Supporting Information

Motility of *Shewanella oneidensis* MR-1 Allows for Nitrate Reduction in the Toxic Region of a Ciprofloxacin Concentration Gradient in a Microfluidic Reactor

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Contents:

Chemicals

All media reagents were of ACS reagent grade or cell culture grade and used without further purification. Solutions were prepared with distilled, deionized water (DDW; 18.2MΩ cm, Millipore Co.). Other reagents include fluorescein sodium salt (Sigma-Aldrich, BioReagent), ciprofloxacin (Sigma-Aldrich, ≥98.0% HPLC grade), sodium nitrate (Sigma-Aldrich, ≥99% BioReagent), and sodium DL-lactate (Sigma-Aldrich, 60 % (w/w)).

MGC Fabrication

The microfluidic gradient chamber (MGC) was fabricated using standard photolithography and inductively coupled plasma reactive ion etching (ICP-RIE). A silicon wafer was initially coated with photoresist (AZ5209E) and the features of the nanoporous barrier were imprinted on the wafer using a chrome mask and a photolithography I-G line mask aligner (SussMicrotec - MA6/BA6). The exposed wafer was then developed (MF-26A Microposit) and hard baked. The nanoporous barrier was then etched to a depth of 200 nm using an RIE (790 Plasma-Therm). After etching the wafer was cleaned using an O_2 descumming process (Asher Nordson March PX-250) and a piranha clean (1:2; hydrogen peroxide: sulfuric acid). The depth of the slits was verified with an optical profilometer (Dektak 150). A second mask contained the features for the boundary channels and hexagonal well array. A similar process was used for the second etch but it was done using a Bosch process on a deep RIE (DRIE, Plasma-Therm Versaline DSE) to ensure an anisotropic deep silicon etch.

MGC Cleaning and Sterilization

After experiments, biomass and organic build-up in the MGC was removed via successive infusion of protease (1X solution Gibco® Trypsin-EDTA), water, surfactant (Alconox, 1% w/v), and water. A Standard Clean 1 (SC-1 clean) was then used as a final step to remove any trace organics. The SC-1 clean consists of 1:1:5 hydrogen peroxide, ammonium hydroxide and water.

The MGC was then purged with sterile D.I. water and stored until used for the MGC nitrate reduction experiments.

Analytical Methods

Nitrate concentrations were quantified using ion chromatography (IC) with a conductivity detector (CS-2100, 4x250 mm IonPac AS-19, Dionex). Nitrite concentrations were measured using a USEPA Diazotization Method (Hach TNT plus 839). Ammonium concentrations were measured using the salicylate method (Hach test kit) at a spectrophotometer wavelength of 655nm. Ciprofloxacin was quantified using HPLC coupled with fluorescence excitation/emission at 485/515 (Shimadzu Prominence-I, UltiMate 3000).

MGC Cell Extraction and Susceptibility Assay Methods

Cells within the well array were extracted from the MGC through the inoculation and extraction ports. Several methods were used to determine the antibiotic susceptibility of MR-1 upon completion of the MGC experiments.

Method 1: 1ml of cells was extracted from the MGC with media buffer. Cells were lawned on agar plates that contained increasing concentrations of ciprofloxacin. This method was used because it was known that the cell density of the MGC extraction was below that needed for a proper MIC susceptibility test. Results showed that there was no resistance for WT-H extracted cells.

Method 2a: 1ml of extracted cells was extracted. 10ul aliquots were diluted to a total of 220ul of solution (200ul media, 10um antibiotic, and 10ul cells extracted from MGC) and run on a 96-well plate at varying ciprofloxacin concentrations (i.e., 0, .5, 1, 2, 3, 5, 10, 20, 30, and 50x MIC_{WT}). This test was conducted without control of the inoculum concentration. The incubation period was extended for 72 hrs to ensure growth of low-density cells, but it should be noted that

within 24hrs cells with no antibiotic grew to normal densities. Results showed that there was no resistance gained for WT-H extracted cells.

