GSA Data Repository 2019187

Formation of ordered dolomite in anaerobic photosynthetic biofilms

Mirna Daye, John Higgins and Tanja Bosak

1. Media and Enrichment Protocol

The minimal photosynthetic culture medium (FGL medium) contained: 0.1 mM KH_2PO_4, 5.61 mM NH_4Cl, 0.9 mM KCl, 0.044 M HCO_3, 1 mM $MnCl_2.2\ H_2O$, 1 mM Na_2SO_4, 1.94 mM $MgCl_2.6H_2O$, 1 mM $CaCl_2.2\ H_2O$, 1ml/L of trace element solution, 1ml/L of vitamin solution. The trace element solution was prepared in 10% (v/v) HCl and contained per liter; 1.5 g $FeCl_2.4H_2O$, 190 mg $CoCl_2.6H_2O$, 100 mg $MnCl_2.4H_2O$, 70 mg $ZnCl_2$, 31 mg Na_2MoO_4, 6 mg H_3BO_3, 2 mg $CuCl_2.2H_2O$, 0.04 mg Na_2SeO_3. The vitamin solution was prepared in nanopure water and contained per liter; 2 mg biotin, 2 mg folic acid, 10 mg pyridoxine-$2H_2O$, 5 mg thiamine-HCl-$2H_2O$, 5 mg riboflavin, 5 mg nicotinic acid, 5 mg D-Ca-pantothenate, 0.1 mg vitamin B12, 5 mg p-aminobenzoic acid, 5 mg lipoic acid. The final background concentration of manganese was 0.7 μM. To inhibit the growth of oxygenic phototrophs, we added 0.01 mM DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) to the initial enrichments, but all analyses described in the main text used later enrichments grown in media that contained no DCMU. Glass serum bottles were capped by butyl rubber stoppers and aluminum seals. The FGL medium was flushed by 20% CO_2:80% N_2, autoclaved and flushed with 20% CO_2:80% N_2 after cooling. The pH of the medium was adjusted to 7 by the addition of NaOH or HCl before autoclaving and was between 7-7.2 after autoclaving. All enrichment cultures were incubated at 27°C with white light and a 12:12h day/night cycle. The chemical composition of modified FGL (MFGL) medium is similar as FGL medium. MFGL does not contain any DCMU, sulfate or ascorbate, and is reduced by 20-50 μM Na_2S. Sterile MFGL contains only traces of sulfate (< 0.9 μM), nitrate (< 0.5 μM) and nitrite (< 0.1 μM). This minimal anaerobic photosynthetic medium was reduced by 20-50 μM Na_2S. The inoculums for all cultures were washed three times by anoxic nanopure water, mechanically dispersed by passing through a syringe, and resuspended in

sterile anaerobic medium, all in an anaerobic chamber under a 5%CO_2: 5%H_2: balN_2 (v/v/v) atmosphere using standard anaerobic techniques (Balch, 1976). The sterile culture medium was equilibrated with an anaerobic atmosphere containing 80% N_2 and 20% CO_2 and titrated to pH 7 with HCl (1N) or NaOH (1M) before inoculation. The initial concentrations of magnesium and calcium, respectively, were 2 and 1 mM, respectively. The medium used for the initial enrichment was reduced by the addition of 4 mM sodium ascorbate instead of sulfide to minimize the growth of organisms that use sulfide as an electron donor. A brown microbial mat formed on the surface of the inoculated sediments after 3-4 weeks of incubation. Fragments of this mat were transferred into the sterile medium with the same composition as described above, incubated in the same conditions for one month and transferred again. After three transfers, all enrichment cultures were grown in MFGL. The composition of the microbial community was analyzed by Illumina high-throughput sequencing of the 16S rRNA genes, 30% of all sequences belonged to a *Chlorobium* sp., there were no other known phototrophic organisms present, and the community was stable among experiments (Daye et al., *submitted*).

Batch enrichment cultures were grown in modified basal FGL (MFGL) medium. To evaluate the influence of light on microbial growth and the precipitation of minerals, one triplicate set of batch cultures was incubated in the light at 27°C for two weeks, another set contained the same inoculum as the photosynthetically growing cultures and were amended by 1 mM $MnCl_2$, but were shielded from the light by aluminum foil. Both sets of cultures were grown in the presence of 1 mM Mn(II) and 50 μM Na_2S. To evaluate the effect of Mn(II), cultures reduced by 50 μM Na_2S were incubated in the light but were not amended by 1 mM Mn(II). Sterile controls were not inoculated by any microbes but were amended by 1 mM $MnCl_2$. The

69 medium was supersaturated with respect to the precipitation of both calcite ($SI_{Calcite}$= 0.85) and
70 dolomite ($SI_{dolomite}$= 2.42) (Supplementary data section 5).
71
72 **2. X-ray Powder Diffraction**
73 X-ray powder diffraction (XRD) patterns were obtained in reflection mode with Ni-filtered Cu
74 Kα radiation (λ= 1.5406 Å) as X-ray source on an X'Pert PRO diffractometer (XRD, X'Pert
75 PRO, PANalytical, Netherlands) equipped with an X'Celerator detector (PANalytical,
76 Netherlands). The patterns were measured in 2Θ range from 3° to 90° with a scanning step of
77 0.008° and a fixed counting time of 600 s at 45 kV and 40 mA. Biofilms were harvested and
78 centrifuged at 14,000 rpm for 5 min in the anaerobic chamber. Microbial paste was smeared on
79 Zero diffraction disk (23.6mm Diameter x2.0 mm thickness, Si Crystal, MTI corporation, CA,
80 USA) and dried in the anaerobic chamber. The samples were analyzed inside the anaerobic dome
81 to maintain the anoxic conditions during the XRD analyses. Data were analyzed and fitted using
82 High Score Plus program version 4.5 (Malvern Panalytical Inc., Netherlands). X-ray powder
83 diffraction data were refined using the Rietveld method (Rietveld, 1969) by High Score Plus
84 program version 4.5. Dolomite phase was determined according to Miller hkl indices which
85 denote planes orthogonal to the reciprocal crystal lattice vector. The presence of superstructure
86 reflections (hkl's of 015, 113, and 021) indicates ordered dolomite (Reeder, 1983).
87 **3. Scanning Electron Microscopy**
88 Scanning electron microscope (SEM) was used to image microbe-mineral associations. Scanning
89 electron micrographs were acquired by a Zeiss Merlin GEMINI II column high resolution
90 scanning electron microscope (SEM, Carl Zeiss microscopy, CA, USA) equipped with a field
91 gun emission and energy dispersive X-ray spectrometer (EDS, EDAX detector; EDAX, NJ,

USA) operating at an accelerating voltage of 5 - 15 kV, probe current of 100 pA and a working distance of 8.5 mm. On-axis in-lens secondary electron (SE-mode) detector was used during imaging. The samples were fixed by 0.2 M sodium cacodylate, 0.1% $CaCl_2$ and 2.5% glutaraldehyde in anaerobic water for 2-3 days at 4°C. The fixed samples were washed by 0.1 M sodium cacodylate, followed by a wash in nanopure water. After washing, the samples were dehydrated with a series of ethanol-water solutions consisting of 30% (20 min), 50% (20 min), 70% (20 min), 80% (20 min), 90% (20 min) and 100% (3×20min) of 200 proof ethanol. After air-drying, the samples were mounted on double-sided carbon tape and coated with a thin layer 5 nm of Au/Pd or 10 nm of carbon before imaging using Hummer V sputter coater. EDS spectra were treated and analyzed using TEAM EDS 2.0 analysis software (EDAX, NJ, USA) and Microsoft Excel 2016.

