

Supplementary Information For:

Marine sediments microbes capable of electrode oxidation as a surrogate for lithotrophic insoluble substrate metabolism

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Supplementary Materials and Methods

Sediment Microprofiling

Sediment microprofiling was performed using Unisense microelectrodes and the micromanipulator (Aarhus, Denmark). A custom attachment for the micromanipulator was constructed to allow attachment to sediment microcosms—maximizing the sediment depths profiled. Microelectrodes were calibrated and used according to the manufacturer's protocols (Unisense, Aarhus, Denmark). Profiles were performed in the sediment water column over a distance of approximately six cm. Profiles were taken in microcosms both prior to addition of electrodes and on a weekly basis during electrode incubations.

Media Supplements

Trace mineral mix added the following components at designated concentrations: FeSO₄ (7.5 μM), H₃BO₄ (48 μM), MnCl₂ (0.5 μM), CoCl₂ (6.8 μM), NiCl₂ (1 μM), CuCl₂ (12 nM), ZnSO₄ (0.5 μM), Na₂MoO₄ (0.15 μM), NaVO₃ (2 μM), Na₂WO₄ (75 nM), Na₂SeO₃ (23 nM), HCl (20 μM). The vitamin mix added a final concentration of 0.1 μg/ml of the following components, unless different concentration designated, to media: riboflavin, biotin (0.03 μg/ml), thiamine HCl, L-ascorbic acid, d-Ca-pantothenate, folic acid, nicotinic acid, 4-aminobenzoic acid, pyridoxine HCl, lipoic acid, NAD, thiamine pyrophosphate, cyanocobalamin (0.01 μg/ml). All solutions were made up at 1000x concentration

Metabolite Analysis

Chemical analysis of soluble redox forms of terminal electron acceptors (chelated ferric iron, nitrate, sulfate), metabolic intermediates (nitrite), and soluble end products of oxidized electron donors (sulfate, chelated ferrous iron) was performed using ion chromatography (IC) and/or spectrophotometric assays. Anion analysis via a Metrohm Ion Chromatograph (Metrohm, Riverview, FL) was performed using an anions separation column (Metrosep A SUPP 250, Metrohm) designed for seawater samples with 3.2 mM sodium bicarbonate 1 mM sodium carbonate running buffer containing 2% acetonitrile as per manufacturer protocol (flow rate 0.7 mL/min). Spectrophotometric assays to quantify iron (Ferrous and Total, Ferrozine Assay) (Stookey, 1970) and sulfide (Cline Assay) (Cline, 1969) were previously described.

Reduction Oxidation Potential Calculations

Standard redox potentials were calculated using the following relationship for pH 0 and corrected for pH 8:

$$\Delta E^\circ = \frac{\Delta G^\circ}{-nF}$$

Standard free energies (25°C, 1 atm, 1 M both products and reactants including or excluding protons depending on pH) were calculated for each reaction as listed above using the SUPCRT92 software (Johnson *et al.*, 1992), with thermodynamic data taken from (Shulte *et al.*, 2001; Shock *et al.*, 1997; Shock and Helgeson, 1988).

The following redox potentials for the given half reactions were calculated:

Electron Acceptors	E_0 (V vs. SHE) pH 0	E_0' (V vs. SHE) pH 8
$O_2 + 4 e^- + 4 H^+ \rightarrow 2 H_2O$	1.23	0.77
$NO_3^- + 2 e^- + 3 H^+ \rightarrow NO_2^- + H_2O$	0.82	0.34
$NO_2^- + e^- + 2H^+ \rightarrow NO + H_2O$	1.06	0.11
$2 NO + 2 e^- + H^+ \rightarrow N_2O + H_2O$	1.7	1.22
$N_2O + 2 e^- + H^+ \rightarrow N_2 + H_2O$	1.72	1.24
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	0.77	0.77
$SO_4^{2-} + 2 e^- + 2H^+ \rightarrow SO_3^{2-} + H_2O$	-0.11	-0.59
$2 SO_3^{2-} + 4 e^- + 6H^+ \rightarrow S_2SO_3^{2-} + 3 H_2O$	0.68	-0.04
$S_2SO_3^{2-} + 4 e^- + 6H^+ \rightarrow 2 S^0 + 3 H_2O$	0.49	-0.23
$S^0 + 2 e^- + 1H^+ \rightarrow HS^-$	0.06	0.11
Electron Donors		
$2 S^0 + 3 H_2O \rightarrow S_2SO_3^{2-} + 4 e^- + 6H^+$	-0.49	0.23
$2 S_2SO_3^{2-} \rightarrow S_4O_6^{2-} + 2 e^-$	-0.02	-0.02
$S_2SO_3^{2-} + 3 H_2O \rightarrow 2 SO_3^{2-} + 4 e^- + 6H^+$	-0.68	0.04
$SO_3^{2-} + H_2O \rightarrow SO_4^{2-} + 2 e^- + 2H^+$	0.11	0.59
$Fe^0 \rightarrow Fe^{2+} + 2 e^-$	0.47	0.47
$Fe^{2+} \rightarrow Fe^{3+} + 1 e^-$	-0.77	-0.77

Scanning Electron Microscopy

ITO electrodes were removed from the electrochemical cells and immediately fixed with 2% glutaraldehyde in basal saltwater media. Electrodes were stored in the glutaraldehyde solution at 4°C until dehydration. Dehydration was performed as described in Weber *et al.*, (Weber, Jr. *et al.*, 1978). In brief,

the fixed samples were washed with basal media and subjected to a series of ethanol washes of increasing concentration with final dehydration step using hexamethyldisilazane (HMDS). Post dehydration, samples were sputter coated with carbon as described in (Hsu *et al.*, 2012). Scanning Electron microscopy was performed using a JEOL 7100 SEM (University of Southern California Microscopy Facility).