Method 2b: 1ml of cells was extracted. 200 µl aliquots were directly amended (without dilution) with different ciprofloxacin concentrations (i.e., 0, 1, 2, 3, and 5x MIC_{WT}) and run in the 96 well plate. The total solution volume in each well was 210ul (10ul of antibiotic and 200ul cells extracted from MGC).

Our imaging results show approximately ~1300 cells/well in Row 1, adjacent to the ciprofloxacin boundary. There are 21 wells in this row, corresponding to 27300 cells. For a 1 ml extraction, this cell number corresponds to $2.73*10^4$ cells/ml. In Method 2a, for example, this corresponds to $2.73*10^2$ cells/well in the 96-well plate (i.e., $1.24*10^3$ cells/ml). In Method 2b, this corresponds to $5.46*10^3$ cells/well in the 96-well plate (i.e., $2.6*10^4$ cells/ml). The cell count is sufficiently high in Method 2b such that if cells in Row 1 were resistant to 50x MIC, they would have visible growth at 1-5x MIC within 24 hrs, which they did not. Only 1x MIC resistance was observed after 48hrs, but a control with WT cells also had visible growth after 48hrs at 1x MIC. After 72 hrs, there was still no observed growth.

Method 3: 1ml of cells was extracted. Extracted cells were grown for 5-8 hrs until cell density was sufficient enough to yield a final concentration of $1*10^6$ cells/mL in each well of the 96-well plate; results showed that there was no resistance gained for WT-H extracted cells.

Method 4: Same procedure as method 3 was conducted. Due to concern that the incubation period would result in selection of non-resistant cells, the incubation vials were spiked with a sub-inhibitory concentration of ciprofloxacin to select possible mutant cells. Results for WT-H showed that a WT control taken through the same process resulted in the same level of enhanced resistance.

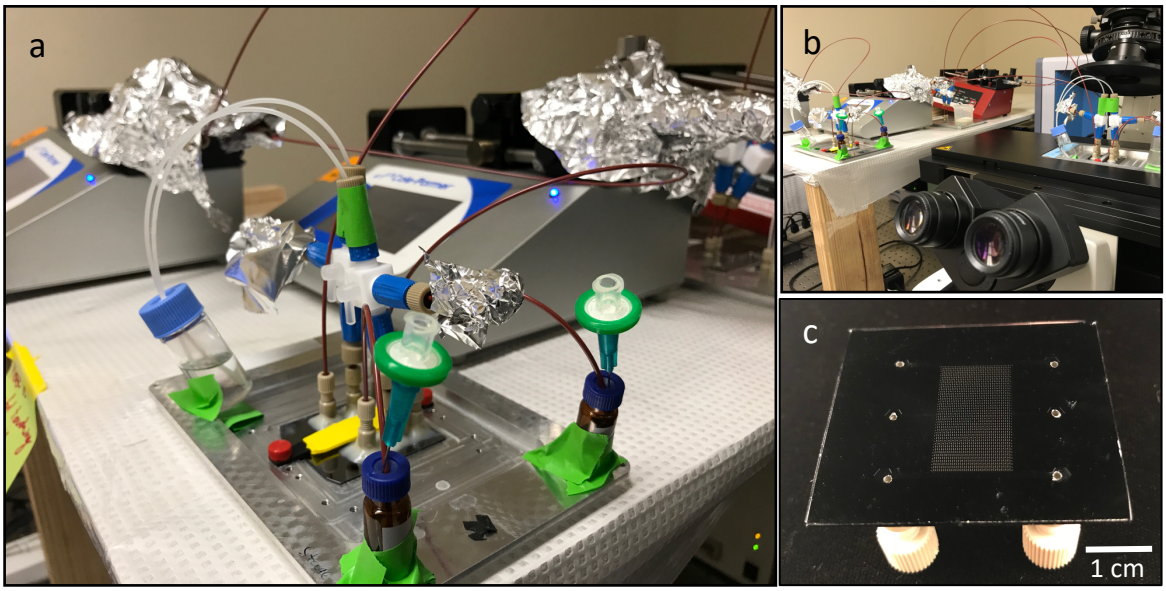

Figure S1: (a) Picture of complete microfluidic assembly depicting syringe pumps, tubing, microscope stage, microfluidic reactor, and sterile effluent collection vials. (b) Picture of the microfluidic assembly mounted on an inverted microscope. (c) Picture of the microfluidic reactor.