Cryo-SEM was performed by harvesting photosynthetic anaerobic biofilms in the anaerobic chamber in molds with Tissue-Tek O.C.T compound resin (Sakura Finetek USA, California, USA) and frozen immediately at - 80 °C. Different vertical sections of 25 µm thickness were cut using Cryostat (Leica CM3050 S) and placed on a microscopic slide. The Tissue-Tek resin was further dissolved by water. The different microbial sections at various depth were coated by 5 nm Au/Pd using Hummer V sputter coater, imaged using Zeiss Merlin microscope and elements were identified using EDS as described above.

4. Transmission Electron Microscopy

Transmission electron microscopy (TEM) was used to characterize mineral-cell associations. Transmission electron micrographs were obtained using FEI Tecnai F20 supertwin microscope (TEM, FEI Tecnai G2, FEI, OR, USA) with a 200 kV Schottky field emission gun. The samples were imaged at 80 kV with a 1024 × 1024 CCD Gatan camera (Gatan, CA, USA). The samples

were fixed by 0.2 M sodium cacodylate, 0.1% $CaCl_2.6H_2O$ and 2.5% glutaraldehyde in aerobic nanopure water for 2-3 days at 4°C. The samples were then washed with washing buffer (0.1 M sodium cacodylate in nanopure water) and postfixed with 1% osmium tetroxide in water for 1 hour. These samples were washed with aerobic nanopure water and stained with 1% uranyl acetate for 1 hour, rinsed with nanopure water and dehydrated with a series ethanol-water solution consisting of: 30% (20 min), 50% (20 min), 70% (20 min), 80% (20 min), 90% (20 min) and 100% (3×20 min) of 200 proof ethanol grade. The samples were further dehydrated with propylene oxide: ethanol solvent (50:50, by vol) for 30 min, then with 100% propylene oxide. The epoxy resin used for embedding consisted of diglycerol ether of polypropylene glycol (EmBed 812, DER 736, Electron Microscopy Sciences, EMS #14130, PA, USA), cycloaliphatic epoxide resin (ERL 4221 Electron Microscopy Sciences, EMS #14300, PA, USA), Nonenyl succinic anhydride (NSA, Electron Microscopy Sciences, EMS#14300, PA, USA) and 2-(dimethylamino) ethanol (DMAE, Electron Microscopy Sciences, EMS#14300, PA, USA). The samples were embedded in resin and cut into 80 nm thick sections with a diamond knife using Leica Reichert Ultracut E microtome (Reichert Ultracut E microtome, Leica, Germany) with a thickness setting of 50 nm. Thin sections were placed on FCF-200 grids (Electron Microscopy Sciences, Cat# FCF-200-Cu, PA, USA).

To determine whether the fixation and embedding protocols introduced any artifacts, photosynthetic biofilms were also harvested without any further processing or staining in the anaerobic chamber. A drop of microbial culture was deposited on LC-200 grid (Electron Microscopy Sciences, Cat#LC-200-Cu, PA, USA) and imaged with JEOL 2010F TEM (JEOL, CA, USA) equipped with a Schottky field emission gun (FEG) operating at 200 kV and a Gatan energy filter (GIF, Gatan 200, Gatan, CA, USA). The 2010F TEM has micro-diffraction,

diffraction pattern in parallel beam and convergent beam electron diffraction features to allow selected area electron diffraction (SAED) on selected areas at high spatial resolution. Gold standard was used as reference for SAED analyses. The high-angle annular dark field detector (HAADF, Gatan, CA, USA) for atomic resolution scanning electron transmission microscopy in the free-lens control mode (STEM) and with an energy dispersive spectrometer (EDS, Bruker silicon drift detector SDD, Bruker, MA, USA) enabled elemental analysis at nanoscale resolution**.** Images in the TEM and STEM mode were taken by digital camera (Gatan Orius, Gatan, CA, USA). SAED patterns were imaged using Gatan digiscan unit (Gatan, CA, USA). TEM, STEM and SAED images were recorded and treated using Gatan digital micrograph software (Gatan, CA, USA). EDS spectra were recorded and treated using INCA program (Oxford instruments, UK).

5. Saturation Indices

Saturation indices (SI) of different carbonate phases were calculated using PHREEQC, Version 2.18 (U.S. Geological Survey, USA) using initial chemical and physical concentrations and properties of the experiments. The following saturation indices (SI: $\log(IAP/K_{sp})$; IAP is the ionic activity product (IAP), K_{sp} is the solubility product) of different carbonate phases is shown in the table below:

Phase	SI
Aragonite	0.70
Calcite	0.85
Dolomite	2.42

6. pH and Alkalinity Measurements

The pH in cultures was determined using a pH meter WTW 315i (Xylem Inc., New York, USA) at 20 °C. Before use, the pH meter was calibrated with two buffer solutions: one at pH 4 and one at pH 7 (The British Drug Houses, London, UK) at 20 °C. Alkalinity of the samples was

159 determined after sample filtration through 0.2 μm pore-size filters (Acrodisc 25 mm syringe
160 filter, PALL corporation, MA, USA). The samples were titrated with a standardized solution of
161 0.02 N sulfuric acid (VWR, PA, USA) to a final pH of 4.5. Total alkalinity was determined by
162 the following equation (Snoeyink, 1982)

163 Total alkalinity: $Va \times Ca \times 50{,}000(\text{mg } CaCO_3/eq)/Vs$

164 Where Va (ml) is the volume of sulfuric acid to titrate the sample, Ca is the normality of the
165 sulfuric acid (eq/L) and Vs is the volume of the sample used (ml).

