Supplementary Material

Enrichment of extremophilic exoelectrogens in microbial electrolysis cells using Red Sea brine pools as inocula

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Location and chemical characterization of the selected brine pools in the Red Sea

The locations of the three brine pools (Atlantis II, Kebrit and Valdivia) used in this study are shown in Fig. S1B. The Atlantis II Deep is located in the middle of the axial rift of the Red Sea at a maximum depth of 2194 m. Its main characteristic is that it is hydrothermally active, showing a continuous increase in temperature from the earliest record of 44.8°C, to the last reported temperature of 68.2°C, due to the influx of hot brine supplied by a geyser spring at the bottom (Bougouffa et al., 2013). According to Antunes et al. (2011), the Atlantis II pool has a high salinity of 25.7% NaCl (wt./vol) and a high concentration of metalliferous sediments that are enriched in iron, copper, zinc, and other heavy metals. It is also a highly acidic brine pool with a pH value of 5.3.

The Kebrit Deep pool is located in its northern section (Fig. S1B) at a maximum depth of 1573 m. Compared with Atlantis II, its temperature is noticeably lower (up to 23.3°C), but with comparable pH and salinity (5.5 and 26%, respectively) (Wang et al., 2013). It is characterized for having substantial amounts of H_2S, with up to 14 mg/L of sulfur (Eder et al., 2001). Unlike Atlantis II, its temperature and physical and chemical structures has remained almost constant over time, suggesting that there is no tectonic or volcanic/hydrothermal activity (Hartmann et al., 1998).

The Valdivia Deep brine pool is located within the same basin as Atlantis II, but encased in a differently located depression that reaches 1,673 m in depth (Backer and Schoell, 1972). While it presents a similar salinity of 24.2%, its temperature at the bottom reaches 33.7°C (Anschutz et al., 1999). Its pH is higher than the latter two deeps, reaching a value of 6.21. The most distinguishable feature of this pool is the high concentrations of sulfate and magnesium (Antunes et al., 2011) (Table S1). Also, according to Anschutz et al. (1999) the temperature and salinity of Valdivia has increased over the years, suggesting hydrothermal activity.

The concentrations of the most important metals in the brine pools were measured using an inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 8300, Perkin Elmer),

equipped with a custom designed solid-state charge-coupled device (CCD) array detector. The samples were analyzed using direct inductively coupled plasma analysis on axial view to detect elements present in parts per million (ppm), and in pre-concentration mode for trace elements.

The concentration of anions in the brine pools, mainly chloride and sulfate, were analyzed by ion chromatography. The samples were first filtered through 0.45 μm pore diameter syringe filters (Corning Incorporated), and then diluted with deionized water. The diluted samples were analyzed using the Dionex ICS-1600 ion chromatography system (Thermo Scientific) equipped with a high-performance conductivity detector and IonPac AS15 Capillary column. KOH solution (30 mM) was used as the mobile phase. The system was operated for 15 minutes per sample at a flow rate of 0.3 mL/min. The resulting peak areas were converted to concentrations using a standard curve prepared with a Seven Anion Standard solution (Thermo Scientific).

The concentration of total organic carbon (TOC) present in the brine pool samples was measured using an on-line TOC analyzer (TOC-V_{CSH}, Shimadzu), utilizing combustion catalytic oxidation at 680°C, nitrogen as carrier gas and non-purgeable organic carbon (NPOC) method (i.e. sparging following addition of 2 M HCl).

The microbial cell density in the brine pool samples was measured using flow cytometry according to El-Chakhtoura et al. (2015). Briefly, samples (700 μL) of each brine pool was added into 1.5 mL centrifuge tubes and pre-heated at 35°C for 10 min, stained with 10 μL/mL SYBR Green I (Molecular Probes, Eugene, OR, USA) and incubated in the dark at 35°C for 10 min. Triplicate samples from each tube was added into a 96-well plate. The plate was loaded into an Accuri C6 Flow Cytometer (BD Biosciences).