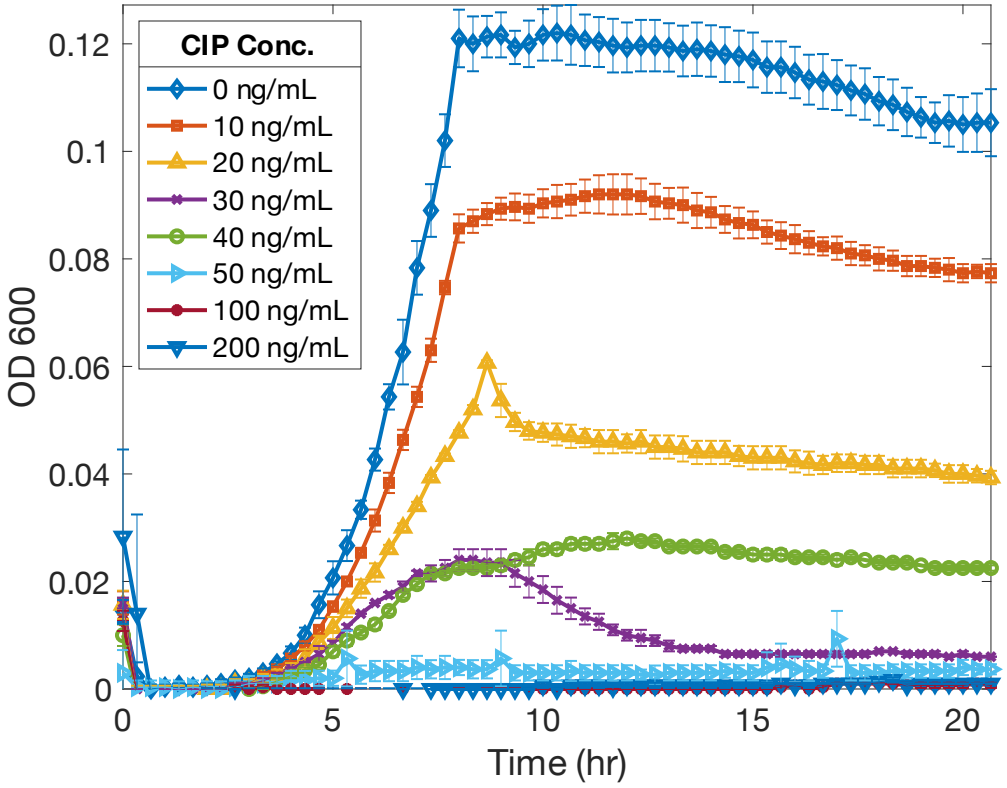

Figure S2: Minimum Inhibitory Concentration (MIC) determination of WT *S. oneidensis* MR-1 under anoxic conditions using a 96-well plate assay. Legend depicts ciprofloxacin concentrations. Error bars are as standard deviation, n=3.