166 **REFERENCES**

167 Beukes, N. J., 1983, Ooids and oolites of the Proterophytic Boomplaas Formation, Transvaal
168 Supergroup, Griqualand West, South Africa, *in* Peryt, T. M., ed., Coated Grains: Berlin
169 Heidelberg Springer-Verlag p. 199-214.
170 Murphy, M. A., and Sumner, D. Y., 2008, Variations in Neoarchean microbialite morphologies:
171 Clues to controls on microbialite morphologies through time: Sedimentology, v. 55, p.
172 1189-1202.
173 Pruss, S. B., Bosak, T., Macdonald, F. A., McLane, M., and Hoffman, P. F., 2010, Microbial
174 facies in a Sturtian cap carbonate, the Rasthof Formation, Otavi Group, northern
175 Namibia: Precambrian Research, v. 181, p. 187-198.
176 Siahi, M., Hofmann, A., Hegner, E., and Master, S., 2016, Sedimentology and facies analysis of
177 Mesoarchaean stromatolitic carbonate rocks of the Pongola Supergroup, South Africa:
178 Precambrian Research, v. 278, p. 244-264.
179 Sim, M. S., Liang, B., Petroff, A. P., Evans, A., Klepac-Ceraj, V., Flannery, D. T., Walter, M. R.,
180 and Bosak, T., 2012, Oxygen-dependent morphogenesis of modern clumped
181 photosynthetic mats and implications for the archean stromatolite record: Geosciences, v.
182 2, p. 235-259.
183 Simonson, B. M., and Jarvis, D. G., 1993, Microfabrics of oolites and pisolites in the early
184 Precambrian Carawine Dolomite of Western Australia, *in* Rezak, R., and Lavoie, D., eds.,
185 Carbonate microfabrics: Heidelberg, Springer-Verlag, p. 227–237.
186 Wright, D. T., 2000, Benthic microbial communities and dolomite formation in marine and
187 lacustrine environments—a new dolomite model: Society for Sedimentary Geology
188 Special Publication, v. 66, p. 7-20.
189 Wright, D. T., and Altermann, W., 2000, Microfacies development in Late Archaean
190 stromatolites and oolites of the Ghaap Group of South Africa: Geological Society of
191 London Special Publication, v. 179, p. 51-70.

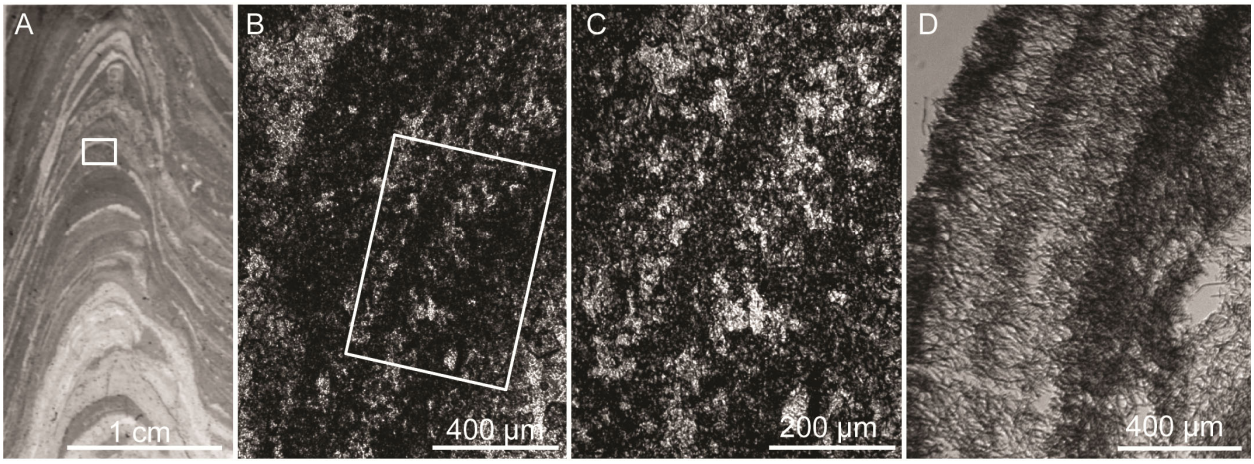


Figure DR1. Comparison of laminae in a conical Archean stromatolite from the Pongola Supergroup, South Africa (2.98 Ga) (Bosak et al., 2013) and textures in a modern cyanobacterial cone. A. Photograph of a stromatolite thin section. White rectangle outlines the area magnified in panel B. The light areas at the bottom are heavily silicified and do not preserve textures well, but dark laminae on the sides and in the top 2/3 of the image contain microcrystalline dolomite and variable amounts of silica. B. Micrograph of the area from panel A showing darker and lighter laminae preserved by microcrystalline dolomite. The lighter laminae contain more silica, the dark laminae consist primarily of microcrystalline dolomite. White rectangle shows the area magnified in panel C. C. Darker and lighter laminae preserved by microcrystalline dolomite. D. Laminae in a modern conical structure formed by cyanobacteria. The dark laminae contain dense filaments that are oriented parallel to the laminae, the lighter laminae contain more exopolymeric substances and unoriented filaments or filaments oriented orthogonally to the laminae (Sim et al., 2012). Additional reports of microbialites and grains with fine dolomitic laminae can be found in Beukes (1983); Murphy and Sumner (2008); Pruss et al. (2010); Siahi et al. (2016); Simonson and Jarvis (1993); Wright (2000); Wright and Altermann (2000).