The majority of the measured metals and anions were present at concentrations within an order of magnitude of those previously reported in the literature (Table S1). One exception was iron in water from the Atlantis II site (786 μM), which was higher by 3 orders of magnitude than values previously reported (1.6 μM) (Antunes et al., 2011). There were also slightly higher salinities (based on Na^+ and

K^+ ions) for the Atlantis II and Valdivia brine pool samples, and considerably higher sulfate concentrations in the Atlantis II and Kebrit samples.

16S rRNA gene pyrosequencing

Triplicate polymerase chain reaction (PCR) reactions were performed for each extracted DNA sample in a 25 µL reaction volume using the HotStarTaq Plus Master Mix (QIAGEN, Valencia, CA), 0.25 µM of each primer and 20 ng of template DNA. Bacterial 16S rRNA genes were amplified using the bacteria-specific forward primer 341F (5'-Adaptor A-Barcode-CA Linker-CCTACGGGNGGCWGCAG-3') and reverse primer 805R (5'-Adaptor B-TC Linker-GACTACHVGGGTATCTAATCC-3'). PCR was performed using life technologies veritus thermocycler with the following PCR conditions: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds; 53°C for 40 seconds and 72°C for 1 minute; after which a final elongation step at 72°C for 5 minutes was performed. Following PCR, all amplicon products from different samples were mixed in equal concentrations, purified using Agencourt Ampure beads (Agencourt Bioscience Corporation, MA, USA), and pyrosequenced on the Roche 454 FLX Titanium genome sequencer (Roche, Indianapolis, IN) according to the manufacturer's instructions.

The 16S rRNA gene sequences were processed using a proprietary analysis pipeline (www.mrdnalab.com, MR DNA, Shallowater, TX). Raw reads were first demultiplexed to trim the barcodes and primers and then low quality sequence reads outside the bounds of 200 and 1000 bp, sequences containing more than 6 ambiguous base or 6 homopolymers, and sequences with quality score less than 25, were removed. Sequences were then denoised and chimeras removed. Sequences were clustered into operational taxonomic units (OTUs) after removal of singletons with a 97% sequence identity threshold. A representative sequence from each OTU was phylogenetically assigned to a taxonomic identity using BLASTn against a curated GreenGenes database (DeSantis et al., 2006).

Table S1
Reported and measured chemical composition and microbial cell density of the studied brine pools. Reported values are obtained from Antunes et al. (2011), Bougouffa et al. (2013), Backer and Schoell (1972) and Anschutz et al. (1999).

Chemicals	Atlantis		Valdivia		Kebrit	
	Reported	This study	Reported	This study	Reported	This study
Na^+ (M)	5.1	5.68	4.1	5.15	NR	5.72
Cl^- (M)	5.5	4.07	4.6	4.45	5.1	4.20
Ca^{2+} (mM)	152.4	151	25.0	23.6	42.4	56.0
Mg^{2+} (mM)	36.9	36.3	95.3	107	98.8	136
K^+ (mM)	76.9	126	52.0	99.6	NR	67.3
SO_4^{-2} (mM)	10.8	49.0	72.0	76.5	22.9	58.4
B (mM)	1.2	0.90	NR[a]	0.96	NR	2.40
Sr (mM)	0.7	0.44	NR	0.17	NR	0.52
Fe (μM)	1.6	786	0.1	0.27	0.1	0.30
Mn (μM)	1.8	NM[b]	0.1	NM	165.0	NM
Zn (μM)	165.7	NM	NR	NM	NR	NM
Cu (μM)	6.3	NM	Traces	NM	NR	NM
Li (μM)	563.4	NM	98.1	NM	NR	NM
Ba (μM)	10.9	NM	0.5	NM	NR	NM
Rb (μM)	25.3	NM	3.1	NM	NR	NM
F (mM)	NR	ND[c]	NR	ND	NR	ND
NO_2^- (mM)	NR	ND	NR	ND	NR	ND
Br (mM)	NR	ND	NR	ND	NR	ND
NO_3^- (mM)	NR	ND	NR	ND	NR	ND
PO_4^{3-} (mM)	NR	ND	NR	ND	NR	ND
TOC (mg/L)	NR	160	NR	171	NR	219
Microbial cell density ($\times 10^4$/mL)	0.83[d]	0.16[e]	NR	28.00	NR	6.36