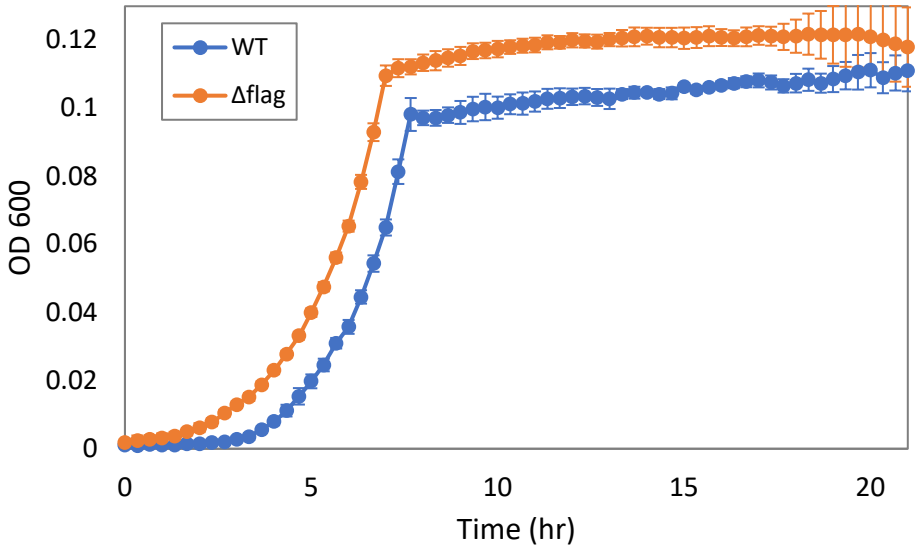

Figure S3: Kinetic run comparing the rate of nitrate reduction of the wild-type strain and the flagella deficient (Δ*flag*) strain. Error bars are as standard deviation, n=5.

