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

Noura A. Shehab [a,c], Juan F. Ortiz-Medina [a], Krishna P. Katuri [a], Ananda Rao Hari [a], Gary Amy [a], Bruce E. Logan [b], Pascal E. Saikaly [a,*]

[a] Biological and Environmental Sciences and Engineering Division, Water Desalination and Reuse Research Center, King Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia
[b] Department of Civil and Environmental Engineering, The Pennsylvania State University, University Park, PA 16802, USA
[c] Research Product Development Innovations, The Business Gate Qurtubah, Riyadh 13244, Saudi Arabia

## HIGHLIGHTS

- Brine pools in the Red Sea were used as inocula in MECs.
- High current density was generated under hypersaline and thermophilic conditions.
- Exoelectrogens are present in extreme environments in the Red Sea.
- Members of *Bacteroides* seemed to play an important role in electricity generation.

## GRAPHICAL ABSTRACT
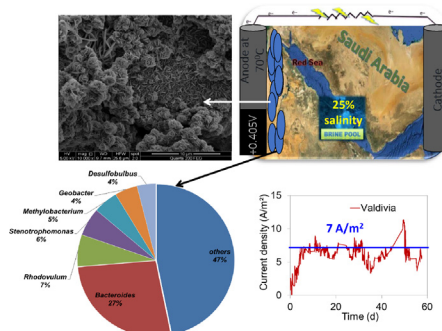
## ABSTRACT

Applying microbial electrochemical technologies for the treatment of highly saline or thermophilic solutions is challenging due to the lack of proper inocula to enrich for efficient exoelectrogens. Brine pools from three different locations (Valdivia, Atlantis II and Kebrit) in the Red Sea were investigated as potential inocula sources for enriching exoelectrogens in microbial electrolysis cells (MECs) under thermophilic (70 °C) and hypersaline (25% salinity) conditions. Of these, only the Valdivia brine pool produced high and consistent current $6.8 \pm 2.1$ A/m$^2$-anode in MECs operated at a set anode potential of +0.2 V vs. Ag/AgCl (+0.405 V vs. standard hydrogen electrode). These results show that exoelectrogens are present in these extreme environments and can be used to startup MEC under thermophilic and hypersaline conditions. *Bacteroides* was enriched on the anode of the Valdivia MEC, but it was not detected in the open circuit voltage reactor seeded with the Valdivia brine pool.

© 2017 Elsevier Ltd. All rights reserved.

## 1. Introduction

Microbial electrochemical technologies (METs) such as microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) utilize a unique group of microorganisms, called exoelectrogens, that are capable of extracellular electron transfer (EET) to a solid anode (Doyle and Marsili, 2015). Successful application of METs for wastewater treatment requires the identification and characterization of robust exoelectrogens capable of producing high current densities (>1 A/m$^2$ anode), and are able to operate at various environmental conditions such as various pH, temperature and saline

---

* Corresponding author.
  *E-mail address:* Pascal.saikaly@kaust.edu.sa (P.E. Saikaly).

conditions (Zhuang et al., 2010; Li et al., 2013). Thermophilic and halophilic exoelectrogens are particularly useful for the treatment of wastewaters that are generated from certain industries, such as oil and natural gas production [1.2–2.9 g COD/L, 10% salinity, 80–100 °C], seafood processing [1–32 g COD/L, 1.3–3.9% salinity], and textile dyeing [0.83–4.7 g COD/L, 3–15% salinity, 40–70 °C] (Badani et al., 2005; Santos et al., 2006; Xiao and Roberts, 2010; Ha et al., 2012; Monzon et al., 2014; Fu et al., 2015). Under typical neutral pH conditions and relatively low salinities, bacteria capable of high current densities are mostly *Geobacter* spp., with bacteria most similar to *Geobacter sulfurreducens* commonly identified on the anodes (Shehab et al., 2013). To date, few extremophiles isolated from extreme natural environments have been tested to be capable of high rates of EET to the anode of MECs operated under extreme conditions, such as highly alkaliphilic (*Geoalkalibacter ferrihydriticus*), halophilic (*Geoalkalibacter subterraneus*), and thermophilic (*Thermincola ferriacetica* and *Thermoanaerobacter pseudethanolicus*) conditions (Badalamenti et al., 2013; Parameswaran et al., 2013; Lusk et al., 2015). Several studies reported the enrichment of mixed cultures of efficient extremophilic exoelectrogens on the anode of METs with inocula from diverse natural sources such as salt marsh (Rousseau et al., 2013), saline microbial mat (Miceli et al., 2012) and salt lake (Pierra et al., 2015). Taken together, these studies suggest that extreme natural environments are likely to harbor microorganisms capable of EET to the anode.