References

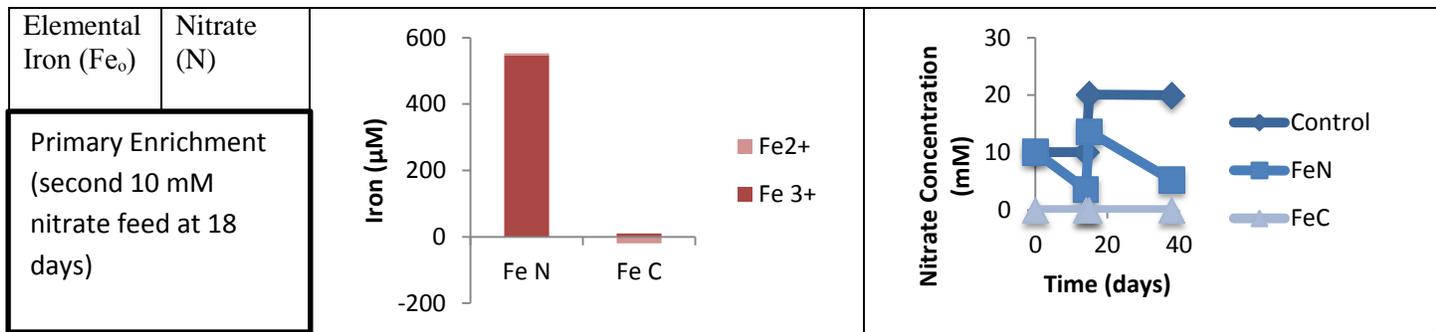
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Supplemental Table S1. Sequences used for tagged sequencing of 16S rRNA genes from the following samples (see Materials and Methods for details).

Sample ID	Barcode Sequence	Linker Primer Sequence
Original Nitrate Electrode Enrichment	AGACGAGT	AGRGTTCGATCMTGGCTCAG
Elemental Iron/Nitrate Enrichment	AGACTGTC	AGRGTTCGATCMTGGCTCAG
Elemental Sulfur/Nitrate (SN) Enrichment	AGACCACT	AGRGTTCGATCMTGGCTCAG
SN transfer 1	AGACCAGA	AGRGTTCGATCMTGGCTCAG
SN transfer 2	AGACCTCA	AGRGTTCGATCMTGGCTCAG
Iron Sulfide/Nitrate (FeSN) Enrichment	AGACTCAC	AGRGTTCGATCMTGGCTCAG
FeSN transfer 1	AGACTCTG	AGRGTTCGATCMTGGCTCAG
FeSN transfer 2	AGACTGAG	AGRGTTCGATCMTGGCTCAG

Supplemental Table S2. Metabolite analysis of replicate solid substrate enrichment cultures (1-3), controls (C) and transfer cultures (data for first transfer only shown [A]). Enrichments were seeded with biomass from sediment free electrochemical reactors. Iron quantification was performed via Ferrozine assay and nitrate/nitrite analysis was performed via ion chromatography (described in Supplementary Materials and Methods).

Electron Donor (ED)	Electron Acceptor (EA)	Oxidation of ED (i.e., Sulfate or Fe ³⁺ production relative to control)	Reduction of EA (i.e., Nitrate consumption relative to control)
Elemental Sulfur (S ₀)	Nitrate		
Primary Enrichment (second 10 mM nitrate feed at 18 days)			
Elemental Sulfur (S ₀)	Nitrate		
First Transfer (second 10 mM nitrate feed at 18 days)			
Reduced Iron-sulfide (Fe _x S _x)	Nitrate		
Primary Enrichment (second 15 mM feed at 14 days)			
Reduced Iron-sulfide (Fe _x S _x)	Nitrate		
First Transfer (second 15 mM feed at 14 days)			