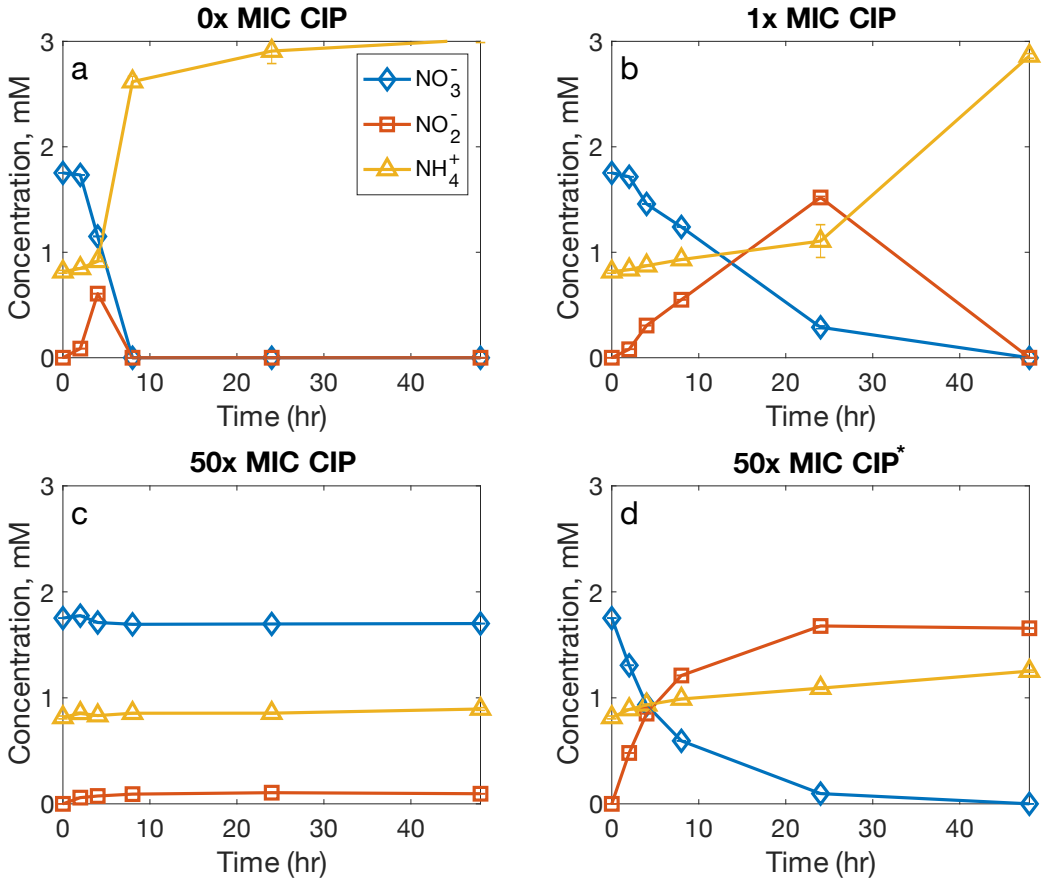

Figure S4: Comparison of NO_3-, NO_2^- , and NH_4^+ concentrations over time in batch cultures under ammonifying conditions (a) without ciprofloxacin, (b) 1x MIC ciprofloxacin, (c,d) and 50x MIC ciprofloxacin. Cultures were inoculated at a concentrations of ~$1*10^6$ cells/ml and incubated at 30 ˚C for 48 hr. * Indicates a 50x MIC sample inoculated at a concentration of ~$1*10^7$ cells/ml. Error bars are as standard deviation, n=3.

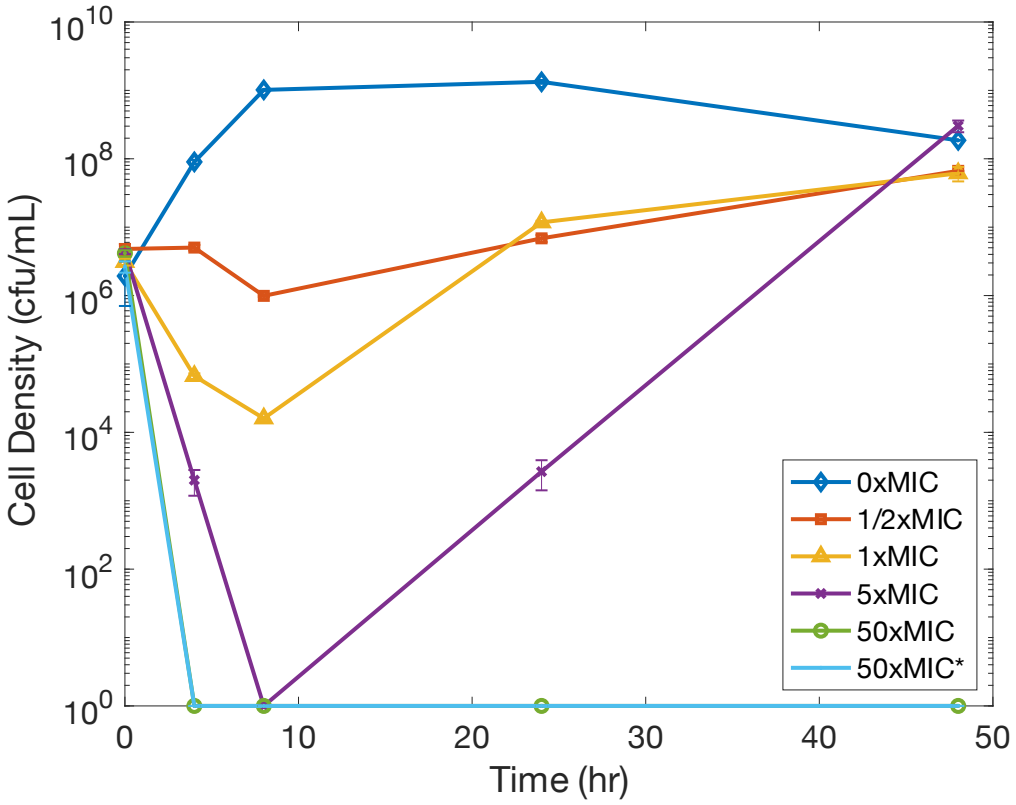

Figure S5: Viable cell counts for cultures of WT *S. oneidensis* MR-1 vs time. Results are shown for experiments of control cultures and cultures treated with ciprofloxacin concentrations corresponding to 0, 0.5, 1, 5, and 50 times the MIC value determined. Cultures were inoculated at a concentration of ~$1*10^6$ cells/ml and incubated at 30 ˚C for 48 hr. * Indicates a 50x MIC sample inoculated at a concentration of ~$1*10^7$ cells/ml. Error bars are as standard deviation, n=3. Limit of detection is $1*10^3$ cells/mL due to required dilutions.