The Red Sea brine pools represent one of the most extreme natural saline environments, with salinities reaching as high as 25% (Antunes et al., 2011), and temperatures that can average 46 °C or higher (Batang et al., 2012). All brine pools in the Red Sea form characteristically sharp brine-seawater interfaces with typically steep gradients of salinity, temperature, density, dissolved oxygen, and pH (Antunes et al., 2011). Therefore, a single brine pool can contain many different microenvironments depending on the number of interfaces in the water column, with differing physical and chemical characteristics at each site. Early analyses of brine pools failed to find viable microorganisms in these systems using traditional cultivation methods (Antunes et al., 2011). This led to a conclusion that the environment was sterile due to synergies between high temperature, salinity, and high metal concentrations. Analysis of these brine pools using modern molecular biology tools based on phylogentic analysis of the 16S rRNA gene, however, has shown that they have diverse microbial communities (Antunes et al., 2011).

The objective of this study was to demonstrate that exoelectrogens could be enriched from brine pools in the Red Sea under both thermophilic and hypersaline conditions. While exoelectrogenic biofilms have been enriched on the anode of MFCs operated under either thermophilic (55–98 °C) (Jong et al., 2006; Mathis et al., 2008; Ha et al., 2012; Fu et al., 2015) or hypersaline (using extremely saline synthetic wastewater of 10% salinity) (Monzon et al., 2014) conditions, there have been no studies showing current generation under both thermophilic and hypersaline conditions. Dissolved oxygen can inhibit growth of exoelectrogens, such as *G. sulfurreducens*, or reduce current generation, as bacteria will use oxygen for respiration as opposed to the anode (Shehab et al., 2013). MECs operated at a set anode potential can be a useful enrichment strategy for exoelectrogens from anaerobic environments (Miceli et al., 2012). MECs were therefore used to avoid oxygen intrusion into the anolyte solution that can occur using MFCs. Water samples were obtained from several brine pool locations in the Red Sea, and used to inoculate single-chamber MECs operated at a set potential, and electrochemical and molecular biology tools were used to characterize the anodic biofilm communities.

## 2. Material and methods

### 2.1. Collection of brine pool samples

The brine pool samples were collected in March 2013 during the R/V Aegeo expedition in the Red Sea. Samples from Kebrit (depth = 1,495 m; T = 23.4 °C; salinity = 25.8%; $O_2$ = 0.11 mg/L), Atlantis II (depth = 2,108 m; T = 68.0 °C; salinity = 26.0%; $O_2$ = 0.10 mg/L) and Valdivia (depth = 1,580 m; T = 34.2 °C; salinity = 24.9%; $O_2$ = 0.16 mg/L) sites in the Red Sea were collected anaerobically using 10 L Niskin water sampling bottles, with a 24-bottle Rosette water sampling system (Fig. S1A). Samples (0.5 L) were immediately transferred into anaerobic bottles and stored at 4 °C (two weeks) until MEC experiments were initiated. The location (Fig. S1B) and chemical characterization of the brine pools are provided in the Supplementary Material.

