Supplementary Online Material

Materials and Methods

Electron microscopy. Biofilm samples were prepared for HRTEM by drying small samples onto copper mesh support grids coated with formvar. Cloudy water samples were filtered on a 0.2 micron polycarbonate filter, then scraped onto TEM grids. TEM samples were lightly coated with carbon and examined in a 200 kV Philips CM200UT transmission electron microscope equipped with an energy dispersive x-ray detector. SEM samples were coated with gold and examined in LEO 1530 field emission scanning electron microscope (FESEM) operated at 3 kV, except for the sample in Figure S1, main image, which was Pt/C coated and examined with a Hitachi S-5000 FESEM (3 kV).

X-ray PhotoElectron Emission spectroMicroscopy (X-PEEM).

X-PEEM analysis was performed using the SPHINX (Spectromicroscope for PHotoelectron Imaging of Nanostructures with X-rays) instrument at the University of Wisconsin Synchrotron Radiation Center.

For this analysis, biofilm samples were deposited directly onto a Si wafer. Cloudy water samples were filtered with a 0.2 micron polycarbonate filter, removed from the filter with a small amount of water and deposited on a Si wafer. Both samples were air-dried and sputter-coated with 10 Å Pt/Pd. For C and Fe analysis we acquired stacks of images while scanning the photon energy across the edges, then mounted the stacks into "movies" from which the relevant, microchemical information was retrieved. A region of interest (ROI) is

selected with the mouse in specific features (filaments, sheaths, substrate areas, etc.) evident from the image.

XANES (x-ray absorption near edge structure) spectra were extracted from isolated filaments because they were difficult to analyze due to excessive topography when associated with other microbial mat components, or when occurring in bundles.

A carbon XANES spectrum from each filament or fibril was normalized to the beamline transmission curve and to the surrounding substrate spectrum. The latter contains C signal originating from C-containing molecules in the water (e.g. carbonate, humic molecules, proteins, etc.), which during air-drying are deposited on and around filaments. Normalization to these spectra removes the contribution of contaminating carbon, and guarantees that the C signal detected originates solely from filaments or fibrils. These C spectra and the reference standards from ref. S1 were acquired on different beamlines. The energy scales were accurately calibrated acquiring an alginate spectrum on both beamlines. For all data presented here SPHINX was installed on the HERMON beamline (60-1200 eV photon energy). The broad x-ray energy range enabled analysis of all relevant elements in the same sample region. During analysis the samples were kept at high voltage in ultra-high vacuum (-20 kV and 10^{-10} Torr).

The ferrihydrite standard for Fe2p analysis was synthesized as described by Schwertmann and Cornell (*S2*).

Biomimetic synthesis of iron oxides. For the first synthesis experiment, we chose alginate, a well-characterized acidic microbial polysaccharide, as a template for mineralization. Iron

was added to a solution of alginate (5 g/L medium viscosity, sodium salt) as dissolved ferric chloride (filtered with a 0.2 micron syringe filter), in an Fe:carboxylic group ratio of ~ 1:100. Excess alginate carboxylic groups were used in order to prevent the acidic $FeCl_3$ solution from decreasing the pH of the mixture (~4.4) below the pKa of alginate (3.38 for mannuronic acid, 3.65 for guluronic acid). This ensured the presence of deprotonated carboxylic acid groups that could bind iron, as expected in the natural sample. The iron-alginate suspensions were incubated in a shaker at 37°C to accelerate mineralization for 2 to 4 days.

In the second synthesis experiment, we synthesized the polymer template, a chitosan (Chit) chondroitin sulfate (Chon) alginate (Alg) gel, prior to mineralization. All or significant parts of their chains possess spatially complementary β-(D)-(1-4) linked molecular conformation, which enabled thermodynamically driven filament formation (Fig. S5). Chon (~50 kDa), Chit (~600 kDa), and Alg (~250 kDa) were combined in the appropriate ratio to form a fibrillar polymer network (Fig. S5C). An amphiphilic surfactant (TWEEN 80) was used to promote an ordered aggregation. In the relevant pH range and in oxic environments, iron-containing species may exist predominantly in a polynuclear form, stabilized by organic molecules (e.g. *S3*). Consequently, we used a very dilute solution of Fe(III) 2,4-pentanedion (10^{-4}-10^{-5} M) buffered at pH 9 to provide a steady influx of hydrolysed polynuclear iron species. The gels were mineralized in a glass column for up to 4 weeks.