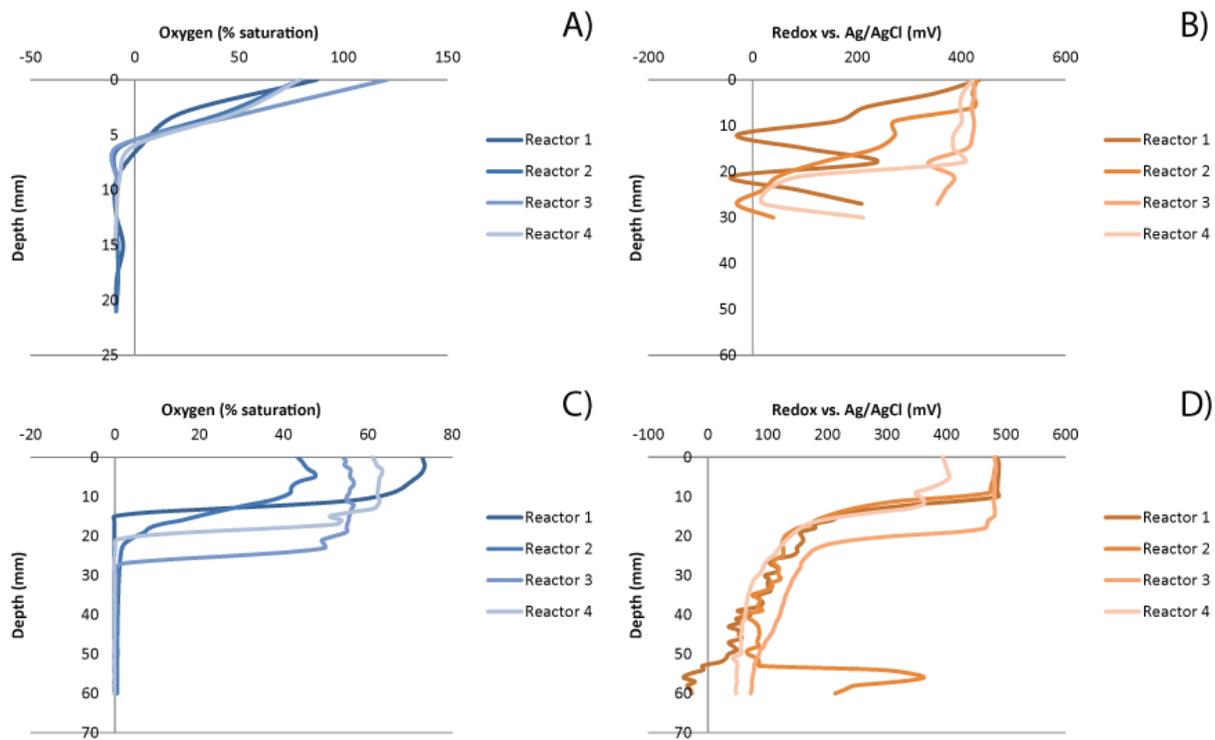


Figure S1. Example oxygen (A&C) and Redox (B&D) microprofiles from marine sediment microcosms displayed. Quadruplicate reactors incubated with electrodes and various potentials. Initial (A&B) and Final (C&D) profiles are illustrated. Initial profiles are shorter due to depth constraints on micromanipulator. Modification of microprofiling equipment eliminated this limitation for later profiles (See Supplementary Materials and Methods).

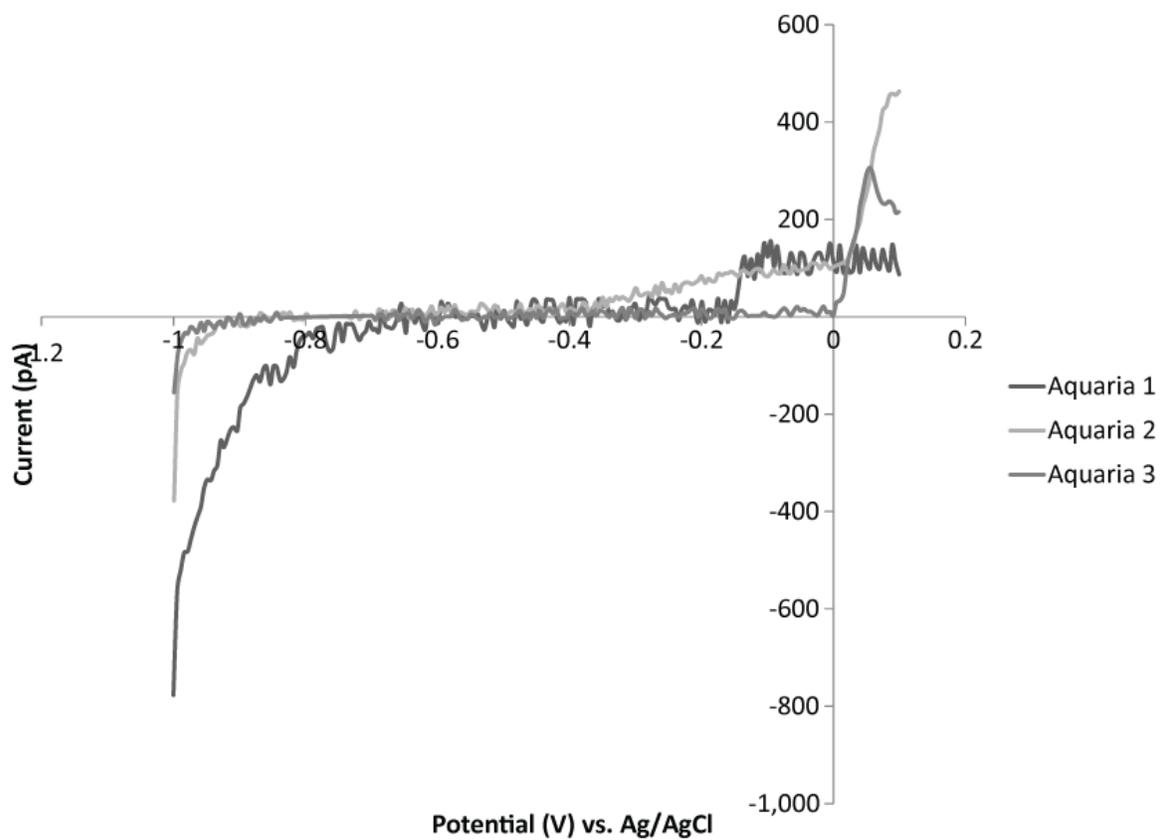


Figure S2. Linear Sweep Voltammetry (LSV) performed on replicate reactors constructed with newly sieved sediments from Catalina Harbor and UV treated Seawater. LSV run from -1.0 to 0.1 V at a scan rate of 0.3 mV/s.