### 2.2. MEC construction, inoculation and operation

The MECs were single-chamber, glass bottles (Adams & Chittenden Scientific Glass, USA) with an anode (working electrode), cathode (counter electrode), and reference electrode (Ag/AgCl, Bioanalytical Systems, Inc., −0.205 V vs SHE) placed in different openings of the reactor (Fig. S2). The working electrodes were graphite rods of 7.5 cm length (7 cm inside the reactor) and 0.5 cm diameter. The counter electrodes were stainless steel mesh (5 cm × 5 cm) rolled into cylinders to avoid contact with the working electrode. Reactors and working and counter electrodes were sterilized by autoclaving prior to start of the experiments. The reference electrodes were sterilized by soaking in 3 M NaCl overnight and rinsing with sterile medium. MECs were sparged with pure nitrogen gas before being assembled. After the reactors were assembled, epoxy glue was used to seal every opening in the reactor to avoid leakage. A gas bag (0.1 L Cali -5 -Bond. Calibrate, Inc.) was connected to the reactors to collect any gas generated.

Nine MECs (three for each brine pool sample) were used for the samples from the three different brine pools. Bottles (duplicates) were filled with 100 mL of the respective brine pool sample (as inoculum and electrolyte) in an anaerobic glove box (Coy Laboratory, USA), and fed acetate (10 mM), and then operated at a set potential of +0.20 V vs. Ag/AgCl (+0.405 V vs SHE) using a VMP3 potentiostat (Biologic, USA). This potential was chosen to avoid the use of other potential electron acceptors by bacteria for anaerobic respiration, such as sulfate or iron (III) that are naturally present in the brine pool samples. One abiotic (autoclaved) control was operated for each brine pool sample.

The MECs were initially operated in a water bath at 70 °C for Atlantis II, 30 °C for Valdivia, and 23 °C for Kebrit sites, that were chosen to be as close to the corresponding temperatures of sampled brine pools. Preliminary tests did not detect any current generation for the Valdivia and Kebrit seeded-MECs when operated at these temperatures. To test whether current could be generated for these two samples at higher temperatures, these reactors with their autoclaved controls were then placed in the same water bath (set at 70 °C) containing the Atlantis II-seeded MECs.

### 2.3. Chemical analyses

Residual acetate concentration in the MECs was measured every week using a high performance liquid chromatograph (HPLC, Accela System, Thermo Scientific) equipped with a photo-diode array (PDA) detector, with peaks detected at 210 nm (Katuri et al., 2014). The column utilized was an Aminex HPX-87H Ion Exclusion Column (Bio-Rad Laboratories) and the mobile phase

was a 5 mM sulfuric acid solution, flowing at 0.55 mL/min. Since sulfate originally present in the brine pool samples could act as a potential electron acceptor, its concentration in the reactors was analyzed at the end of the experiment using an ion chromatography (details in the Supplementary Material).

### 2.4. Chronoamperometry and cyclic voltammetry

Amperometric current was monitored continuously using a VMP3 potentiostat (Biologic, USA), with measurements recorded every two minutes. At the end of the continuous measurement tests (day 58), cyclic voltammetry (CV, 1 mV/s) was performed for the anodic biofilms in the same electrolyte and under turnover conditions, to determine their redox behavior. Current was normalized to the geometric anode surface area. Midpoint potentials for the anode biofilms were calculated from the CVs. Also, CVs were performed on the cell-free spent brine pool solution (filtered using a 0.2 μm pore diameter filter) at the end of the experiment in a separate 3-electrode electrochemical cell, using a new set of electrodes, in order to determine the presence of extracellular secreted redox mediators by anodic communities.

### 2.5. Microbial analysis

Biofilm samples for bacterial analysis were collected from the anode at the end of the experiment and genomic DNA was extracted using the PowerSoil DNA extraction kit (MO BIO Laboratories, Inc., Carlsbad, CA) according to the manufacturer's instructions. Genomic DNA was also extracted from each of the three seed samples. The microbial community in the extracted DNA was characterized using 16S rRNA gene pyrosequencing according to El-Chakhtoura et al. (2015). The details of 16S rRNA gene pyrosequencing are provided in the Supplementary Material. All sequences from this study were deposited in the Short Read Archive (SRA) of the European Nucleotide Archive (ENA) under study accession number PRJEB9638.