Scanning Transmission X-ray Microscopy (STXM)

STXM analysis was performed on the newly commissioned beamline 11.0.2 STXM at the Advanced Light Source (*S4*).

For this analysis, a droplet of sample (~0.5 µl) was placed between two x-ray transparent Si_3N_4 windows (100 nm thick). To prevent water evaporation during imaging, the sample assembly was sealed and placed in the microscope which is filled with helium at atmospheric pressure. Collection of images was started immediately after the samples were placed in the microscope. Images were obtained by raster scanning a thin sample through an x-ray beam focused by zone plate optics. The transmitted beam intensity was recorded at fixed energy as the sample was scanned so that variations of the photoelectric absorption of x-rays across the sample could be mapped in two dimensions. STXM imaging uses the near-edge x-ray absorption as a chemical contrast mechanism, therefore contrast is dependent on the elemental composition of the sample. Spectral and spatial resolution were less than 0.1 eV and 30 nm, respectively, during these analyses.

Filament and Core Mass Calculations

To calculate the mass of a mineralized filament, we assumed a diameter of 100 nm, a length of 5000 nm (as was the length used for extracting the spectrum from the filament of Fig. 3B) and the density of ferrihydrite ~4 g/cm^3. For the mass of the saccharide core, we assumed that one half of the core nanocrystal volume (2 nm wide, as measured by TEM)

was occupied by saccharide and one half by akaganeite. For this mass calculation we also assumed a 5000 nm length and the density of typical saccharides to be 1.5 g/cm^3. The resulting masses (200 fg and 10 ag, respectively) cannot be used to calculate the C concentration detected, because not all the volume of the filament was probed by X-PEEM. Although the penetration depth of photons with ~300 eV energy is on the order of 100 nm, and the whole filament volume is illuminated, only a fraction of the filament is probed. This is due to the limited escape depth of secondary electrons, on the order of 5 nanometers at 300 eV (*S5*). Since the ferrihydrite nanoparticles surrounding the akaganeite-saccharide core are extremely porous, a detectable signal is collected from all portions of the filament, but a quantitative estimate of the detection volume is not possible.

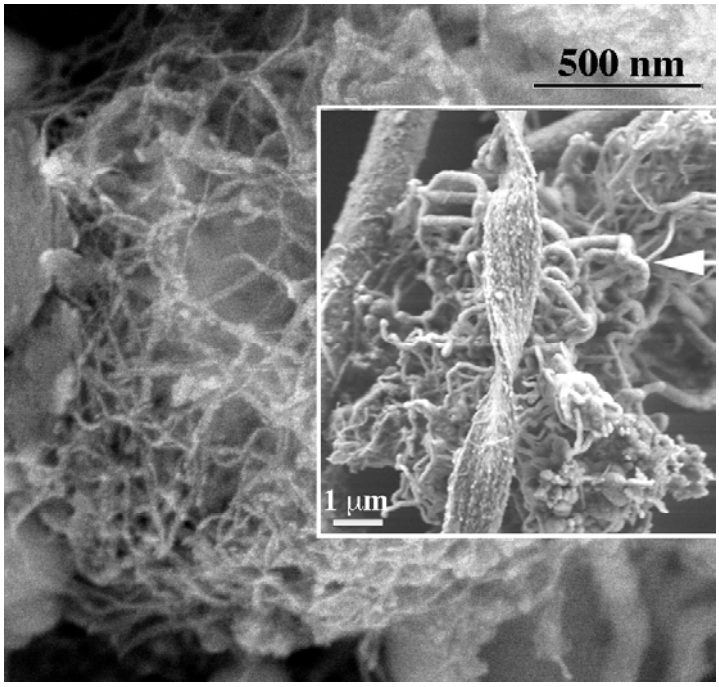


Figure S1

SEM images of mineralized filaments possibly surrounding a cell and (inset) tangled filaments (arrow) associated with sheaths and a twisted stalk (center) in a natural biofilm.