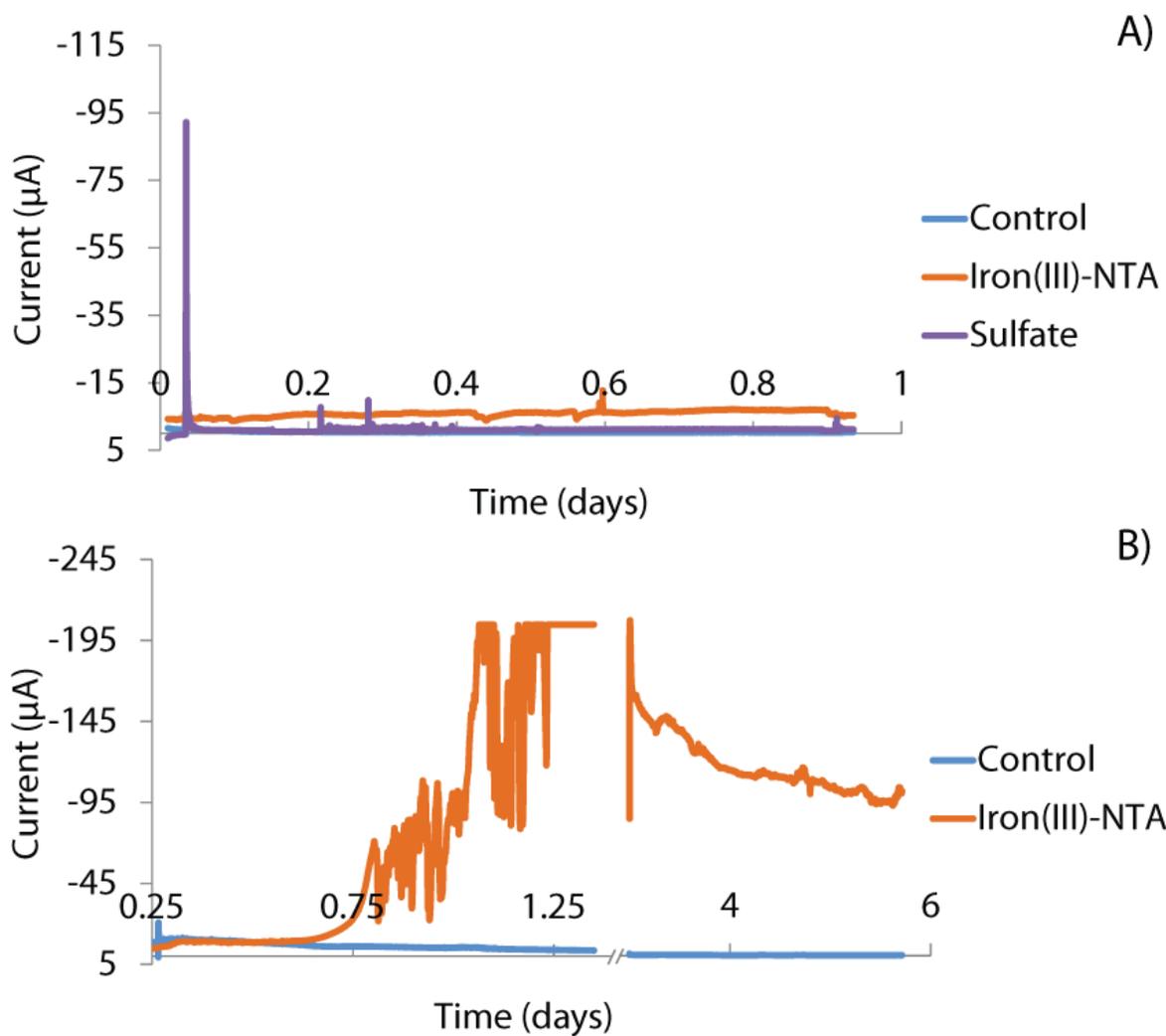


Figure S3. Chronoamperometry profiles for iron and sulfate amended sediment free bioreactors relative to control (sterile electrode). A) Represents early time points in the reactor while B) demonstrates current production after five consecutive feeds of 250 μM Ferric Iron NTA. In both of these profiles amendments were added to reactor at approximately time zero.

