Biological fuel cells produce bioelectricity with *in-situ* brackish water purification

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**ABSTRACT**

Biological fuel cells, namely microbial desalination cells (MDCs) are a promising alternative to traditional desalination technologies, as microorganisms can convert the energy stored in wastewater directly into electricity and utilize it *in situ* to drive desalination, producing a high-quality reuse water. However, there are several challenges to be overcome in order to scale up from laboratory research. This study was conducted in order to better understand the performance of MDCs inoculated with marine sediments during the treatment of brackish water (5.0 g L\(^{-1}\) of NaCl) under three different configurations and cycles of desalination, envisaging the future treatment of saline wastewaters with conductivities lower than 10 mS cm\(^{-1}\). Results have shown that by increasing the desalination cycle three times, the efficiency of salt removal was improved by 3.4, 2.4 and 2.3 times for 1-MDC, 3-MDC, and 5-MDC, respectively. The same trend was observed for electrochemical data. Findings encourage further development of the MDC for sustainable brackish water and wastewater purification and future on-site utilization.

**Key words** | biological fuel cells, desalination, water reuse

**INTRODUCTION**

Compared to traditional chemical fuel cells, biological fuel cells use low-cost and self-sustaining bacteria to oxidize organic and/or inorganic electron donors, mainly from waste materials (Pant *et al.* 2010; Pandey *et al.* 2016) to generate energy. The basic principle is the reaction of microbial oxidation. However, the way these electrons are used on the cathode shows how attractive this technology is: any reduction reaction can be performed in the cathode chamber, creating numerous application options (Wang & Ren 2013) such as the generation of bioelectricity, hydrogen, methane, acid, and alkali compounds. Also, it is possible to produce clean water from desalination mediated by microbial desalination cells (MDCs) (Shannon *et al.* 2008; ElMekawy *et al.* 2014).

MDCs have the capacity to convert the energy stored in organic matter (for example in wastewater) directly into electricity and utilize it *in situ* to drive desalination in a process similar to electrodialysis (Cao *et al.* 2009; Ping *et al.* 2015). The production of protons at the anode and consumption of protons at the cathode drives the desalination of saltwater in the middle chamber, once salt ions migrate through the cation and anion exchange membranes to balance charge (Morel *et al.* 2012).

The potential benefits of MDC are due to their versatility for treating wastewater (e.g. food processing, shrimp farming, petroleum refineries), brackish or produced water, seawater, and also in remediating polluted environments (Patil *et al.* 2009; Sevda *et al.* 2017; Stoll *et al.* 2013; Vilajeliu-Pons *et al.* 2016; Sevda & Abu-Reesh 2017; Mohanakrishna *et al.* 2018). These systems may benefit small, rural, and remote communities as they require little to no energy input, treat wastewater, and produce fresh water (Guerra 2014). MDCs can be used for simultaneous organic matter and salt removal with energy production, or as a pre-treatment step for conventional desalination processes reducing the salt concentration, minimizing energy consumption and membrane fouling. In contrast to traditional desalination techniques that demand a higher energy input, MDCs do not use an extra source of energy (Forrestal *et al.* 2012; Kim & Logan 2013a, 2013b; Wang & Ren 2015).

However, as a novel technology, there are several challenges to be overcome (Logan & Rabaey 2012; Hernández-Fernández *et al.* 2015) in order to scale up MDC systems,
mainly in terms of the total desalination rate and efficiency improvements. This study was conducted in order to better understand the performance of MDCs during the purification of brackish water under different reactor configurations by varying the membrane pair numbers and cycles of desalination. Marine sediment was used as MDC inoculum due to its potential source of exoelectrogenic microorganisms (Bond et al. 2002; Scott et al. 2008; Malik et al. 2009; Teleken et al. 2017). In addition, the reactor configuration and the saline solution load (5.0 g NaCl L\(^{-1}\) and conductivity <10 mS cm\(^{-1}\)) were studied in order to understand the behavior of the desalination rate and efficiency at lower saline loads, aiming in the future to treat saline wastewater with similar characteristics, such those from petroleum refineries (conductivity <10 mS cm\(^{-1}\)).