## 3. Results and discussion

### 3.1. Current generation under extremophilic conditions

Valdivia-seeded MECs operated at 70 °C, hypersaline medium (25%) and set anode potential of +0.405 V vs. SHE generated a stable current of $6.8 \pm 2.1$ A/m$^2$ (relative to anode area, hereafter the same) over 58 days (Fig. 1A). No appreciable current ($0.02 \pm 0.01$ A/m$^2$) was generated by the autoclaved control. Current densities for the MECs inoculated using water from the other two samples were much lower, and they decreased over time (Fig. 1B and C). The MECs inoculated with the Atlantis II samples (at 70 °C) produced current of $1.3 \pm 0.08$ A/m$^2$ over the first 5 days of the experiment (Fig. 1B). However, this current density was lower than that generated by the autoclaved control, and current rapidly decreased, suggesting that the current generation did not have a biological origin. The lowest current (maximum value of 0.05 A/m$^2$) was produced in the Kebrit reactors (at 70 °C). The current produced by this sample also decreased over time, although it was higher than the autoclaved control (Fig. 1C), suggesting that the initial biological activity was not sustainable under hypersaline and thermophilic conditions.

In addition to the autoclaved control, an open circuit voltage (OCV) control was operated for the Valdivia brine pool samples as it was the only reactor showing a significant current at 70 °C. The OCV reactor was also placed in the water bath at 70 °C. Negligible change in acetate concentration was observed for the OCV reactor. The generation of current for the reactor with the Valdivia
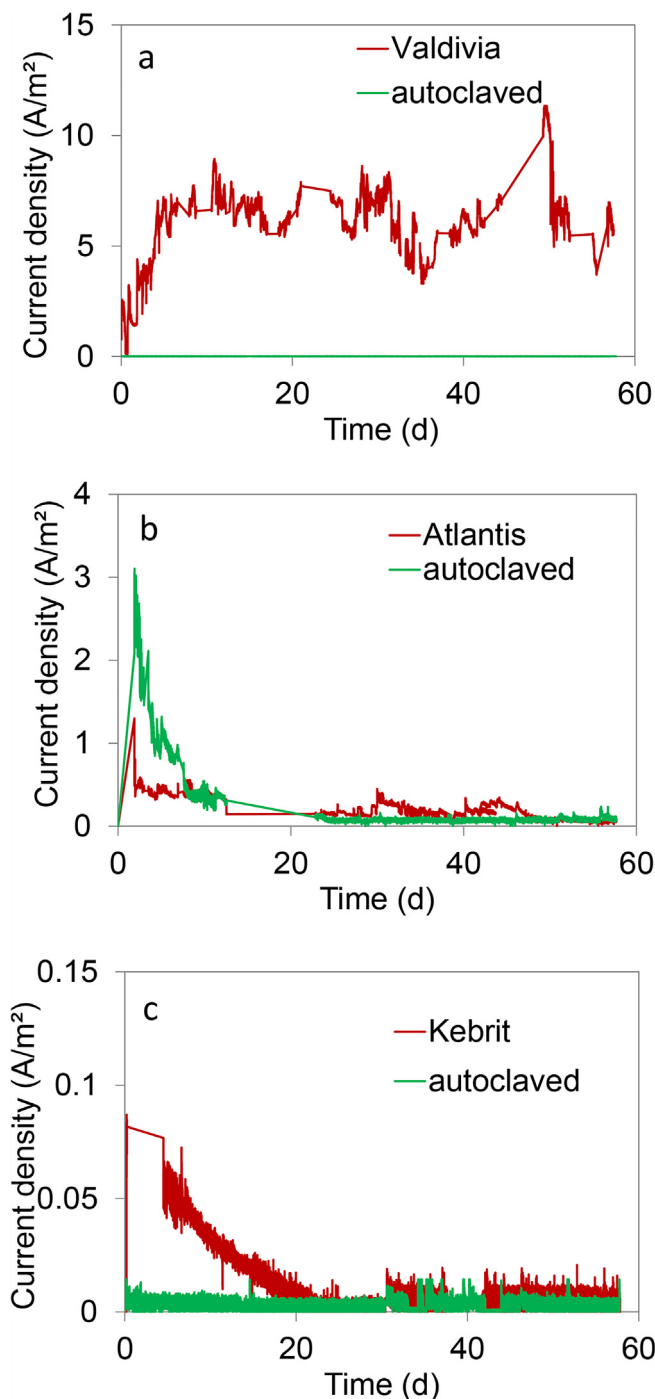