[a]NR not reported in the literature
[b]NM not measured
[c]ND not detected
[d]measured using epifluorescence microscope. Depth of 2,139 m
[e]measured using flow cytometry in this study. The depths of the brine pools are provided in the main text of the manuscript

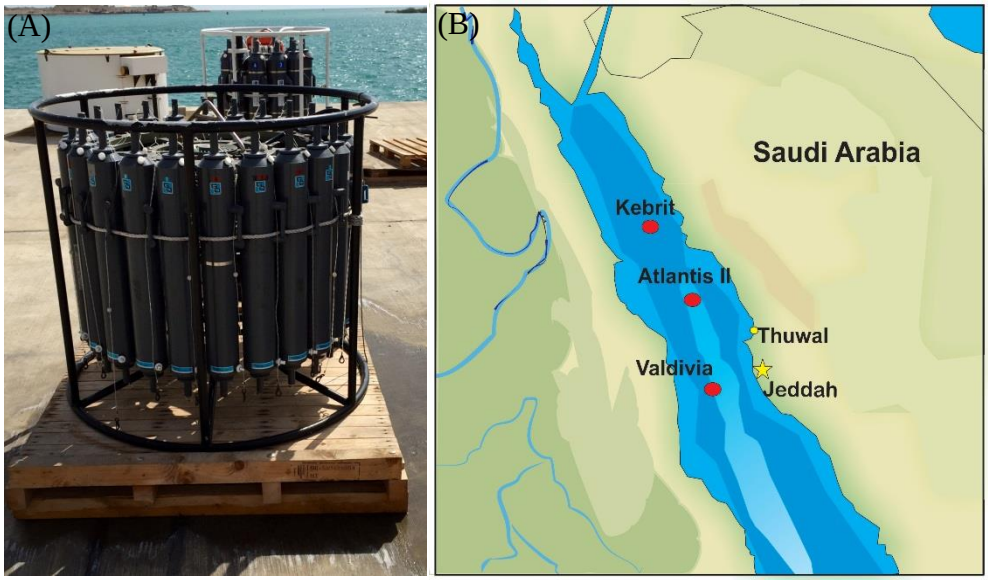

Fig. S1. (A) Photo of the Rosette/Niskin multi-bottle assembly. (B) Geographic location of the different brine pools on the Red Sea. Atlantis II, Valdivia and Kebrit (bold red marks) are the brine pools selected for this study.

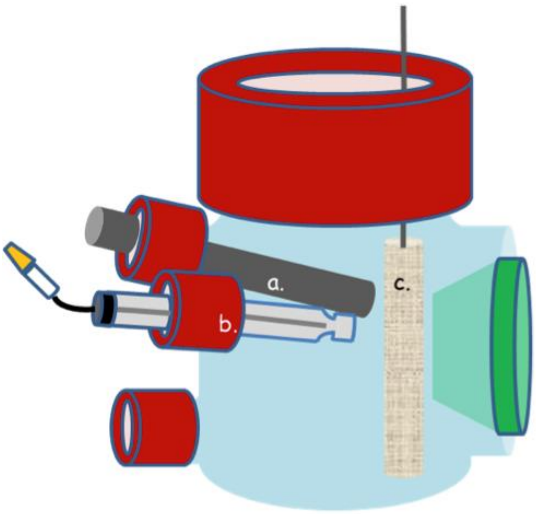

Fig. S2. Graphical representation of (a) the MEC anode or working electrode, (b) reference electrode, and (c) the cathode or counter electrode.