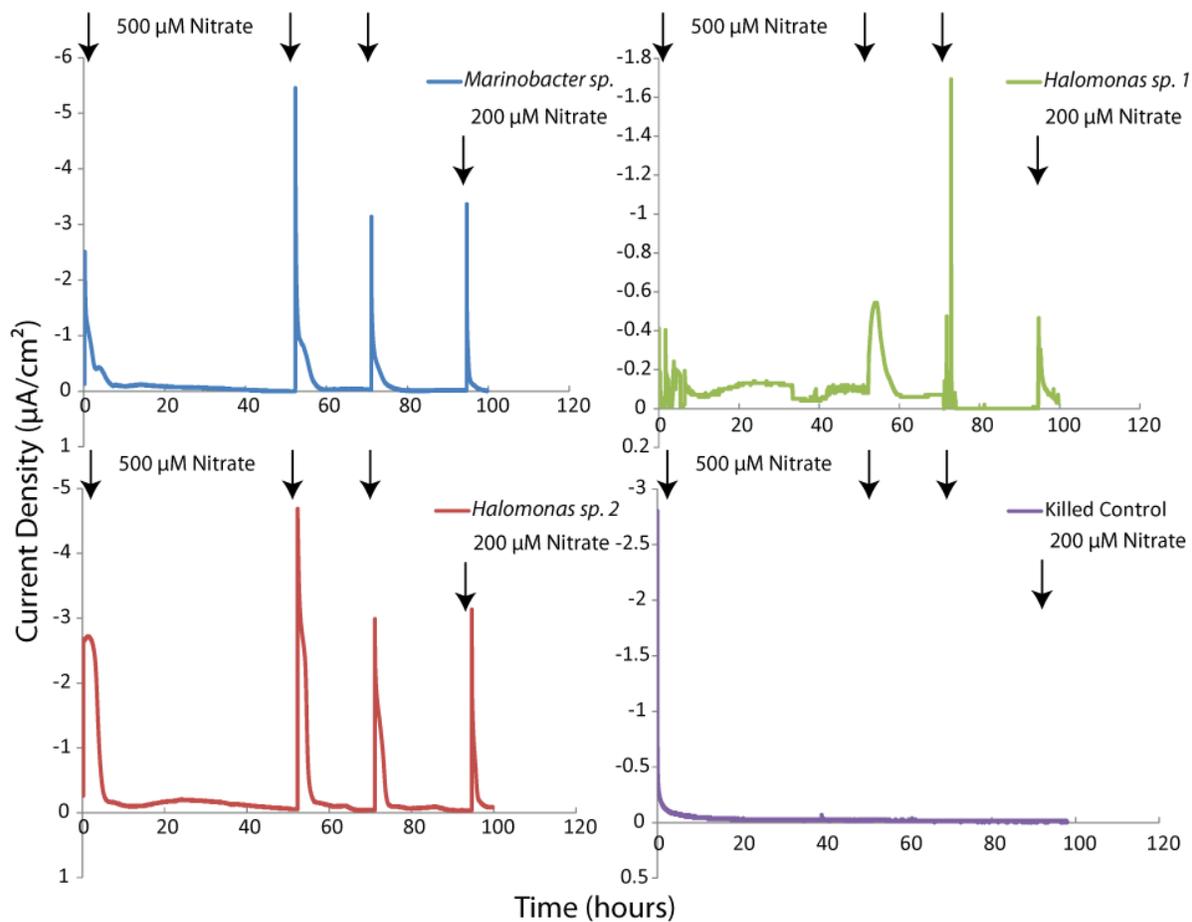


Figure S4. Selected chronoamperometry results for isolated culture from the *Marinobacter* and *Halomonas* genera of the *Gammaproteobacteria*. A killed control consisting of an autoclaved *Halomonas* culture was also tested. Periodic batch feeds of nitrate (first three 500 μM and last 200 μM) were added to stimulate current production as indicated by arrows.

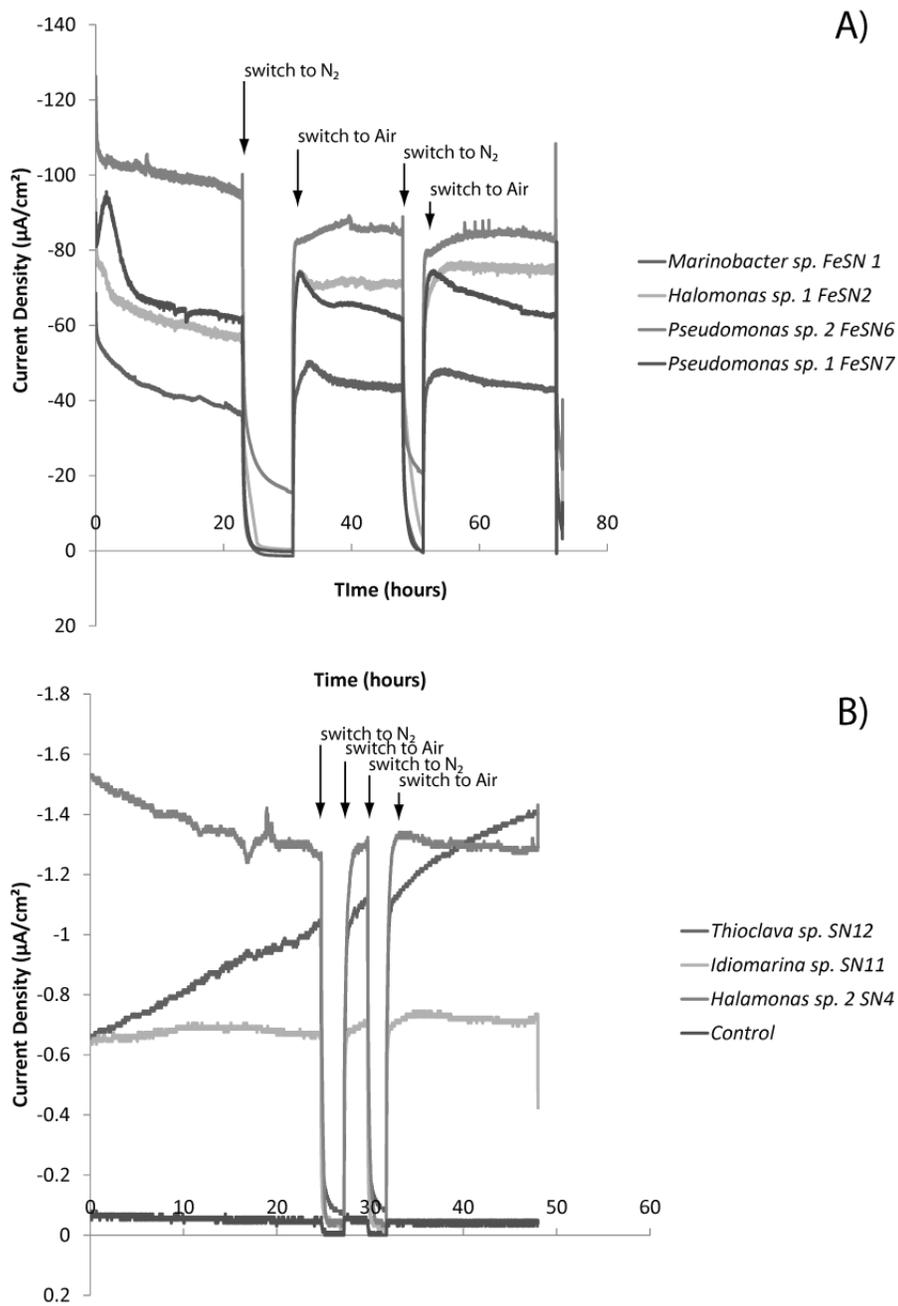


Figure S5. Chronoamperometry data for electrochemically active cultures at various stages of inoculation on either graphite (A) or ITO (B) electrodes. Current production in electrochemical cells linked to the presence of oxygen