**Fig. 1.** Current densities for the MEC reactors (average of duplicate reactors) and the autoclaved control operated at 70 °C and inoculated with samples from (a) Valdivia, (b) Atlantis II, and (c) Kebrit brine pools.

inoculum was linked to a decrease in the acetate concentration over time, with acetate decreasing by $68\% \pm 1.2\%$ (from $820 \pm 1$ to $262.4 \pm 1.2$ mg/L over 58 days). There was negligible removal of sulfate at 70 °C, supporting loss of acetate primarily linked to current generation. Acetate concentrations decreased by only $8 \pm 1.2\%$ in the OCV controls, and by $5 \pm 1.2\%$ in autoclaved controls over the same period of time. Small changes in acetate concentrations could have been due to acetate oxidation linked to sulfate or iron reduction, but they are well within experimental error for chemical measurements.

The current densities (6.8 ± 2.1 A/m² over 60 days) measured for the Valdivia-seeded MECs were within the range of those reported by others for other saline or thermophilic environments (Miceli et al., 2012; Badalamenti et al., 2013; Parameswaran et al., 2013), although different set anode potentials and culture media were used in these studies. A maximum current of 7–8 A/m² was reported for *Thermincola ferriacetica* using dual-chamber MECs operated at 60 °C, with the graphite-rod anodes set at −0.06 V vs. SHE (Parameswaran et al., 2013). Sustained current by *T. ferriacetica* was obtained with a buffer (10 mM bicarbonate) relatively low in salinity compared to the medium used here (Parameswaran et al., 2013). Using single-chamber MECs operated at set anode potential of −0.30 V vs Ag/AgCl (−0.095 V vs. SHE), Miceli et al. (2012) obtained current densities of 1.5 A/m²–10.8 A/m² from various anaerobic environmental samples including saltwater samples (4.2–4.5 A/m²). Their saltwater samples where enriched in a media with progressively increased salt concentrations (20 g/L NaCl and 3 g/L MgCl₂). Current densities of 5.0–8.3 A/m² were generated by acetate-fed pure culture of alkaliphilic *Geoalkalibacter ferrihydriticus* under alkaline (pH 9.3) and slightly saline conditions (0.1%) in dual-chamber MECs using
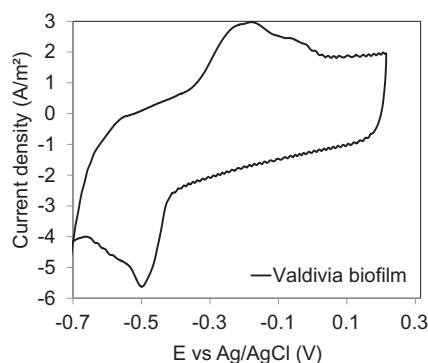
anodes poised at +0.07 V vs. SHE (Badalamenti et al., 2013). In the same study, *Geoalkalibacter subterraneus* produced current densities of 2.4–3.3 A/m² under a higher saline solution (1.7%) and anode poised at +0.04 V vs. SHE (Badalamenti et al., 2013).