**MATERIAL AND METHODS**

**Reactor design**

MDC bioreactors were constructed of polymethylmethacrylate (5.0 × 5.0 cm), consisting of three blocks: anode (20 mL), cathode (20 mL) and desalination cells (20 mL each). Desalination cells were composed of diluted and concentrated chambers with compartmental anion exchange membranes (AEMs; AMI-7001S, Membranes International) and cation exchange membranes (CEMs; CMI-7000S, Membranes International), with 1.5 cm spaces between them. For each desalination chamber, the AEM was on the side close to the anode while the CEM was on the other side close to the cathode. The 1-MDC configuration was composed of one dilute chamber, the 3-MDC configuration was composed of two dilute chambers and one concentrated chamber, and the 5-MDC configuration was composed of three dilute chambers and two concentrated chambers.

Granular graphite (15 g, surface area of each particle: 0.039 ± 0.001 cm\(^2\)) with a surface area of 84.01 ± 1.05 cm\(^2\) acted as the anode electrode and a graphite plate (5.0 × 2.0, surface area of 10.05 cm\(^2\)) was used as the cathode. Both electrodes were supported by a stainless-steel mesh 304, used as the electricity collector. The configuration of the MDC bioreactors is shown in Figure 1.

**Enriched exoelectrogenic culture and medium**

The anode compartments were inoculated with marine sediment (total carbon: 15.75 g kg\(^{-1}\) of dry weight) from Patos...
Lagoon Estuary (Rio Grande City, Brazil). The nutrient medium (anolyte) contained sodium acetate (5.0 g L\(^{-1}\)) as the carbon source and the following nutrients (per litre): 2.5 g NaHCO\(_3\), 0.1 g CaCl\(_2\).2H\(_2\)O, 0.1 g KCl, 1.5 g NH\(_4\)Cl, 0.6 g Na\(_2\)HPO\(_4\).12H\(_2\)O, 0.1 g NaCl, 0.1 g MgCl\(_2\).6H\(_2\)O, 0.1 g MgSO\(_4\).7H\(_2\)O, 0.005 g MnCl\(_2\).4H\(_2\)O, 0.001 g Na\(_2\)MoO\(_4\).2H\(_2\)O and 0.05 g of yeast extract. Potassium ferricyanide (16.46 g L\(^{-1}\)) was used as the catholyte. The simulated brackish water in the desalination chamber contained NaCl at 5.0 g L\(^{-1}\).

**Operational conditions**

The anodic and cathodic chambers were fed daily, with a replacement volume of 25% (5.0 mL), hydraulic retention time (HRT): 96 h\(^{-1}\) and 100% (20.0 mL, HRT: 24 h\(^{-1}\)), respectively. In order to investigate the influence of time on the efficiency of salt removal, three different desalination cycles of 24, 48 and 96 h\(^{-1}\) were assessed. The external resistance (Re) was fixed at 560 \(\Omega\). The reactors were operated in duplicate at a controlled temperature of 35 \(^\circ\)C (Eletrolab, EL101/2, Brazil), in order to stimulate exoelectric growth (Logan 2008).

**Analysis and calculations**

Total and organic carbon content was analyzed by TOC analyser (TOC-V, Shimadzu). Chemical oxygen demand (COD) was determined by the colorimetric method \((r^2 = 0.999)\), according to APHA (2012). pH was determined using a pH meter (TEKNA, T-1000). The conductivity was measured using a conductivity meter at the beginning and end of each desalination cycle. It was experimentally confirmed that the conductivity and concentration of a NaCl solution fitted a linear correlation within the extent of concentrations \((r^2 = 0.998)\).