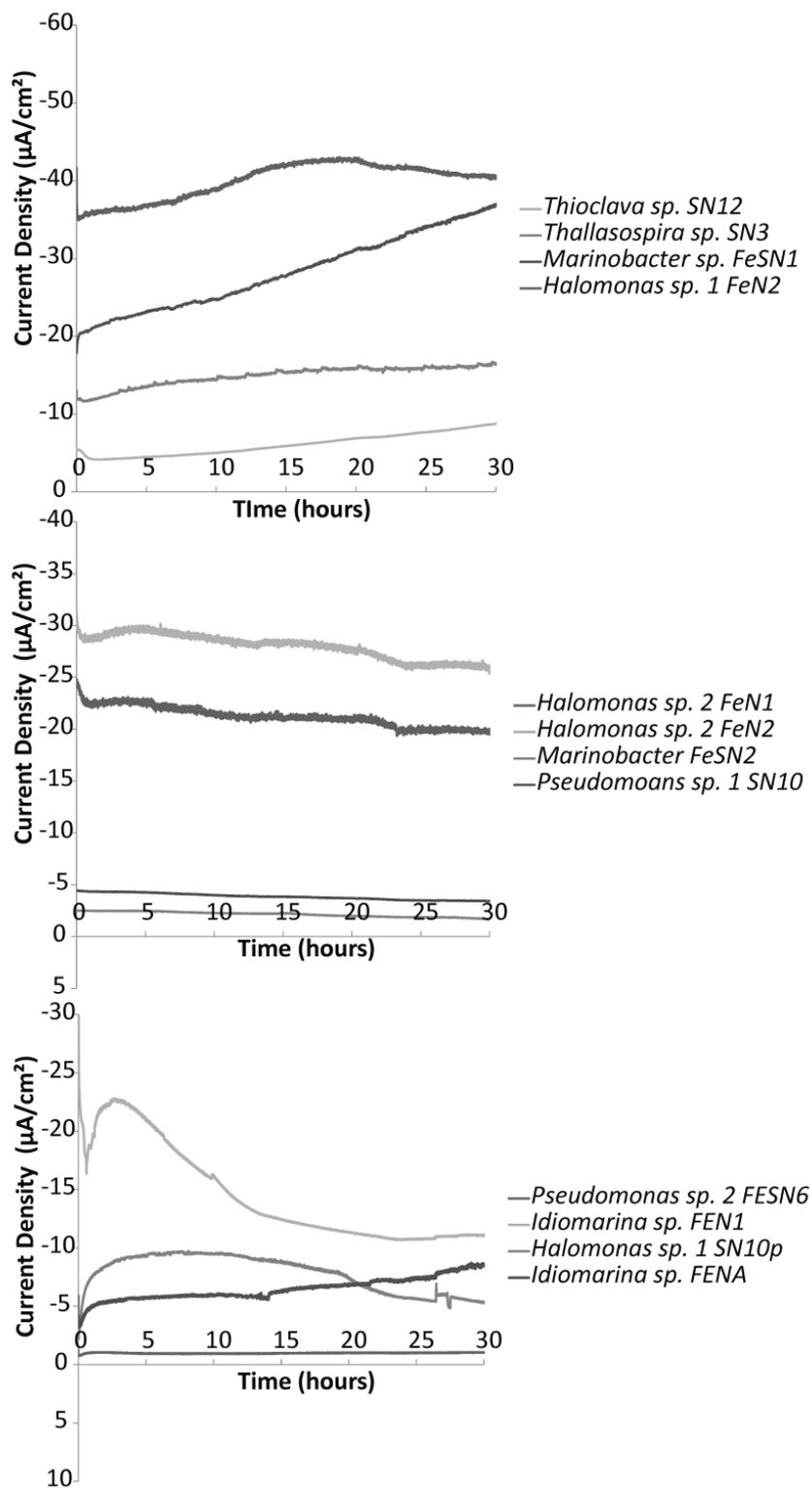


Figure S6. Chronoamperometry profiles for electrochemically active isolates grown in an electrochemical cell with oxygen as a terminal electron acceptor in the absence of organic carbon from two to four weeks. Electrodes were poised at -400 mV vs. Ag/AgCl.