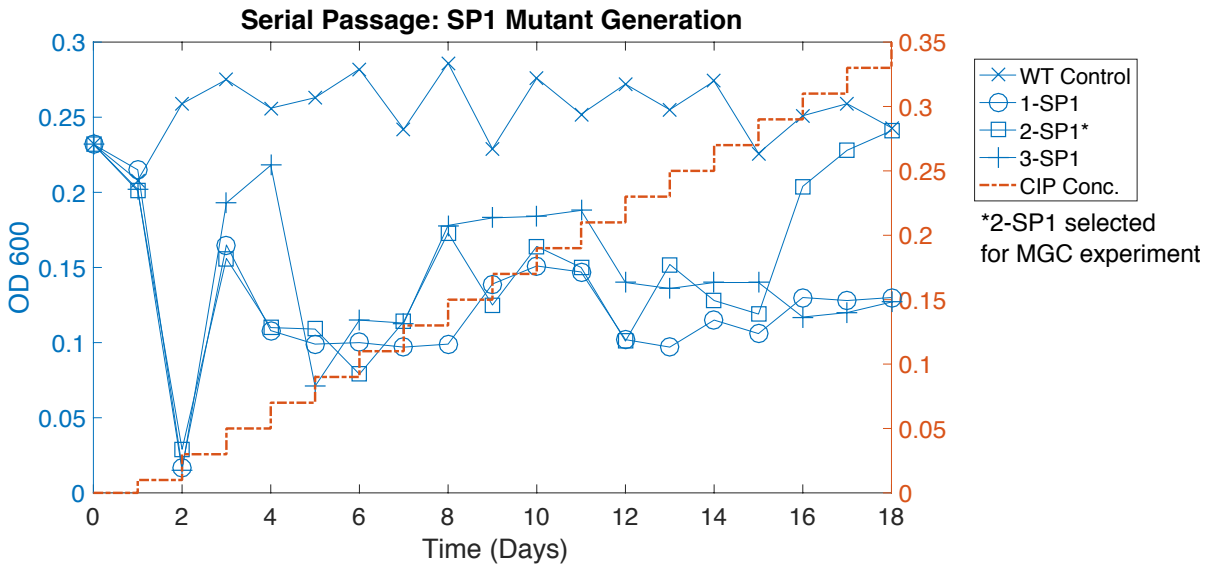

Figure S6: SP-1 mutant generation through a serial passage experiment. Three replicates were monitored over the course of 18 days. OD 600 measurements are on the first y-axis and daily increments of ciprofloxacin are represented on the second y-axis. *A single colony from this triplicate was selected on day 18 for MGC experiment, SP-L.