Cell voltage was monitored by a microcontroller (Arduino Due) combined with a data acquisition system (Cool Term Data) every 60 seconds during the experiments. Current density \((I)\), power density \((P)\), normalized by the area of the electrode in the anode chamber were derived from the cell voltage measurements according to Ohm’s laws as follow:

\[
I \ (\text{mA m}^{-2}) = \frac{U}{\text{Re} \times A} \tag{1}
\]

\[
P \ (\text{mW m}^{-2}) = \frac{U^2}{\text{Re} \times A} \tag{2}
\]

where: A is the electrode area, U is the cell voltage, Re is the external resistance.

Coulombic efficiency (CE) in one feed cycle is described as the ratio of the output charge \((Q_{out})\) to the input charge \((Q_{in})\) and is calculated as follows:

\[
CE = \frac{Q_{out}}{Q_{in}} \times 100\% \tag{3}
\]

\[
Q_{in} = 96485 \left( \frac{C_{mol}}{\text{mol}} \right) \times 4 \ (\text{mol}) \times \Delta\text{COD} \times V_a/M_{O_2} \tag{4}
\]

\[
Q_{out} = \sum \left( \frac{U}{\text{Re} \times t} \right) \tag{5}
\]

where: t is the time of one feed cycle, \(\Delta\text{COD}\) is the concentration of COD during one feed cycle, and \(M_{O_2}\) is the molecular weight of \(O_2\).

In order to calculate the total desalination rate (TDR; Equation (6)), the conductivity of the salt solution in each desalination chamber was measured in the end of one desalination cycle. TDR was computed by the average of the desalination rate observed in the diluted chambers. Salt removal efficiency (R) was calculated according to Equation (7).

\[
\text{TDR (mg h}^{-1}\text{)} = (C_{in} - C_{out}) \times V_d/t \tag{6}
\]

\[
R(\%) = \frac{(C_{in} - C_{out})/C_{in}}{100\%} \tag{7}
\]

where: \(C_{in}\) and \(C_{out}\) are the initial and the final salt concentrations in one desalination cycle, \(t\) is the time of one desalination cycle and \(V_d\) is the volume of the desalination chamber.

**Scanning electron microscopy**

To investigate the biofilm formation, scanning electron microscopy (SEM) analysis was performed. Samples of the anode chamber were prepared according to the method proposed by Nation (1985) and adapted by Varesche et al. (1997). Briefly, samples were fixed in 0.1 M phosphate buffer \((pH 7.3)\) containing 2.5% glutaraldehyde, for 12 hours at 4 \(^\circ\)C. After fixation, samples were rinsed three times with 0.1 M phosphate buffer, allowing 30 min between each rinsing. Next, the samples were gradually dehydrated with successive immersions in ethanol solutions with increasing concentrations (30, 50, 70, 80, 90 and 95%), for 10 min each. Afterwards, samples were rinsed three times with 100% ethanol and dehydrated by critical point method. Finally, the samples were placed in the microscope supports and coated with gold powder. The samples were examined under a JEOL microscope (SEM JSM-6610LV), operated at 15 kV at CEME-SUL, FURG, Brazil.
RESULTS AND DISCUSSION

Scientists around the world have documented the existence of organism naturally found in marine sediments with the ability to react with minerals to convert organic nutrients into biological fuel (Bond et al. 2002; Bond & Lovley 2005; Malik et al. 2009; Lovley & Nevin 2011; Zhang et al. 2015). We identified an opportunity to study the potential for bioelectricity production from marine sediment with concomitant desalination in order to produce clean water by using biological fuel cell technology.

Desalination and energy production

One of the most important parameters of MDC performance is the desalination rate, which is greatly dependent on the salt concentration (Saeed et al. 2015). Several studies have been reported a desalination efficiency >90% with NaCl ranging from 10.0 to 35.0 g L\(^{-1}\) (Cao et al. 2009; Mehanna et al. 2010a; Ping & He 2013). However, few studies assessing the desalination of low-salinity water and wastewaters (<10 mS cm\(^{-1}\)) in traditional MDCs have been conducted, mainly due to the high internal resistance in these systems. In our study, the saline solution load was chosen with the future aim of treating saline wastewater, for example from petroleum refineries with conductivity lower than 10 mS cm\(^{-1}\).