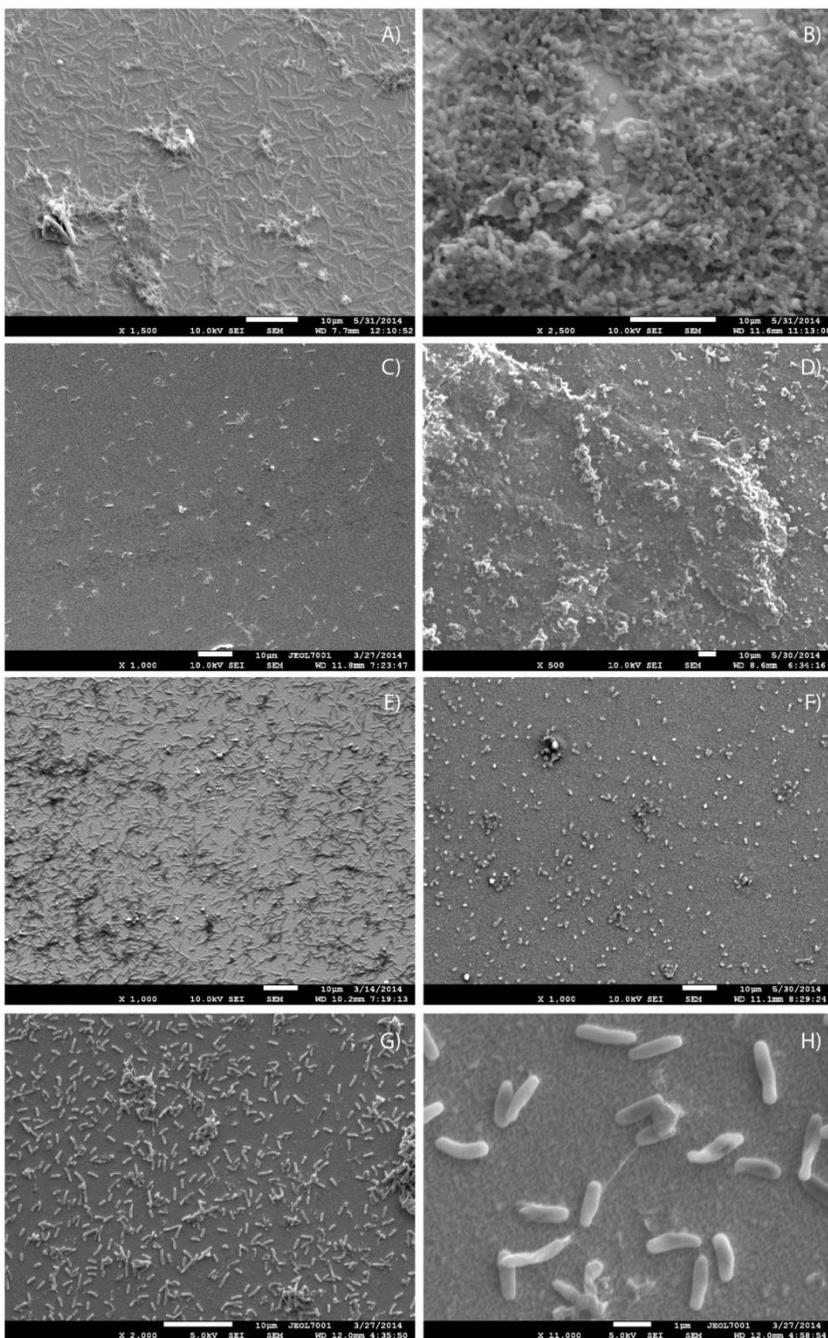
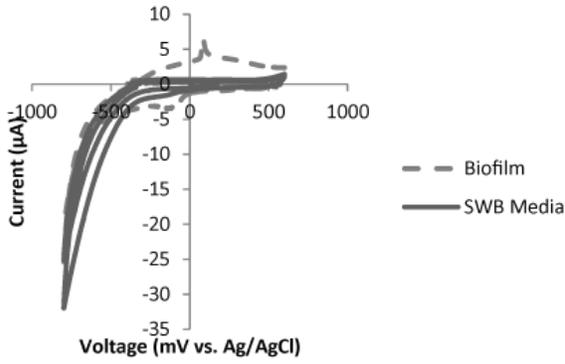
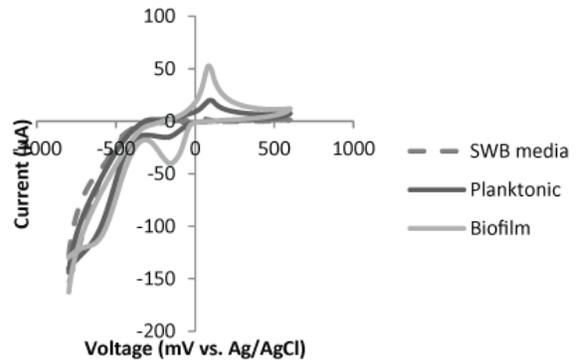


Figure S7. Representative Scanning Electron Micrographs of select glutaraldehyde fixed ITO electrodes. Electrodes taken from electrochemical cells for: A) *Pseudomonas sp.* 2 SN 5, B) *Halomonas sp.* 2 FeSN 2, C) *Marinobacter sp.* FeSN 1, D) *Idiomarina sp.* SN 11, E) *Thalassospira sp.* SN 3, F) *Thioclava sp.* SN 12, G) *Halomonas sp.* 1 FeN 2 and H)