209

210

211

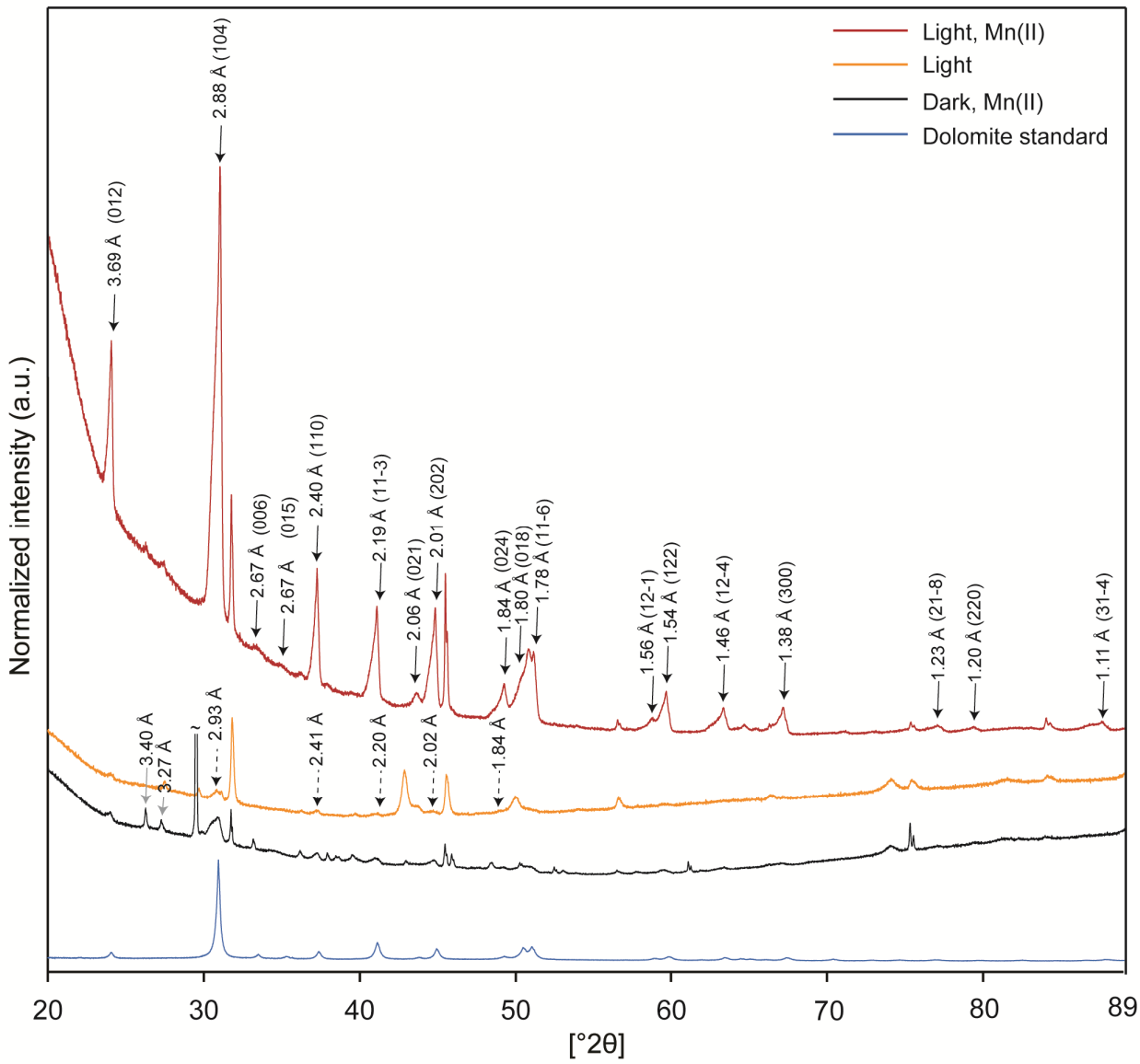


Figure DR2. XRD patterns of samples incubated in different conditions for two weeks. Red: photosynthetic biofilms incubated in the light and amended with 1 mM Mn(II). Dolomite peaks and superstructure reflections are noted with black arrows and labeled by *hkl* indices. Orange: biofilms incubated in the light but not amended by Mn(II). Black: biofilms incubated in the dark and amended with 1 mM Mn(II). Blue: Dolomite standard (Azkarate quarry, Eugi/Eugui, Spain). Dashed arrows mark calcium carbonate peaks in microbial cultures grown without Mn(II). Grey arrows mark aragonite peaks that precipitated in microbial cultures grown in the dark. The

220 presence of hkl (015), (113) and (112) and the peak at 2Θ° = 30.9 corresponding to the (104)
221 plane indicate ordered dolomite in microbial cultures amended with Mn(II) and incubated in the
222 light. Dolomite peaks in the samples match the dolomite standard from Azkarate quarry,
223 Eugi/Eugui, Spain. Dolomite and calcium carbonate precipitated in microbial cultures grown in
224 the light without Mn(II) and microbial cultures grown with Mn(II) in the dark. Calcium
225 carbonate matched with the standard ICDD-04-018-4704 and precipitated at 2Θ° = 30.4 with
226 basal reflections of (104), (110), (113), (202) and (018). Aragonite peaks that precipitated in
227 microbial cultures grown in the dark with basal reflections of (111) and (021) that matched the
228 aragonite standard (ICDD-01-075-9987).
229

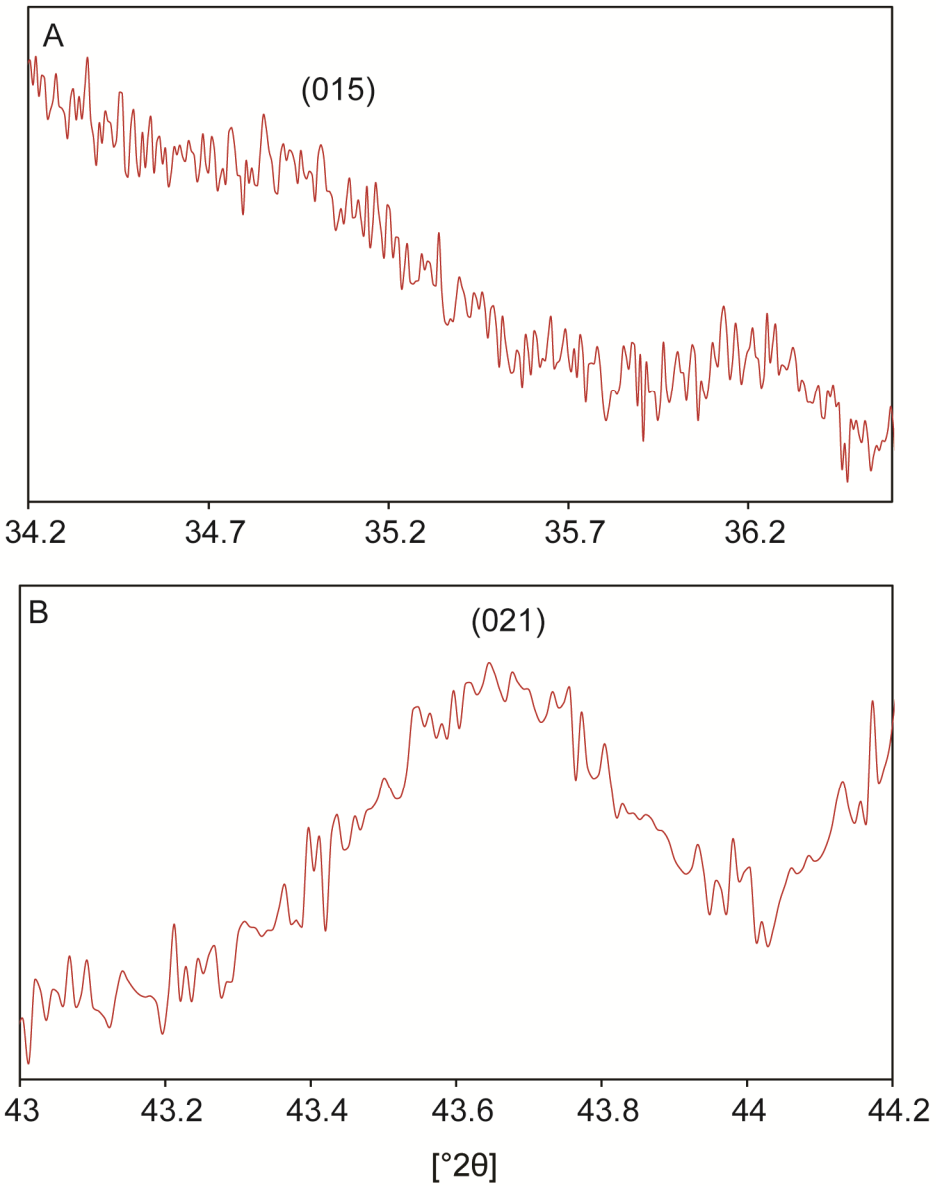


Figure DR3. XRD patterns of the sample incubated in the light for two weeks shown in figure DR2. A: XRD spectrum detail between 34.2° and 36.2° 2Θ showing the 015 basal reflection characteristic for ordered dolomite. B: XRD spectrum detail between 43° and 44.2° 2Θ showing the 012 basal reflection characteristic for ordered dolomite.