In this study, the desalination efficiency was higher in the studied configurations with the increase from one (1-MDC) to three desalination chambers (3-MDC). However, with five desalination chambers (5-MDC), we observed a decrease in desalination efficiency compared to 3-MDC configuration. These findings could be a function of the increase of internal resistance, which limits desalination performance. As shown in Figure 2, by increasing the desalination time from 24 to 96 hours desalination efficiency (R, %) was improved by 3.4 times for 1-MDC (from 12.4 ± 2.5 to 42.5 ± 6.0), 2.4 times for 3-MDC (from 27.1 ± 5.1 to 64.8 ± 2.0) and 2.3 times for 5-MDC (from 25.2 ± 0.9 to 58.0 ± 0.1).

Also, increasing the number of desalination cells improved TDR. The same trend was observed by Chen et al. (2011), but the TDR in our study was lower. This can be explained mainly by factors that may have contributed to the increase in the systems’ internal resistances, such as higher resistor charge (560 Ω), saline concentration solution and reactor design (i.e.: volume of desalination cells, distance between electrodes and membranes, among others). According to Gude et al. (2015), desalination performance of a MDC can be increased using multiple pairs of ion-exchange membranes, inserted between the anode and cathode chambers, to improve the charge transfer efficiency and allow the saline water to flow through a series of MDCs, leading to more salt removal. In addition, an increase in the number of cell pairs reduces the voltage required in each cell, allowing a greater net energy gain. Moreover, with the use of thin ion exchange membranes (IEMs) and desalination chambers, the internal resistance is reduced and more efficient separation of ions and water desalination can be achieved (Kim & Logan 2011).

A similar trend was observed for current density (Figure 3) across an external resistor of 560 Ω.

High COD removal in the anode chamber (>95%) was observed for all MDC configurations (Figure 4). However, the CE was around 9.74%, 15.47% and 16.01% for 1-MDC, 3-MDC, and 5-MDC, respectively.

![Figure 2](https://example.com/fig2.png) | Total desalination rate and salt removal efficiencies of 1-, 3- and 5-MDC configuration obtained during three consecutive desalination cycles of 24, 48 and 96 hours, respectively.

![Figure 3](https://example.com/fig3.png) | Mean current density for 1-, 3- and 5-MDC configuration (fed every 24 hours) obtained during three consecutive desalination cycles of 24, 48 and 96 hours, respectively.
This means that not all of oxidized organic matter was converted into electricity indicating that most of the COD was removed through non-electrogenic mechanisms (Brown et al. 2014). It could also be due to the possible competition between the exoelectrogenic and other bacteria, probably anaerobic fermenters and methanogens, for organic substrates (Capodaglio et al. 2015; Capodaglio et al. 2017, 2018; Molognoni et al. 2018). According to Vilajeliu-Pons et al. (2016), an alternative for improving the CE could be the application of a variable resistance control to enhance the electron transfer, once intermittent electric connection allows higher current production since both capacitive and faradaic currents are harvested.

**Biofilm formation**

The biofilm growth in the anodic chamber of a MDC acts as an anodic catalyst and helps in the rapid respiration of the bacteria, which in turn produces more current (Kramer et al. 2012; Yang et al. 2012; Baranitharan et al. 2015). Bacteria that colonize the electrode are defined as exoelectrogenic bacteria, although they have been described using other terms, such as electrochemically active bacteria, anode respiring bacteria, and electricigenes. These bacteria have the capability of extracellularly transferring the electrons gained by the respiration process towards an exogenous electron acceptor. The highest power densities are almost always produced by inoculating the anode with a rich and diverse source of bacteria, such as sludge, soil or sediment (Logan 2009). Also, the application of a variable resistance control can enhance the electron transfer, thus stimulating exoelectrogenic biofilm growth (Vilajeliu-Pons et al. 2016).