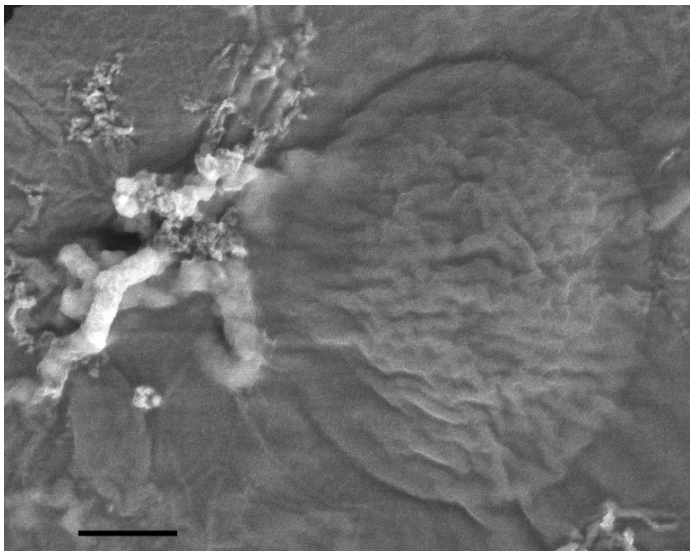
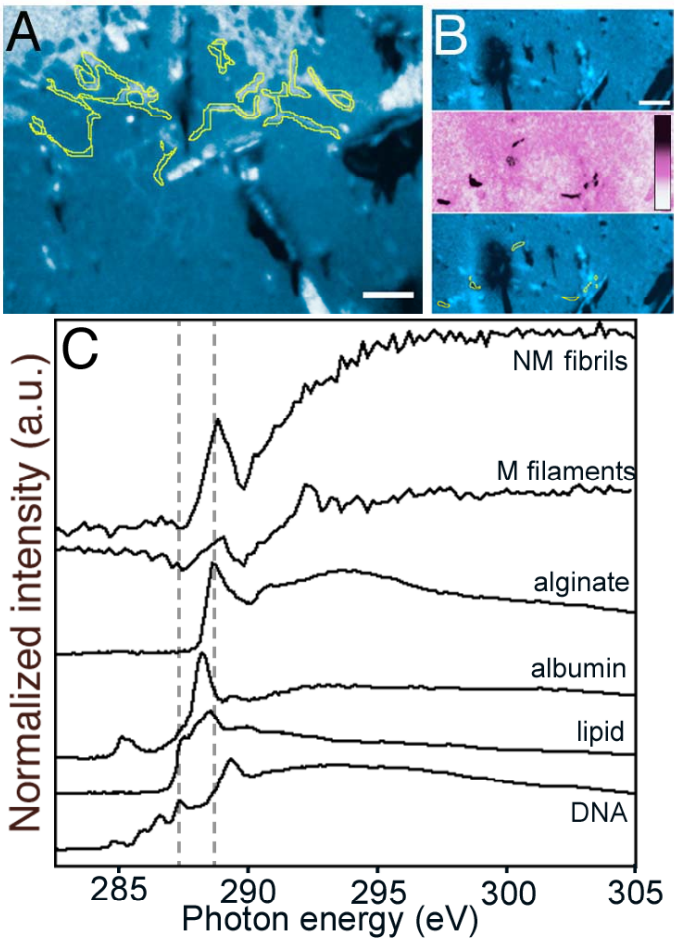


Figure S2

SEM image of a cell associated with mineralized filaments. Scale bar = 0.5 micron.

Figure S3



X-PEEM analyses of mineralized (M) filaments and non-mineralized (NM) fibrils from the biofilm. (**A**) X-PEEM image at 280 eV, showing several mineralized filaments and sheaths. The yellow outlined regions of interest **(**ROIs) indicate the filaments from which the spectrum in (**C**) was extracted. Scale bars in (**A**) and (**B**) are 5 µm. (**B**) Top: image at 282 eV of another portion of the biofilm sample. The dark blurry feature is an object standing out of the surface, as seen from the shadow it projects below. SPHINX has a very shallow depth of field (< 1 µm), this feature, possibly a sheath, is therefore completely out of focus, while its x-ray shadow on the Si substrate is well in focus. Middle: carbon distribution map, obtained by digital ratio of two images of the same sample region, acquired at 295 eV (on peak) and 282 eV (off-peak). Darker color in this distribution map corresponds to higher C concentration. We notice that carbon is present everywhere on the sample, but at higher concentration in specific locations, consistent in size and shape with fibrils. Bottom: the ROIs are shown in yellow, on the same sample image. These were selected on the C-rich fibrils. (**C**) Top to