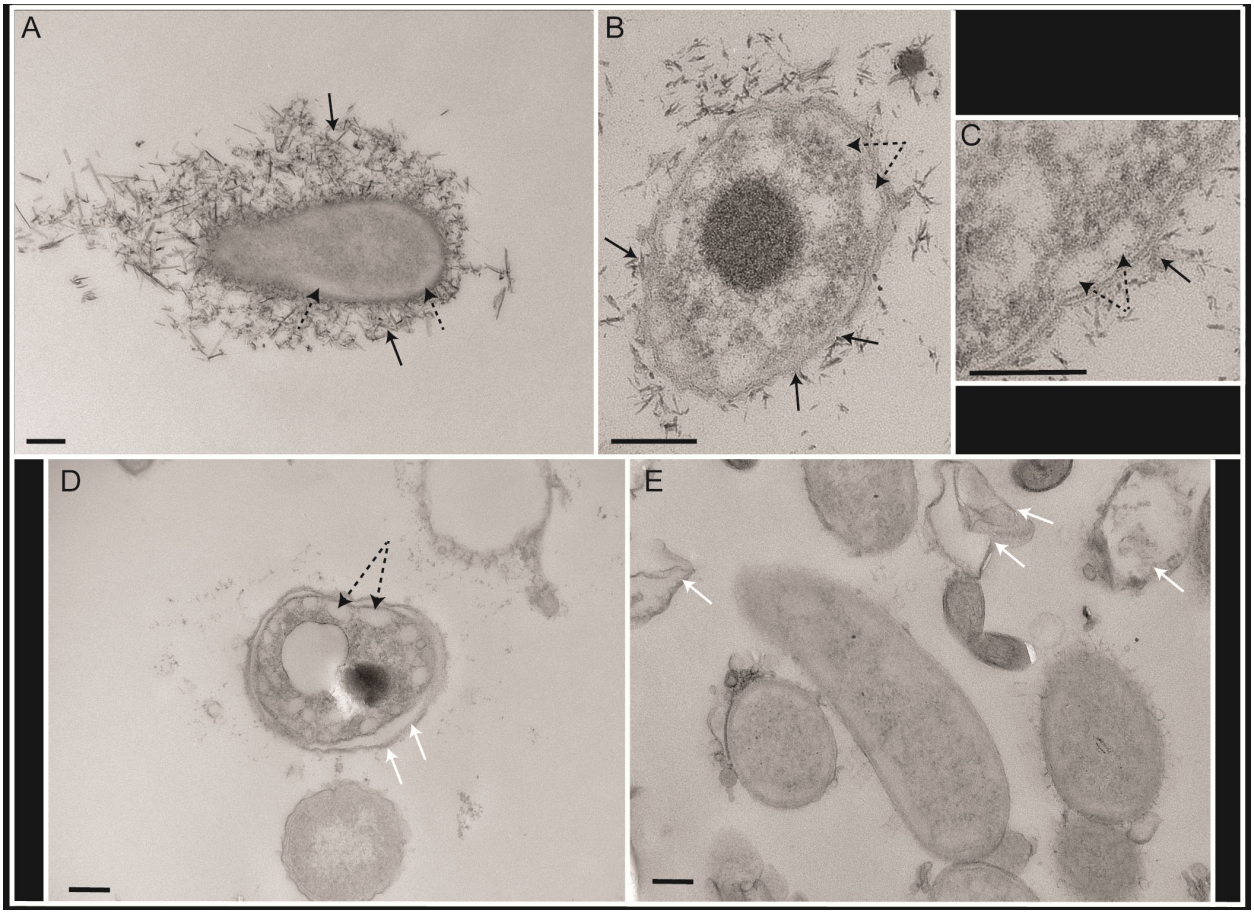


Figure DR4. TEM at 80 kV of stained biofilms incubated in different conditions for two weeks. A-C: *Chlorobium* sp. cells incubated in the light with 1 mM added manganese. Abundant minerals surround the cell surface; black dotted arrows indicate chlorosomes, black arrows indicate minerals that surround the cell. Scale bar: 200 nm. D: A typical *Chlorobium* cell with chlorosomes (dashed arrows) incubated in the dark. The cell is not encrusted by minerals; white arrows indicate signs of cell degradation. E: Different types of cells in a biofilm incubated in the dark, all lacking precipitates; white arrows indicate signs of cell degradation.

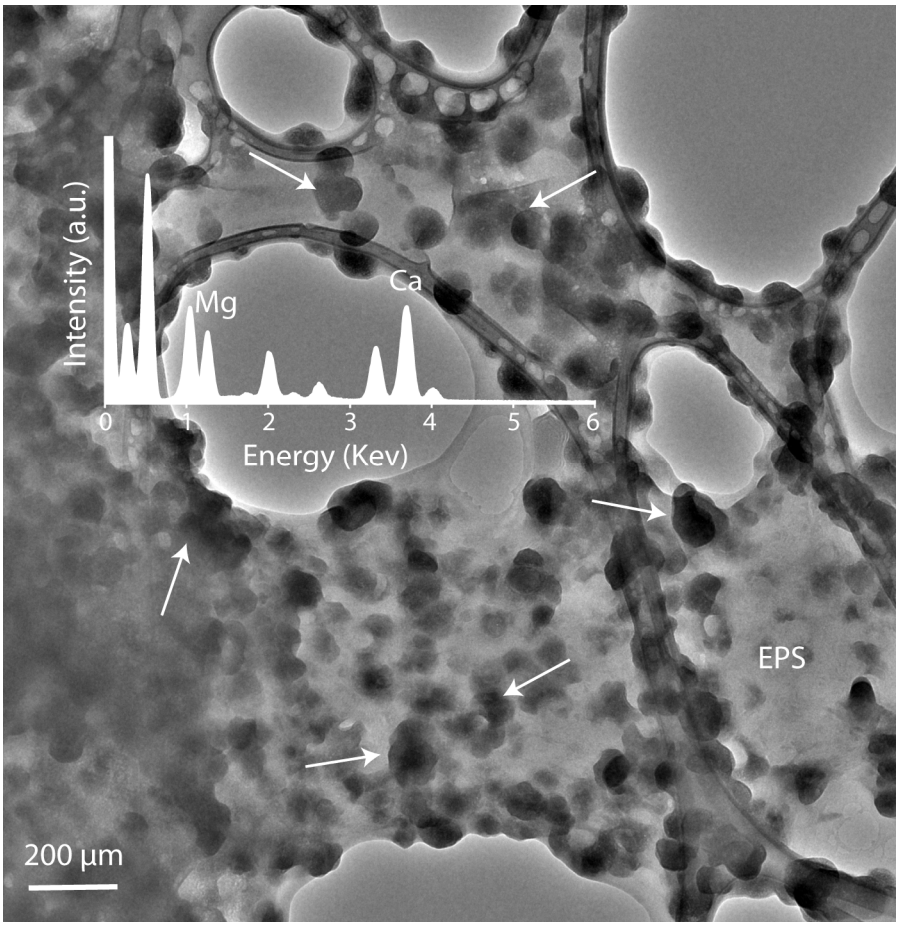


Figure DR5. SEM of a two-week old biofilm incubated in the light with 1 mM Mn(II). White arrows point to dolomite nanocrystals that nucleated on the fibrous EPS. Also shown is the EDS spectrum of these crystals.