In this study, *in nature* marine sediment was used as the microorganism source to inoculate the anode chambers. According to previously studies, the microbial community composition of biofilm is immensely affected by the inoculum source, type of microorganisms (Gram-positive or Gram-negative), nature of microbial culture (pure or mixed) and pre-enrichment and start-up strategies (Molognoni et al. 2014; Molognoni et al. 2016; Saratale et al. 2017). Thus, in order to investigate the morphology of the bacterial biofilm developed on the electrode surface, SEM was conducted. In Figure 5, we can observe the development and enrichment of exoelectrogens with biofilm formation on energy-harvesting granular graphite anodes.

Electron micrographs (Figure 5) revealed unique biofilm structures and cell shapes. The anode in the MDC fed with acetate was covered with a monolayer of short-rod bacteria, which were similar in cell shape and arrangement on the anode with exoelectrogenic bacteria (Bond et al. 2002; Bond & Lovley 2003).

Studies on the bacterial communities present in these biofilms reveal a complex diversity of microorganisms, and those belonging to the Proteobacteria and Firmicutes phyla are the most abundant (Beyenal & Babauta 2015). Currently, *Geobacter, Sheuanella, and Pseudomonas* are the most investigated species and most often used as models for researchers involving exoelectrogenics (Lovley 2006; Logan 2009; Korth et al. 2015; Md Khudzari et al. 2016). Also, several other microorganisms such as *Rhodofex sp.*, *Klebsiella sp.*, *Aeromonas sp.*, *Escherichia coli*, *Geothrix sp.*, *Citrobacter sp.*, *Clostridium sp.*, *Enterobacter sp.* and *Burkholderia sp.* have demonstrated electroactive properties (Dulon et al. 2006; Feng et al. 2014).

**Perspectives**

Several studies have demonstrated the ability of bioelectrochemical systems to treat wastewater with simultaneous production of electricity (Mehanna et al. 2010a, 2010b; Logan & Rabaey 2012; Zhang et al. 2012; Kim & Logan 2013b; Capodaglio et al. 2015; Zhang & He 2015; Molognoni et al. 2018). However, these technologies have been shown to generate a small amount of energy, which would be sufficient only for low-power applications. Thus, the simple production of electricity is not yet economically feasible compared to well-established processes, such as anaerobic digestion.

Alternatively, it would be an advantage to utilize the electricity generated *in situ* to conduct desalination, as presented here. After evaluating the performance of several
MDC reactors, Saeed et al. (2015) proposed a design for a wastewater treatment plant integrating MDC technology, in order to improve the overall performance and reduce the energy consumption. The authors chose a stacked MDC configuration as a model reactor for integration in this plant since they are simple to scale up by adding more MDC units to each other to allow increased desalination rates.

ElMekawy et al. (2014) discussed the possibilities of MDC technology to be used as a stand-alone or as a pre-treatment step for reverse osmosis, reducing the overall load and, consequently, costs associated with water desalination. The authors suggested that MDCs may be incorporated into water softening plants or into a conventional wastewater treatment plant as pre- or post-treatment to deal with different influent and effluent quality needs.

However, as a novel technology, there are several challenges to overcome before the successful applications of MDCs. In a global perspective, the most limiting factors for large-scale systems relate to engineering and reactor design. Hence, research is needed for the development of this technology, including: optimization of stacked MDCs for large-scale studies; widening the lifecycle cost estimates; developing alternative (low-cost) electrode materials; stimulating exoelectrogenic biofilm formation, for example, by applying a variable resistance control enhancing the electron transfer; further optimization of MDCs with real wastewater; substitution of chemicals to a sustainable catholyte (oxygen-based cathode or biological cathode); and promoting the development of continuous flow reactors (Sophia et al. 2016; Vilajeliu-Pons et al. 2016).

CONCLUSIONS

This study demonstrates the great potential of MDCs as a low-cost desalination process with environmental friendly...
benefits, encouraging further development of this technology. Improvements are still needed to be performed in order to translate this technology from laboratory studies to real applications. And, in a near future, MDCs can be applied to treat saline industrial wastewater, producing a high-quality water with simultaneous bioelectricity generation.

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