bottom: Carbon XANES spectra extracted from the M filaments in (**A**) and the NM fibrils in (**B**), compared to the reference spectra from organic compounds (*S1*). The dashed lines at 287.3 eV and 288.6 eV highlight the energy position of the most characteristic peaks for DNA and alginate. The filaments in (**A**) had an Fe spectrum identical to the filament whose Fe spectrum is shown in Fig. 4, while the filaments in (**B**) contained no Fe; we therefore attributed Fe presence to mineralization. As in the filaments of Fig. 3, the C spectrum from NM fibrils is much more intense and matches alginate, while in M filaments this spectrum shows additional structure. In particular, a peak appears at 292.4 eV. The decrease in intensity of the M filaments spectra in Fig. 3 and here corresponds to lower relative concentration of carbon in the presence of Fe.

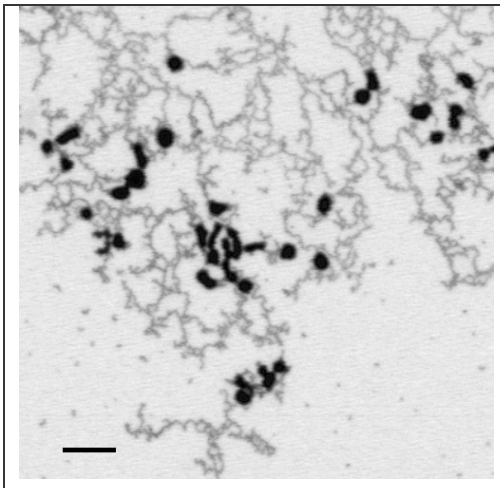


Figure S4 STXM image of synthetic iron-mineralized alginate filaments, taken at 709.5 eV, near the Fe L_3-edge. Scale bar = 1 micron.

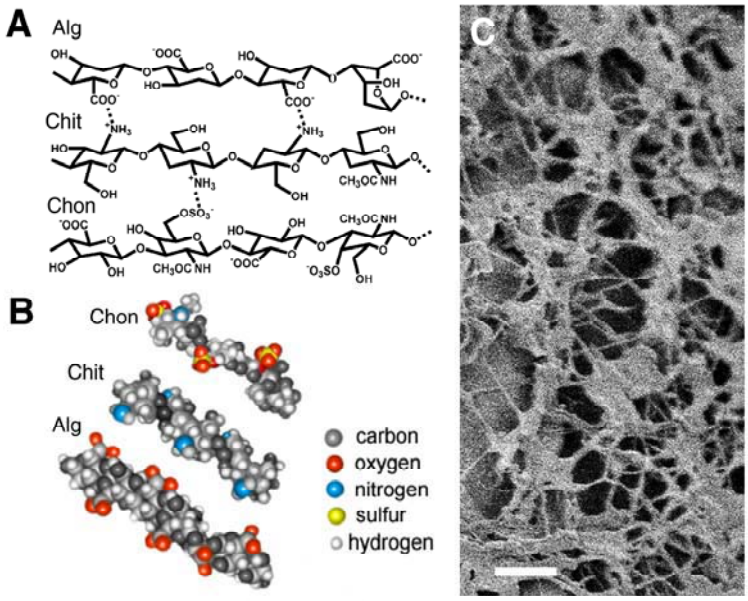


Figure S5 Chitosan-chondroitin sulfate-alginate gel used in the saccharide mineralization experiments (*S6-S8*). (**A**) Diagram of the multiple poly ion pairings between components shown in (**B**). Chon, Chit, and Alg combined in the appropriate ratio form a fibrillar polymer network with stable fibril structures. (**C**) SEM picture of high-pressure freeze-dried sample of gel, showing the self-assembled fibrils. Scale bar = 1 micron.

Figure S6

Possible model of polymer-localized iron oxyhydroxide precipitation and effect on energy metabolism.