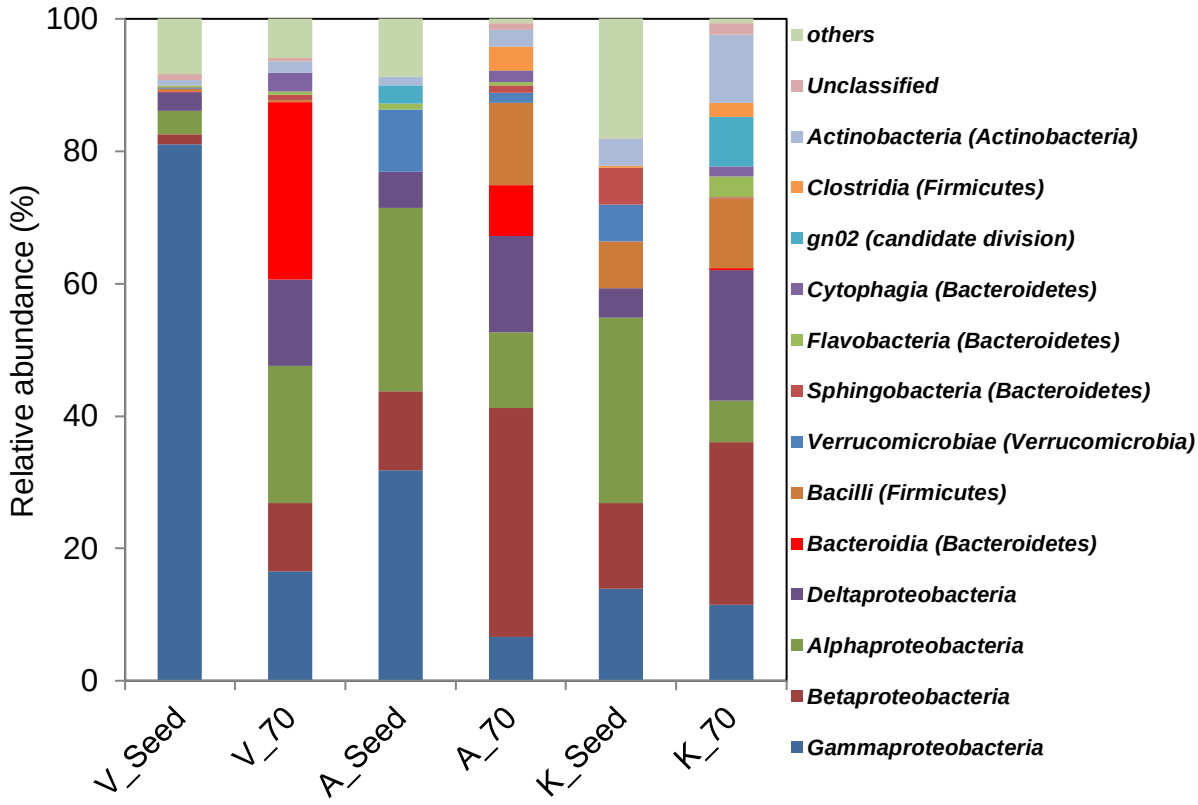

Fig. S3. Relative abundance of bacterial reads classified at the class level for the seed and biofilm samples collected from the Valdivia (V_70), Atlantis (A_70) and Kebrit (K_70) 70 °C anodes. Classes that represent less than 1% of the total bacterial community composition were classified as "others".

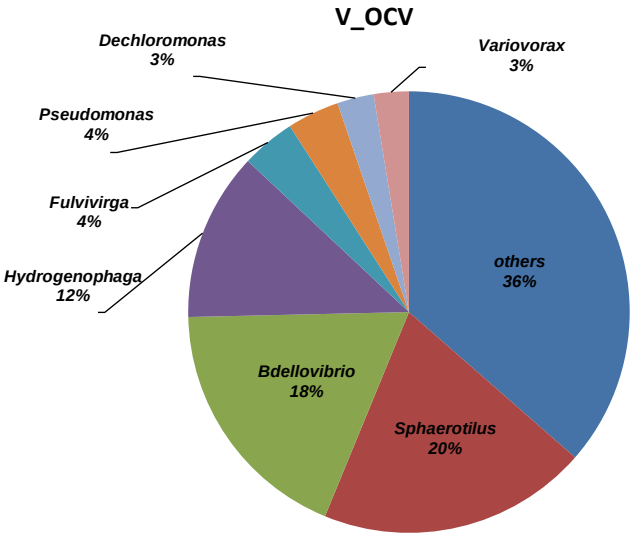

Fig. S4. Relative abundance of bacterial reads classified at the genus level for the anode biofilm sample obtained from the Valdivia-seeded MEC operated at 70ºC and in open circuit voltage (OCV). Genera that represent less than 3% of the total bacterial community composition were classified as "others".

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