### 3.2. Cyclic voltammetry

Cyclic voltammograms were obtained at the end of the experiments (day 58) using the anode and cell-free filtrate (filtered reactor solution) from the Valdivia 70 °C reactors that produced current. The anodes exhibited redox peaks with a midpoint potential of −0.35 V vs. Ag/AgCl (−0.15 V vs. SHE) (Fig. 2). The midpoint potential was calculated based on the oxidation potential of −0.2 V and reduction potential of −0.5 V (Fig. 2). No redox peaks were observed for the filtered solution (i.e. cell-free solution), indicating a lack of mediators for current generation. Thus, the CV analysis suggests that the anodic biofilm was responsible for the current generation in the Valdivia 70 °C reactors.

### 3.3. Bacterial community composition of the anodic biofilm and original brine pool samples

16S rRNA gene sequencing showed that the genus *Bacteroides* (class *Bacteroidia*), which was detected in very low abundance (0.13%) in the Valdivia seed sample (Fig. S3) and was not detected on the anode of the Valdivia OCV control at 70 °C (Fig. S4), was highly enriched (27%) on the anode of Valdivia 70 °C reactor (Fig. 3), which was showing significant current generation. This suggests that members of the genus *Bacteroides* (phylum *Bacteroidetes*) had an important role in electricity generation in the Valdivia-seeded MECs. Ha et al. (2012) reported the enrichment of anodic biofilm communities dominated by *Bacteroidetes* capable of generating high current density (2.3 A/m²) in thermophilic MFCs (55 °C) fed alcohol distillery wastewater. The strain *Anditalea andensis* ANESC-Sᵀ, belonging to the phylum *Bacteroidetes*, was shown to generate electricity in MFCs under alkaline (pH 7.0–11.0) and high salt conditions (4%) (Shi et al., 2015). Taken together, these results suggest that members of the phylum
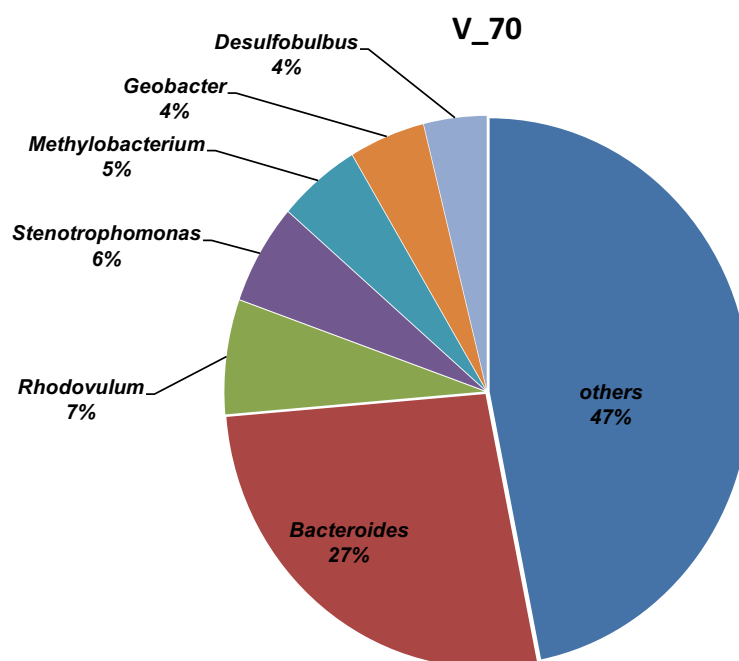


**Fig. 2.** Cyclic voltammogram of the Valdivia 70 °C biofilm at the end of experiment.



**Fig. 3.** Relative abundance of bacterial reads classified at the genus level for biofilm samples collected from the Valdivia (V_70) 70 °C anodes. Genera that represent less than 3% of the total bacterial community composition were classified as "others".

