In situ nuclear magnetic resonance microimaging of live biofilms in a microchannel

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Electronic Supplementary Experimental Details

Device fabrication and interface assembly

The silicon nitride (SiN) membrane was oxidized in an oxygen plasma for 30 sec and immediately employed to conformal contact with the polydimethylsiloxane (PDMS) block.[1] Irreversible bonding was formed by heating the assembly in an oven at 75 °C for 2 hr. Two 1/16" polytetrafluoroethylene (PTFE) tubings (I.D. 0.023") were interfaced to the holes on the PDMS block via two small metal tubings (New England Small Tube Inc. Litchfield, NH, USA). These connections and the original PDMS, except the SiN surface bearing the detection window, were encapsulated in a bigger PDMS block, and then coated with a thin layer of gold to reduce potential gas permeation.[2] A syringe pump (Harvard Apparatus, Holliston, MA, USA) was used to infuse solutions to the microfluidic reactor as needed at a maximum flow rate of 2 μL/min. A 100 nm thick SiN membrane on a silicon (Si) frame (window: 1.5×1.5 mm^2; and frame: 7.5×7.5 mm^2, 200-μm-thick, Norcada, Inc., Edmonton, Canada) was used to enclose the microfluidic channel.

Biofilm growth

All chemicals used in the chemically defined, modified M1 minimal medium were purchased from Sigma-Adrich Chemical Co. (St. Louis, MO, United States) unless otherwise noted. The modified M1 medium solution consists of piperazine-N,N'-bis(ethanesulfonic acid) (PIPES) buffer (30 mM in the starter culture or 3 mM in the microfluidic reactor) at pH 7.2, 7.5 mM sodium hydroxide, 28 mM ammonium chloride, 1.34 mM potassium chloride, 4.35 mM monobasic sodium phosphate, 30 mM sodium chloride, 0.68 mM calcium chloride, 0.005 mM ferric nitrilotriacetic acid, and 0.001 mM sodium selenate. Wolfe's vitamins and minerals solutions were provided as described in Kieft et al.[3], and the amino acids L-glutamic acid, L-arginine, and D,L-serine were supplemented at final concentrations of 2.0 $mg \cdot L^{-1}$. In the starter culture, 30 mM sodium lactate was added as the electron donor and atmospheric O_2 was the terminal electron acceptor. The microfluidic reactor contained 20 mM sodium lactate and 20 mM sodium fumarate as the electron donor and acceptor, respectively.

Prior to inoculating the microfluidic reactor, the reactor was sterilized by flowing a 70% ethanol solution through the system for a minimum of 3 hr. Filtered sterilized (0.22 μm) ultrapure water was passed through the system for a minimum of five volume-changes and a sterile medium solution was passed through the system overnight. To grow the starter culture, 20 mL of modified M1 minimal medium was added to a 60 mL serum bottle and sealed with a thick butyl rubber stopper. The batch starter culture was grown for 24 hours at 30° C with shaking (150 rpm). Cells were harvested by centrifugation for 10 minutes at 5000 x *g* at 23° C. The supernatant was decanted and the cell pellet was resuspended in 10 mL of medium optimized for the microfluidic reactor. The microfluidic reactor was inoculated as described in the main text.

To inoculate the reactor, an overnight culture of *Shewanella oneidensis* MR-1 expressing green fluorescent protein (GFP)[4, 5] was harvested by centrifugation (5000 x *g*, 10 min) and resuspended in an equal volume of sterile medium. The resuspended bacterial culture was flown through the microfluidic reactor at 2 μL/min for 3 hr. Two 10-mL syringes containing sterile growth medium and a drip tube flow break to prevent back-contamination were aseptically attached to the manifold at the end of inoculation period. The medium solution was run through the microfluidic reactor at room temperature for five to six days at a flow rate of 2 μL/min, which was permissive for suboxic bacterial growth. In the microfluidic channel, the biofilm was adherent to the SiN membrane (Fig. 1c) and biofilm growth was confirmed by *in situ* CLSM imaging of GFP (see Fig. S1).[4]