SOM references

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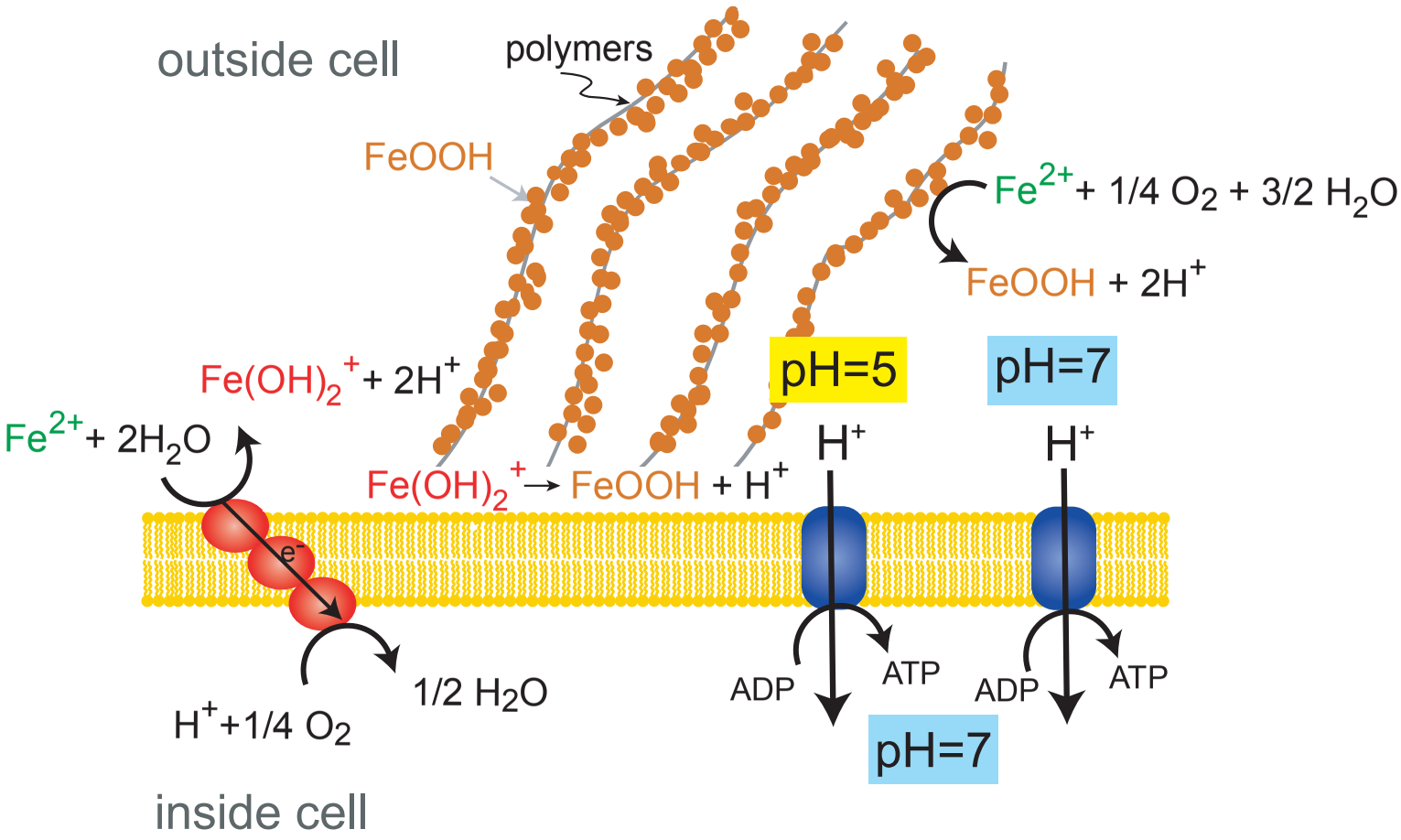
outside cell
polymers
FeOOH
Fe^{2+} + 1/4 O_2 + 3/2 H_2O
FeOOH + $2H^+$
pH=5
pH=7
$Fe(OH)_2^+$ + $2H^+$
Fe^{2+} + $2H_2O$
$Fe(OH)_2^+$ → FeOOH + H^+
H^+
H^+
e^-
1/2 H_2O
H^+ +1/4 O_2
ADP
ATP
ADP
ATP
pH=7
inside cell