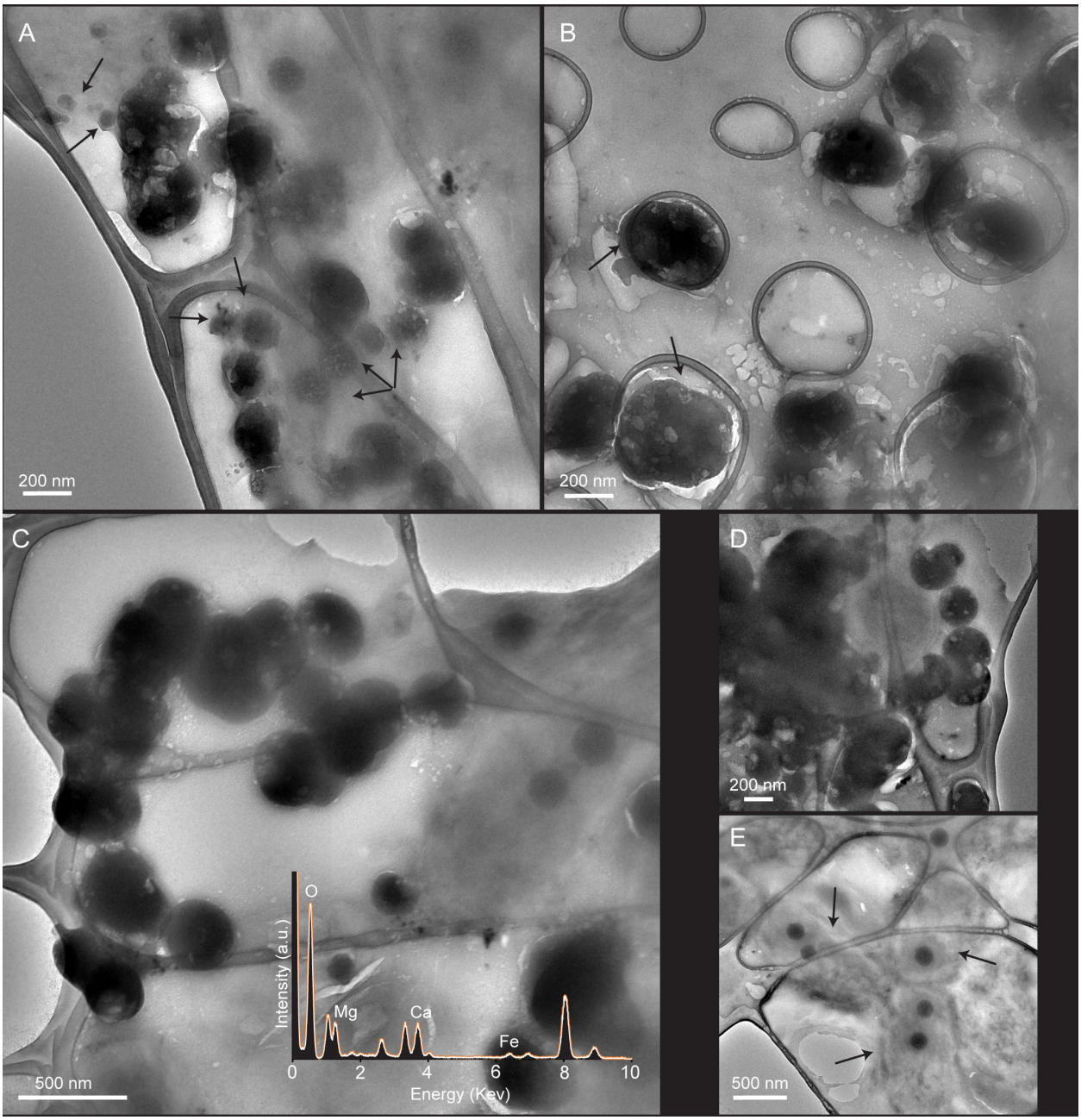
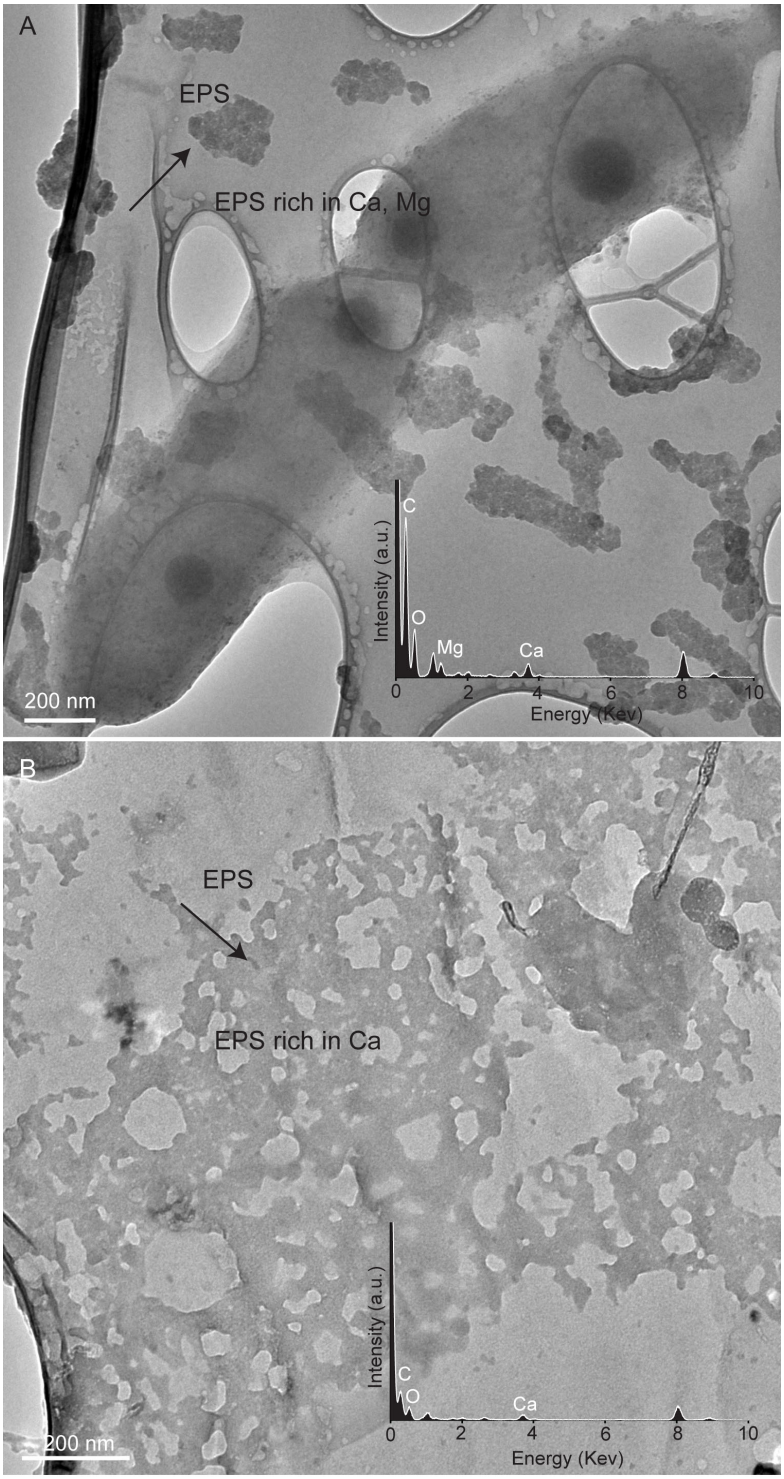


Figure DR6. TEM of photosynthesizing biofilms incubated without Mn(II) for two weeks. A: amorphous minerals in EPS; black arrows indicate dolomite grains < 200 nm wide. B: amorphous phase on EPS network; black arrows indicate dolomite grains > 200 nm wide. C: Aggregates of globular dolomite on an EPS network and the associated EDS spectrum. D: Wide dolomite aggregates on EPS. E: Microbial cells (black arrows) from the same biofilm lack

encrusting minerals. Dark granules inside the cells are rich in Ca and P, as shown by the EDS analyses.