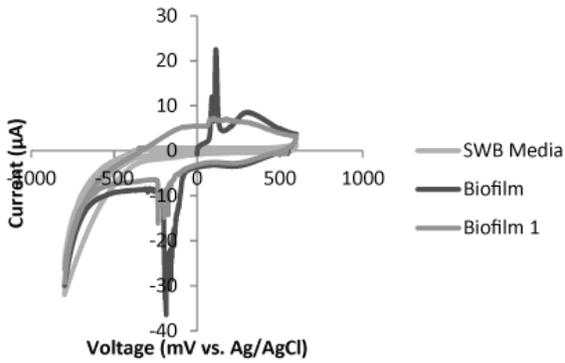
A) *Pseudomonas sp. 1 FeSN6*



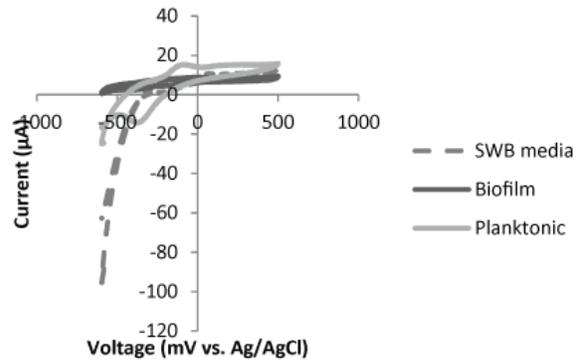
B) *Pseudomonas sp. 2 SN5*



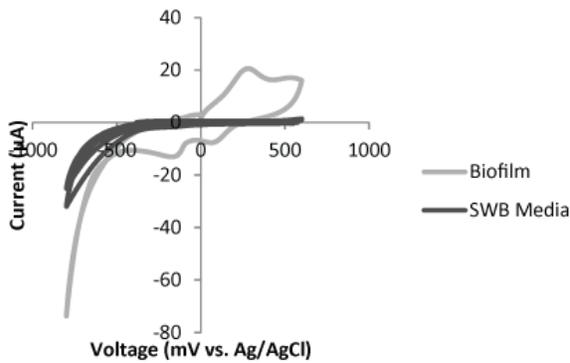
C) *Halomonas sp. 1 FeN1*



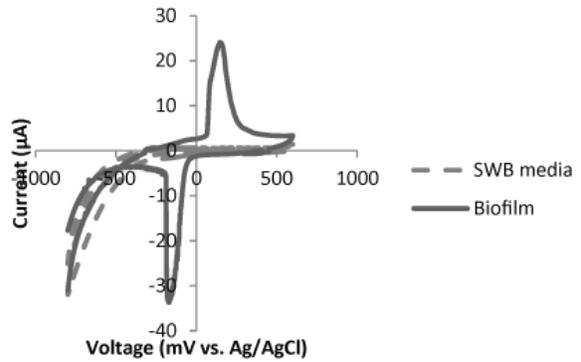
D) *Halomonas sp. 1 FeN2*



E) *Halomonas sp. 2 SN4*



F) *Halomonas sp. 2 SN10*



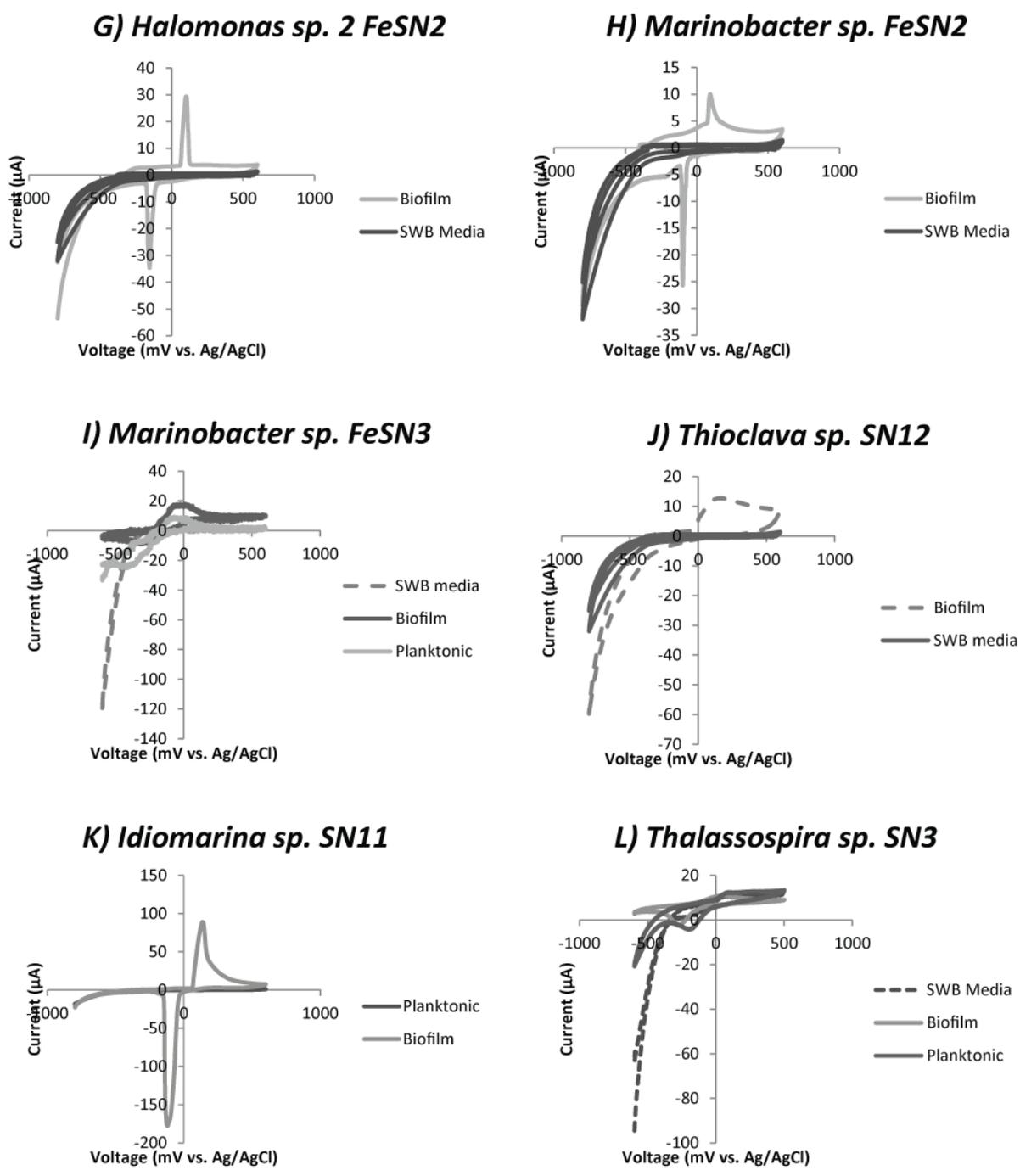


Figure S8. Representative cyclic voltammety plots under non-turnover conditions for organism listed (A-L). Various strains and plots represented for the background media in a sterile cell (SWB) as well as planktonic (suspended cells in spent media) and biofilm (cell biomass attached to electrodes) associated biomass. Scans were run from 5-10 mV/s.