ToF-SIMS instrumentation

A pulsed 25 keV Bi^+ (beam size: ~250 nm) ion beam with an incident angle of 45 degree off the normal was used as the primary ion beam for all measurements, with the beam current of ~1.0 pA, pulse width of 130 ns and a repeated frequency of 20 kHz. No spraying or fast spreading of aqueous solutions from the aperture was observed during operation. Vacuum pressure during measurements was 2.5-5.5×10^{-7} mbar in the main chamber. Before each measurement, a 500 eV O_2^+ beam (~40 nA) was scanned on the SiN window with a 400×400 μm^2 area for ~30 s to remove surface contamination. Also, an electron flood gun was used to compensate for surface charging during all measurements. To determine the location of the aperture, high-quality SIMS images were obtained by using a 10×10 μm^2 imaging mode, in which the Bi^+ beam was scanned with 128×128 pixels with a total integration time of 65.5 s (40 shots per pixel). The average counts per pixel are about ~ 50±10. To collect high mass resolution ($M/\Delta M \approx$ 2000-4000 at m/z > 30 amu) spectra from the liquid surface, a narrow pulse width (~5 ns) Bi^+ beam was scanned in a 2-μm diameter round area at the aperture center. Long measurement time (>20 min) was necessary to obtain spectra with reasonable quality. More ToF-SIMS measurement details were described in our previous papers.[4] The m/z peak intensities were integrated using the IONTOF instrument software.[6, 7]

Electronic Supplementary Figures

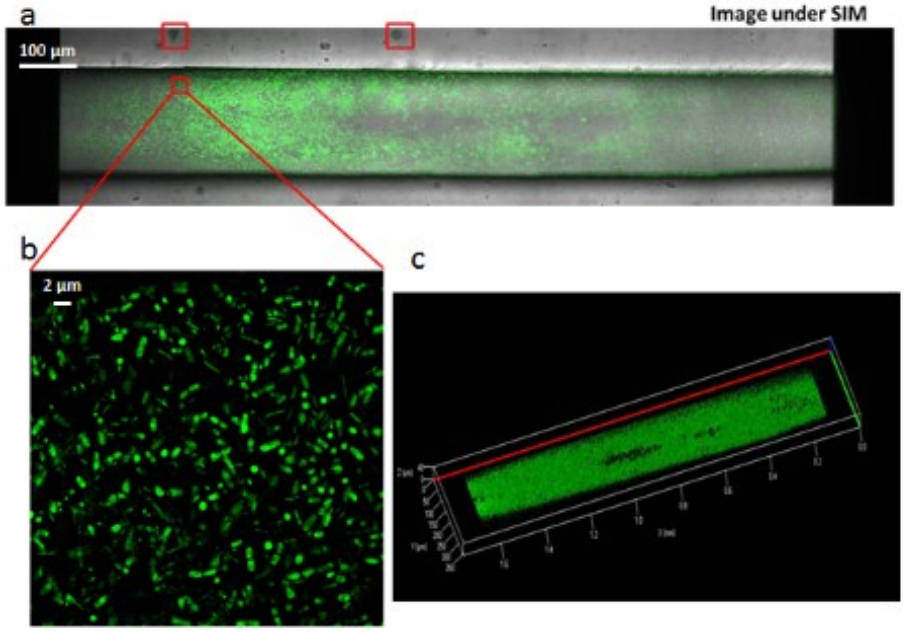

Fig S1a. SIM images of (a) the microfluidic channel; (b) the zoomed-in area in the red square in a; and (c) a view of the channel reconstructed using ImageJ software.

