and scanning electron microscope images of microbial fabrics (sample GeoB12353–11; Fig. 2D). **A:** Lithified filament bundles (arrows) surrounding clotted and fibrous aragonite cements (Cf). P—pore space. **B:** Lithified sheaths resembling *Thioploca* bundles next to clotted and fibrous aragonite (Cf); note preserved individual filaments (arrows). **C:** Lithified bundle (center left) transitioning into bouquet-like sheaths (center right) with preserved segmentation (arrows) typical for *Thioploca* filaments. **D:** Lamina surface showing mineralized extracellular polymeric substance (EPS; arrows) partly covering aragonite crystals (Ara); U—*Umbilicosphaera sibogae* coccolith. **E:** Mineralized rod-shaped cells (arrow) within mineralized EPS; EPS partly covering coccoliths (G—*Gephyrocapsa oceanica*). **F:** Mineralized, unidentified filamentous microorganisms (arrows) partly attached to EPS; EPS partly covering coccoliths (E—*Emiliania huxleyi*; G—*G. oceanica*). See Figure DR1 (see text footnote 1) for sample locations of D–F.

impact the carbonate system in a similar way, it is difficult to unambiguously prove the significance of ND-SO for the preservation of the *Thioploca* filaments. Fossils of sulfide-oxidizing bacteria are overall scarce in seep carbonates. Because of the exclusive occurrence of these bacteria in environments typified by steep redox gradients, the uncommon generation of their body fossils at methane seeps is best explained by the inference that fossilization requires the combined effects of SD-AOM and ND-SO.

Modern marine stromatolites from Shark Bay or the Bahamas grow in well-oxygenated, photic-zone environments. Therefore, they are analogs for some, but not all, stromatolite growth mechanisms, particularly with regard to the pre-late Neoproterozoic oxygen-deficient oceans. Because the chemosynthesis-based Makran stromatolites form in an aphotic, oxygen-deficient environment, they represent a unique modern analog that includes calcifying microbial mats. The Makran stromatolites represent a stromatolite growth mechanism that was feasible on an early Earth, with mainly oxygen-deficient deep water, abundant methanotrophic microbes, and microbial denitrification present (Hinrichs, 2002; Scott et al., 2008; Godfrey and Falkowski, 2009). Interestingly, a nitrate-reduction-based metabolism was suggested for sulfide-oxidizing bacteria fossils preserved in 2.52 Ga, laminated deep-water deposits (Czaja et al., 2016). Based on these considerations, we put forward the suggestion that chemosynthesis-based stromatolite growth might have been more common in oxygen-deprived Archean and Proterozoic oceans than previously recognized.

CONCLUSIONS

The association of stromatolites with mats of sulfide-oxidizing bacteria, the minute preservation of sheaths of filament bundles and individual filaments of these bacteria in the topmost layer of a stromatolite, and the occurrence of mineralized EPS in its layers suggest that nitrate-driven sulfide oxidation contributed to alkalinity production and, thus,

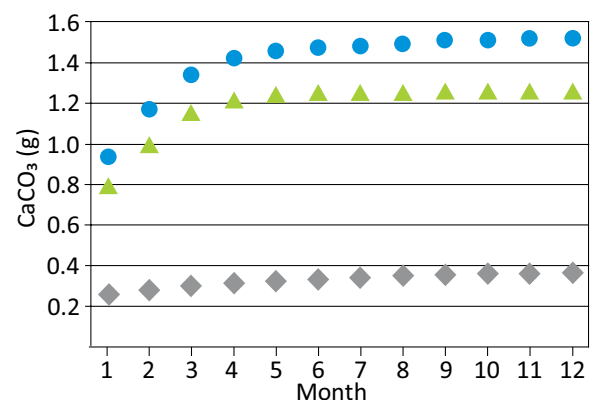


Figure 4. Modeled effect of sulfate-driven anaerobic oxidation of methane (SD-AOM) and nitrate-driven sulfide oxidation (ND-SO) on calcite precipitation (CaCO_3) in grams (g) over time; the experimental products are equivalent to the products suggested by the reactions; circles = SD-AOM + ND-SO; triangles = SD-AOM; diamonds = ND-SO.

stromatolite growth at methane seeps in ~731 m water depth within the oxygen minimum zone of the northern Arabian Sea. The filament bundles resemble the locally abundant sulfide-oxidizing bacterium *Thioploca*, indicating that its nitrate-based metabolism fostered the fossilization of bacterial sheaths and cells by an increase in carbonate alkalinity beyond the increase induced by sulfate-driven anaerobic oxidation of methane. Forming in oxygen-deficient water, the chemosynthesis-based Makran stromatolites may represent a viable analog to some Archean and Proterozoic stromatolites.

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