259

260 **Figure DR7.** TEM of biofilms incubated in the dark for two weeks. A: Minerals on EPS from

261 dark cultures incubated with Mn(II). Black arrow shows mineral grains, their EPS spectra are

consistent with dolomite. B: Biofilms incubated in the dark without Mn(II). Black arrow points to minerals analyzed by EDS. The EDS spectrum shows that the mineral is calcite.

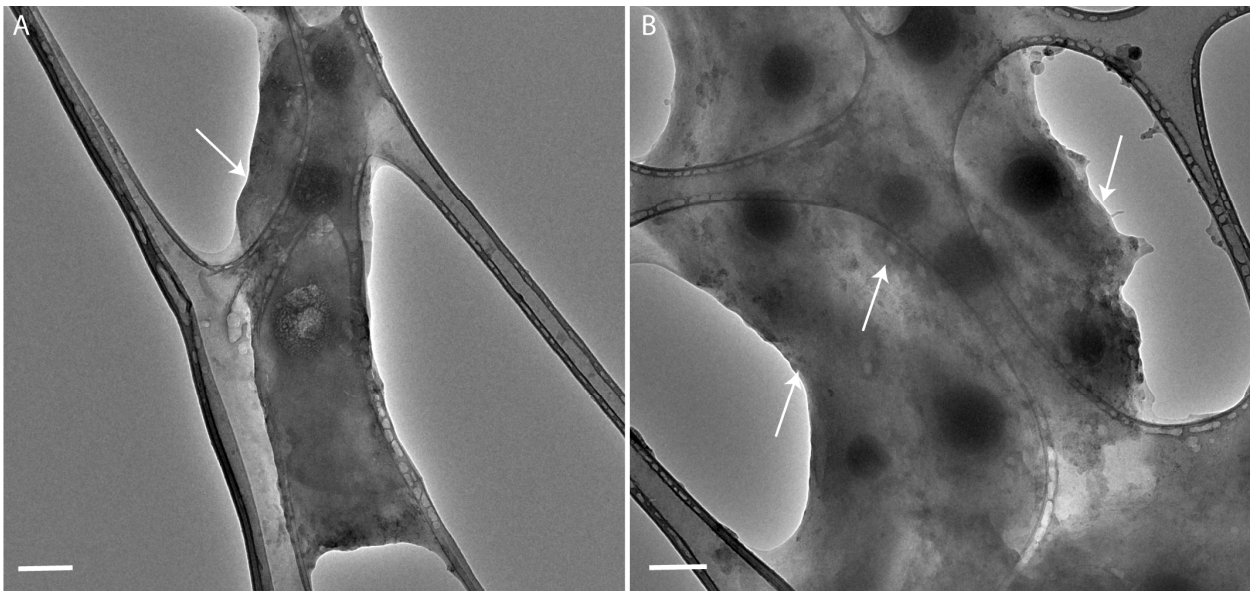


Figure DR8. TEM of cells incubated in the dark for two weeks without any added Mn(II). A: single cell with intracellular dark granules of phosphorus and no minerals precipitated on the surface. B: A layer of cells with dark P granules, but without carbonate precipitates on the surfaces. White arrows point to individual cells. Scale bar is equivalent to 200 μm.