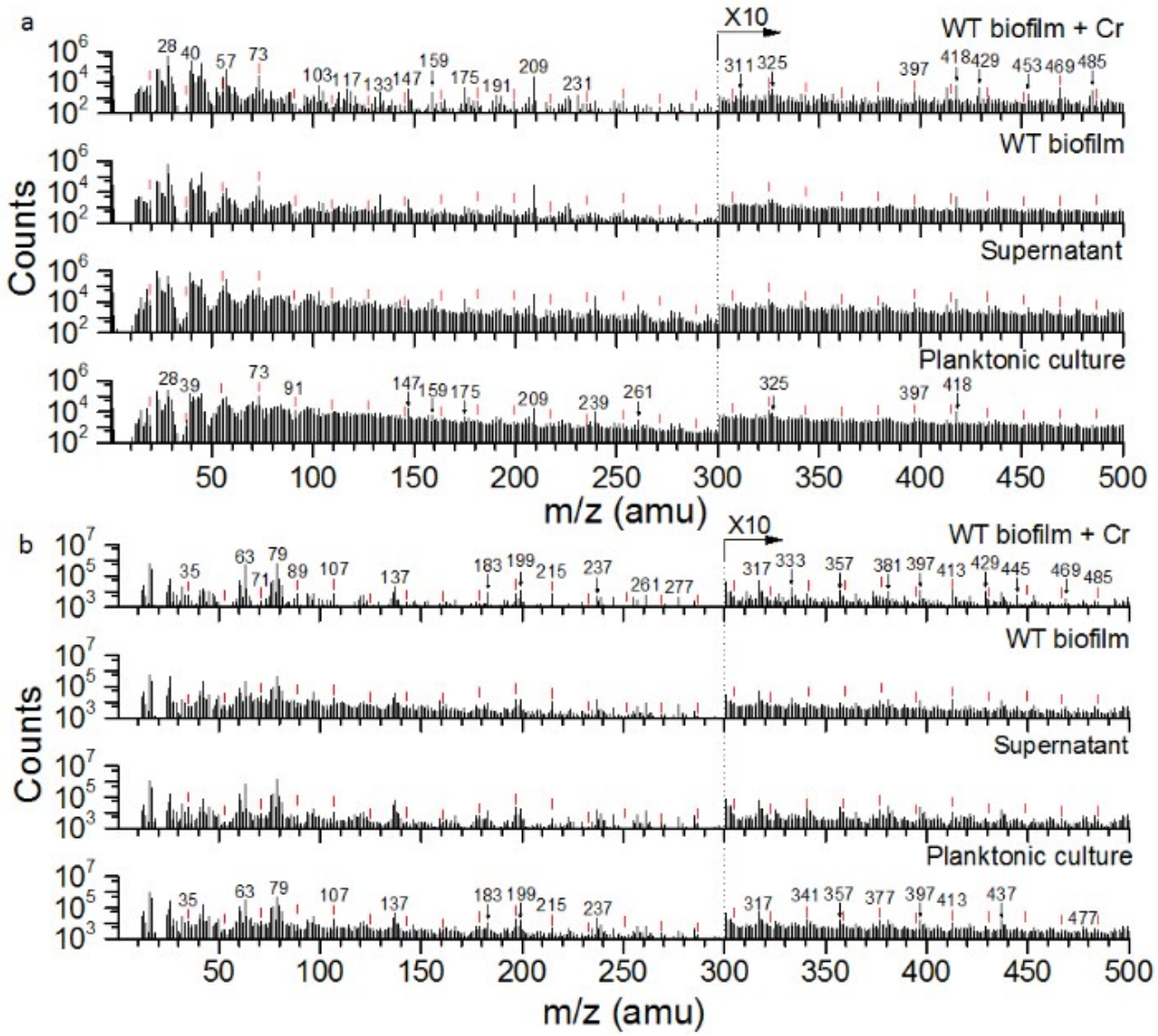

Fig S2a.Representative ToF-SIMS spectra of *Shewanella* biofilms cultured in the microchannel: a) positive and b) negative ion mode.

Electronic Supplementary References

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