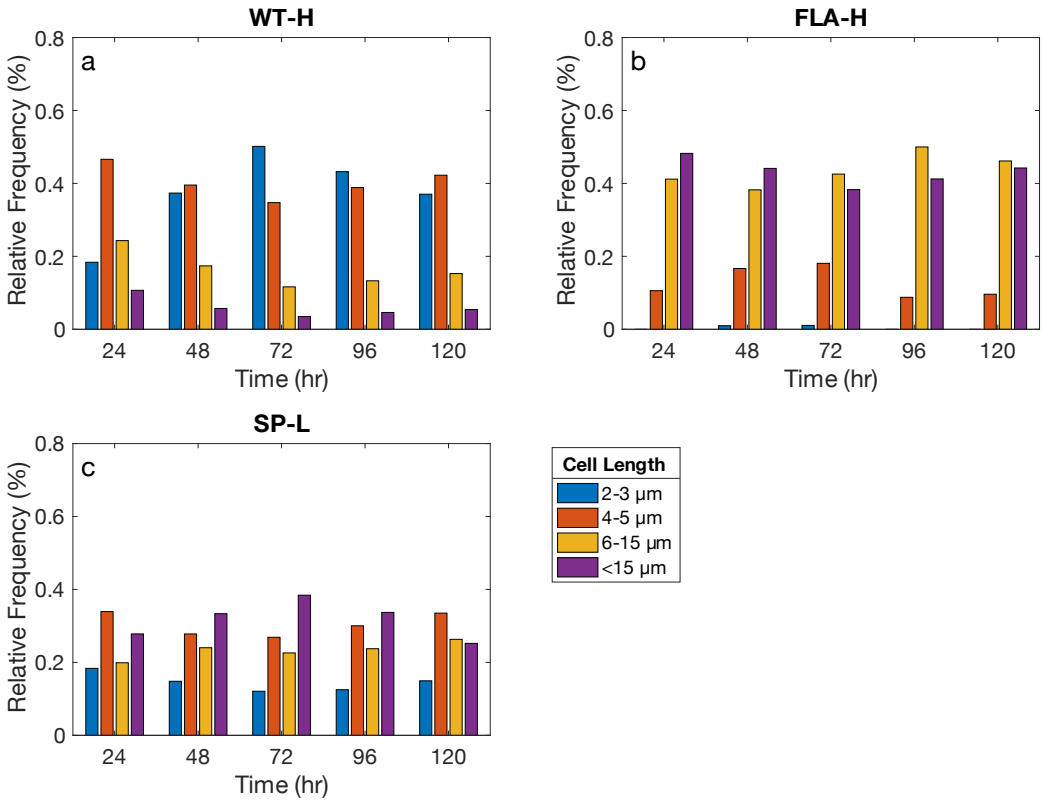

Figure S7: Relative frequency (%) of cell lengths vs time (hr) for MGC experiments. Each bar is a representative range of cell lengths. (a) WT-H (24hr n=4710 cells, 48hr n=5596, 72hr n=4989 cells, 96hr n=4702, 120hr n=4757 cells). (b) FLA-H (24hr n=85 cells, 48hr n=102, 72hr n=94 cells, 96hr n=114, 120hr n=104 cells). (c) SP-L (24hr n=392 cells, 48hr n=1349, 72hr n=1447 cells, 96hr n=1495, 120hr n=2074 cells). Average WT *S. oneidensis* MR-1 cell length is 2-3µm.

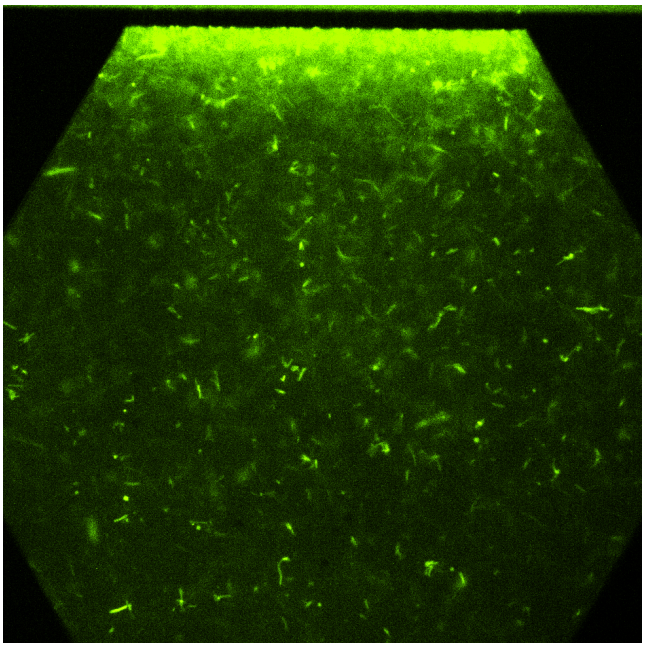

Figure S8: Epi-fluorescent microscopy image with a FITC filter cube at 120hr of a single well for experiment WT-H. Metabolic staining of cells at a well adjacent to BC-1 with RedoxSensor Green.