*Bacteroidetes* might be able to live under extremophilic conditions and contribute to the generation of current. Since extremophilic exoelectrogens are less studied compared to exoelectrogens grown under mesophilic and low salinities, it remains unclear what conditions stimulated the dominance of members of the genus *Bacteroides* in thermophilic (70 °C) and hypersaline (25% salinity) MECs. Further isolation and characterization are needed to provide a better insight on their function. The presence of reads (4% of the total reads) with sequence similarity to *Geobacter* in the Valdivia 70 °C reactor was somehow surprising since already known species of *Geobacter* cannot survive under these extreme environments. It should be noted that the sequences were not long enough (~400 bp) to confidently classify reads down to the species level. Even with the full-length 16S rRNA gene sequencing, it is sometimes not possible to distinguish organisms at the species level. For example, the two exoelectrogens *G. sulfurreducens* and *Geobacter anodireducens* could not be distinguished as different species based on full-length (1457 bp) 16S rRNA gene sequencing due to their high 16S rRNA gene sequence similarity (98%) (Sun et al., 2014). *Geobacter anodireducens* has a higher tolerance to salinity than other species of *Geobacter* including *G. sulfurreducens*. Therefore, we cannot rule out the possibility that some species of the genus *Geobacter* that are not yet discovered, or cannot be distinguished as a new species based on 16S rRNA gene sequencing, could grow under these extreme environments.

## 4. Conclusions

This study is the first to report the enrichment of efficient exoelectrogenic community from Red Sea brine pools capable of producing high current density (~7 A/m$^2$) under both hypersaline (25%) and thermophilic conditions (70 °C). Enriching for efficient exoelectrogens with multiple extremophilic characteristics from natural extreme environments using MECs operated at a set anode potential could offer an opportunity to expand the application of METs for simultaneous treatment and energy generation from high-temperature and high-saline wastewaters generated from many industries such as oil, gas and food industry.

## Acknowledgements

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biotech.2017.04.122.

## References

Antunes, A., Ngugi, D.K., Stingl, U., 2011. Microbiology of the Red Sea (and other) deep-sea anoxic brine lakes. Environ. Microbiol. Rep. 3, 416–433.

Badalamenti, J.P., Krajmalnik-Brown, R., Torres, C.I., 2013. Generation of high current densities by pure cultures of anode-respiring *Geoalkalibacter* spp. under alkaline and saline conditions in microbial electrochemical cells. mBio 4, e00144-13.

Badani, Z., Ait-Amar, H., Si-Salah, A., Brik, M., Fuchs, W., 2005. Treatment of textile waste water by membrane bioreactor and reuse. Desalination 185, 411–417.

Batang, Z.B., Papathanassiou, E., Al-Suwailem, A., Smith, C., Salomidi, M., Petihakis, G., Alikunhi, N.M., Smith, L., Mallon, F., Yapici, T., 2012. First discovery of a cold seep on the continental margin of the central Red Sea. J. Mar. Syst. 94, 247–253.

Doyle, L.E., Marsili, E., 2015. Methods for enrichment of novel electrochemically-active microorganisms. Bioresour. Technol. 195, 273–282.

El-Chakhtoura, J., Prest, E.I., Saikaly, P.E., van Loosdrecht, M.C.M., Hammes, F., Vrouwenvelder, J.S., 2015. Dynamics of bacterial communities before and after distribution in a full-scale drinking water network. Water. Res. 74, 180–190.

Fukushima, Q., Maeda, H., Sato, K., Kobayashi, H., et al., 2015. Bioelectrochemical analysis of a hypersaline microbial fuel cell generating electricity at temperatures above 80 °C. Biosci. Biochem. Biochem. 79, 1200–1206.

Ha, P.T., Lee, T.K., Rittmann, B.E., Park, J., Chang, I.S., 2012. Treatment of alcohol distillery wastewater using a *Bacteroidetes*-dominant thermophilic microbial fuel cell. Environ. Sci. Technol. 46, 3022–3030.

Jong, B.C., Kim, B.H., Chang, I.S., Liew, P.W.Y., Choo, Y.F., Kang, G.S., 2006. Enrichment, performance, and microbial diversity of a thermophilic mediatorless microbial fuel cell. Environ. Sci. Technol. 40, 6449–6454.

Katuri, K.P., Werner, C.M., Jimenez-Sandoval, R.J., Chen, W., Logan, B.E., Lai, Z., Amy, G.L., Saikaly, P.E., 2014. A novel anaerobic electrochemical membrane bioreactor (AnEMBR) with conductive hollow-fiber membrane for treatment of low-organic strength solutions. Environ. Sci. Technol. 48, 12833–12841.