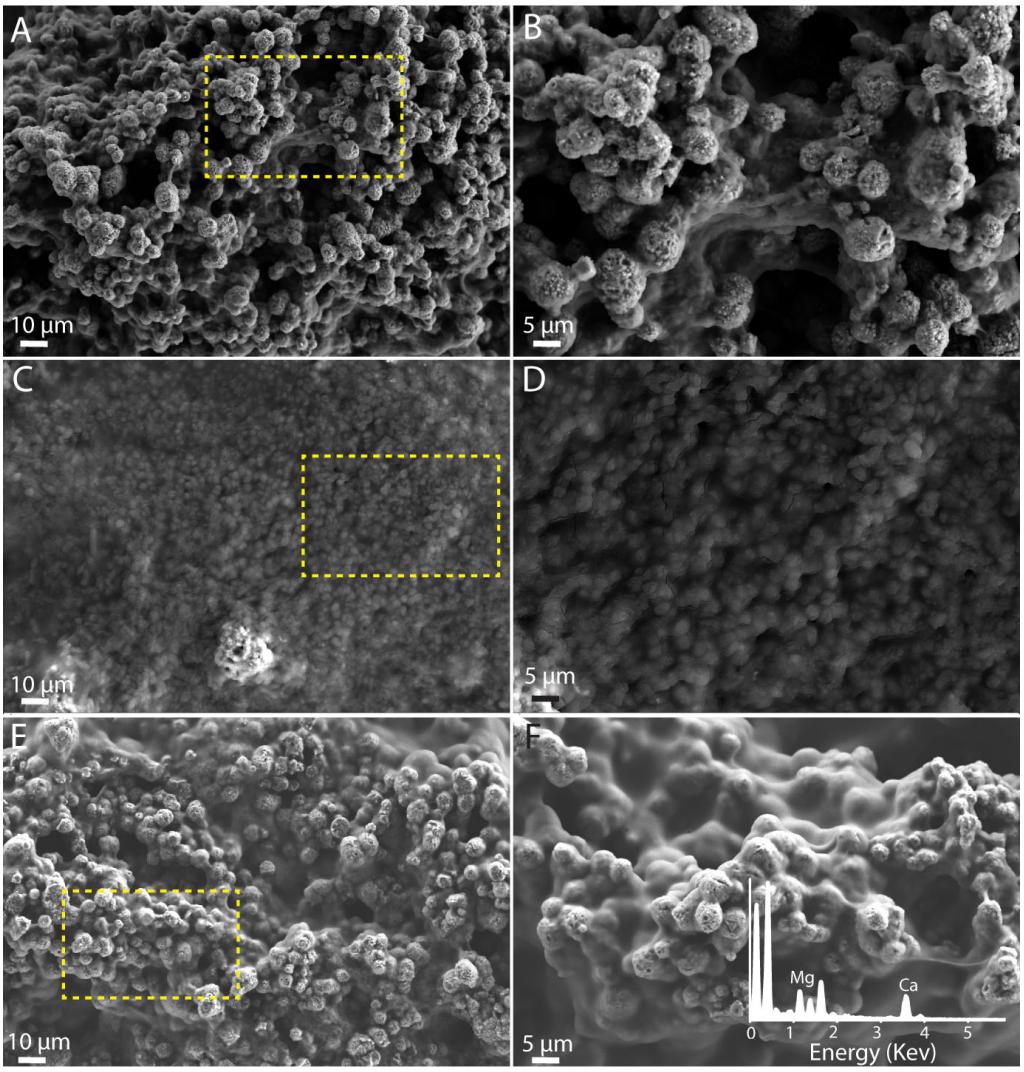


Figure DR9. Cryo-SEM of horizontal sections through a two-month old biofilm. A: Heavily mineralized cell-rich area 30 µm below the surface. The dashed rectangle outlines the area magnified in B. B: Round dolomite crystals in the cell-rich area 30 µm below the surface of a two-week old biofilm. C: Heavily encrusted area 300 µm below the surface. Cells are not apparent in this area. The dashed rectangle outlines the area magnified in D. D: Fine-grained microcrystalline dolomite. E: Heavily mineralized cell-rich area 400 µm below the surface. The dashed rectangle outlines the area magnified in F. F: Globular dolomite and cells in the area shown in E with respective EDS spectrum. EDS analyses demonstrated that the Ca, Mg contents in precipitates from all analyzed areas were the same.

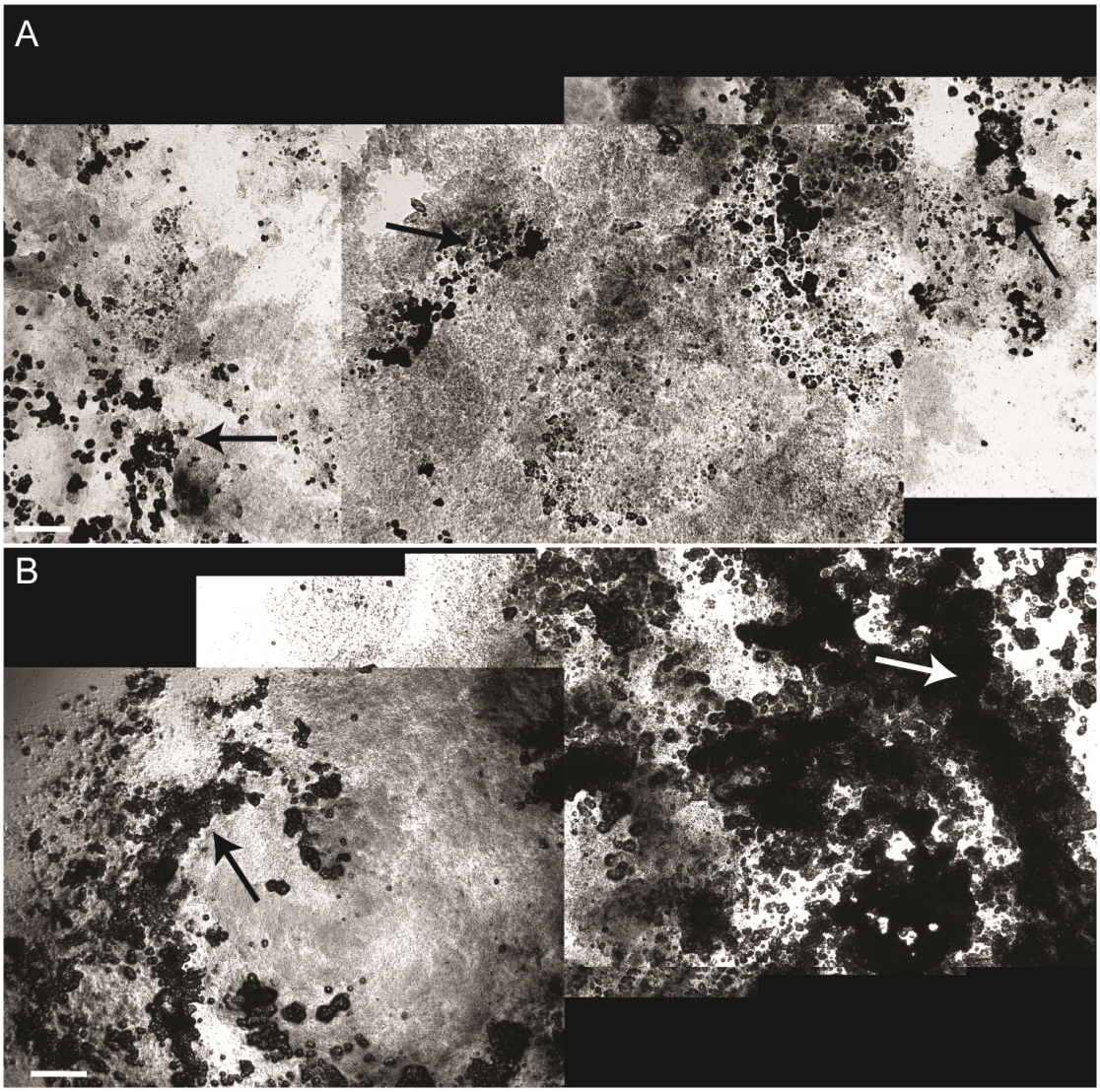


Figure DR10. Transmitted light micrographs of the cryo-sections of a two-week old biofilm incubated in the light with Mn(II). A: Horizontal section of a cell-rich area 25 µm below the surface. Black grains are dolomite minerals. B: Horizontal section 30 µm below the surface. Black and white arrows point to heavy mineral cover. Note the increase of mineral cover with depth. The scale bar is 50 µm.

Table DR1. REFINED UNIT-CELL PARAMETERS USING RIETVELD ANALYSES OF SAMPLES AND STANDARD REPRESENTED IN FIGURE 1.

Sample	Phase*	Percentage (%)	Unit cell (Å)	d-spacing$_{104}$ (Å)	Formula
Light, Mn	Dolomite^{o} 1	12.1	a= 4.801662 b= 4.801662 c= 16.01951	2.87784	$Ca_{3.42}Mg_{2.58}C_6O_{18}$
	Dolomite^{o} 2	39.8	a= 4.825161 b= 4.825161 c= 16.07935	2.89015	$Ca_{3.42}Mg_{2.58}C_6O_{18}$
	Dolomite^{o} 3	30.6	a= 4.844679 b= 4.844679 c= 16.1918	2.90622	$Ca_3Mg_3C_6O_{18}$
Light, no Mn	Dolomite^{d} 1	8.1	a= 4.806 b= 4.806 c= 16.006	2.88460	$Ca_1Mg_1C_2O_6$
	Dolomite^{d} 2	1.7	a= 4.822 b= 4.822 c= 16.111	2.89903	$Ca_{1.14}Mg_{0.86}C_2O_6$
Dark, no Mn	Dolomite^{d} 1	34.2	a= 4.839679 b= 4.839679 c= 16.02784	2.89182	$Ca_{3.42}Mg_{2.58}C_6O_{18}$
Standard	Dolomite^{o}	100	a= 4.8160 b= 4.8160 c= 16.0790	2.89431	$Ca_1Mg_1C_2O_6$

*Notes the dolomite phase; ^{o}is an ordered phase of dolomite, ^{d}is a disordered phase of dolomite