Li, X.M., Cheng, K.Y., Wong, J.W., 2013. Bioelectricity production from food waste leachate using microbial fuel cells: effect of NaCl and pH. Bioresour. Technol. 149, 452–458.

Lusk, B.G., Khan, Q.F., Parameswaran, P., Hameed, A., Ali, N., Rittmann, B.E., Torres, C.I., 2015. Characterization of electrical current-generation capabilities from thermophilic bacterium *Thermoanaerobacter pseudethanolicus* using xylose, glucose, cellobiose, or acetate with fixed anode potentials. Environ. Sci. Technol. 49, 14725–14731.

Mathis, B.J., Marshall, C.W., Milliken, C.E., Makkar, R.S., Creager, S.E., May, H.D., 2008. Electricity generation by thermophilic microorganisms from marine sediment. Appl. Microbiol. Biotechnol. 78, 147–155.

Miceli III, J.F., Parameswaran, P., Kang, D.-W., Krajmalnik-Brown, R., Torres, C.I., 2012. Enrichment and analysis of anode-respiring bacteria from diverse anaerobic inocula. Environ. Sci. Technol. 46, 10349–10355.

Monzon, O., Yang, Y., Yu, C., Li, Q., Alvarez, P., 2014. Microbial fuel cells under extreme salinity: performance and microbial analysis. Environ. Chem. 12, 293–299.

Parameswaran, P., Bry, T., Popat, S.C., Lusk, B.G., Rittmann, B.E., Torres, C.I., 2013. Kinetic, electrochemical, and microscopic characterization of the thermophilic, anode-respiring bacterium *Thermincola ferriacetica*. Environ. Sci. Technol. 47, 4934–4940.

Pierra, M., Carmona-Martinez, A.A., Trably, E., Godon, J.J., Bernet, N., 2015. Specific and efficient electrochemical selection of *Geoalkalibacter subterraneus* and *Desulfuromonas acetoxidans* in high current-producing biofilms. Bioelectrochemistry 106 (Part A), 221–225.

Rousseau, R., Dominguea-Benetton, X., Delia, M.L., Bergel, A., 2013. Microbial bioanodes with high salinity tolerance for microbial fuel cells and microbial electrolysis cells. Electrochem. Commun. 33, 1–4.

Santos, A.B., Madrid, M.P., de Bok, F.A.M., Stams, A.J.M., van Lier, J.B., Cervantes, F.J., 2006. The contribution of fermentative bacteria and methanogenic archaea to azo dye reduction by a thermophilic anaerobic consortium. Enzyme Microb. Technol. 39, 38–46.

Shehab, N., Li, D., Amy, G.L., Logan, B.E., Saikaly, P.E., 2013. Characterization of bacterial and archaeal communities in air-cathode microbial fuel cells, open circuit and sealed-off reactors. Appl. Microbiol. Biotechnol. 97, 9885–9895.

Shi, W., Wang, V.B., Zhao, C.-E., Zhang, Q., Loo, S.C.J., Yang, L., Xu, C., 2015. *Anditalea andensis* ANESC-S$^T$ – an alkaliphilic halotolerant bacterium capable of electricity generation under alkaline-saline conditions. PLoS One 10, e0132766.

Sun, D., Wang, A., Cheng, S., Yaates, M., Logan, B.E., 2014. *Geobacter anodireducens* sp. Nov., an exoelectrogenic microbe in bioelectrochemical systems. Int. J. Syst. Evol. Microbiol. 64, 3485–3491.

Xiao, Y., Roberts, D.J., 2010. A review of anaerobic treatment of saline wastewater. Environ. Technol. 31, 1025–1043.

Zhuang, L., Zhou, S., Li, Y., Yuan, Y., 2010. Enhanced performance of air-cathode two-chamber microbial fuel cells with high-pH anode and low-pH cathode. Bioresour. Technol. 101, 3514–3519.