Strategies for merging Microbial Fuel Cell Technologies in Water Desalination processes: start-up protocol and desalination efficiency assessment

Zulema Borjas [1], Abraham Esteve-Núñez[1,2] and Juan Manuel Ortiz [1,3*]

[1] Madrid Institute for Advanced Studies Water. Scientific-Technological Park of Alcalá, Madrid 28805, Spain; zulema.borjas@imdea.org

[2] Department of Chemical Engineering, University of Alcalá, Madrid 28871, Spain; abraham.esteve@uah.es

[3] FCC Aqualia, Gestión Integral del Agua S.A., FCC Citizen Services. Avenida del Camino de Santiago, 40, Building 3, 4tth Floor, 28050, Madrid (Spain)

* Corresponding author: juanma.ortiz@imdea.org; Tel.: +34-918 30 59 62-

Abstract

Microbial Desalination Cells constitute an innovative technology where microbial fuel cell and electrodialysis merge in the same device for obtaining fresh water from saline water with no energy-associated cost for the user. In this work, an anodic biofilm of the electroactive bacteria *Geobacter sulfurreducens* was able to efficiently convert the acetate present in synthetic waste water into electric current (j=0.32 mA cm^{-2}) able to desalinate water. .Moreover, we implemented an efficient start-up protocol where desalination up to 90 % occurred in a desalination cycle (water production:0.308 L m^{-2} h^{-1}, initial salinity: 9 mS cm^{-1}, final salinity: <1 mS cm^{-1}) using a filter press-based MDC prototype without any energy supply (excluding peristaltic pump energy). This start-up protocol is not only optimized for time but also simplifies operational procedures making it a more feasible strategy for future scaling-up of MDCs either as a single process or as a pre-treatment method combined with other well established desalination technologies such as reverse osmosis (RO) or reverse electrodialysis.

Introduction

Shortage of fresh water is one of the major challenges for societies all over the world. In this sense, current water desalination technologies can significantly increase water resources for human consumption, industrial use and irrigation, but require significant electric or thermal energy. On top of the environmental impact due to brine disposal [1], reverse osmosis (RO) consumes around 3.5 $kWhm^{-3}$ of electric energy [2] for seawater desalination with only a recovery of 50%, while thermal technologies could reach more than 7 $kWhm^{-3}$ to drive desalination processes. The installed capacity of systems in year 2015 was about 86.8 million m^3 day^{-1} of desalted water, this is expected to increase drastically in the next years. The desalination market is mostly dominated by reverse osmosis (RO), compared to competition between multi-stage flash (MSF) and multi-effect distillation (MED). These systems require electric or thermal energy to drive the desalination process, and the equivalent amount of fossils fuel to produce the energy related to desalination processes (i.e. 86.8 million m^3 day^{-1}) is around 793 million tons per year, according to Kalogirou [3]. Thus, if desalination is accomplished by such technologies, environmental pollution would be a major concern because of green-house gases emissions. On top of that, conventional desalination technologies spend an important amount of energy to produce desalted water, so innovative desalination systems are required to be developed.

Microbial Electrochemical Technologies (METs) constitute a platform [4] of emerging technologies based on the interaction between electroactive bacteria and electrodes. Indeed, a variant of the fuel cell, the microbial fuel cell (MFC), allows the direct transformation of soluble organic matter into electric current [5]. The produced power (in the range of 0.001-19 W m^{-2} (referenced to cross section) [6]) could be used in the same system to enhance organic matter degradation or drive other processes requiring

electrochemical energy, and operate in a self-sufficient and decentralised fashion as a passive system.

In this microbial electrochemical context, the Microbial Desalination Cell (MDC) is the integration of a MFC and an electrodialysis (ED) cell in order to treat wastewater and desalinate seawater. By using the energy provided by the oxidation of organic matter, contained in the wastewater, this system drives the migration of ions and the desalination process. Thus, MDC technology is able to desalinate saline water without consuming electric or thermal energy and allowing the use of the energy for any other processes. In this sense, MDC technology could be employed to save energy and avoid the greenhouse gases related to the conventional processes (seawater RO produces 1.78 kg of CO_2 per m^3 using 600 g CO_2 kWh^{-1} in the average European Union (EU) energy mix). Indeed, the versatile and simultaneous applications of MDC have made it a real and feasible alternative for both desalination and wastewater treatment [7].

The MDC unit is composed of at least three chambers (figure 1): 1) an anaerobic anodic chamber that contains the electroactive microbial community which first oxidises the organic matter (fuel) contained in wastewater and then transfer electrons to the anode; 2) a central desalination compartment separated from the others by an anion exchange membrane (AEM) and a cation exchange membrane (CEM); 3) a cathodic chamber where the reduction counter-reaction occurs. In a MDC reactor, CEM and AEM are alternatively placed between the cathodic and the anodic compartment, as indicated in Figure 1. Moreover, an external load is placed between anode and cathode collector, allowing the flow of electric current. If organic matter is used to feed the anodic compartment, and the cathodic compartment is fed with a catholyte (for example, Fe^{3+} or oxygen in acidic solution), then a potential difference is established between both electrodes. Due to the differential charges of the two chambers, anions and cations

migrate through the respective membranes raising the salt concentration in the anodic and cathodic compartments while decreasing it in the saline compartment (central compartment). Thus, the MDC constitutes a sustainable process since it does not require external energy input because microbes harvest it from waste.

Different MDC designs have been reported in the literature from the first proof-of-concept study [7,8], including cubic and tubular reactors [9-12], stacked cells [13, 14], using batch recirculation [15, 16], biocathodes MDCs [17], increasing water production by applying external voltage [18], or integrating innovative membranes (Forward Osmosis) [19, 20] or ion exchange resins in the compartments [21]. Although most of the MDC prototypes studied in the literature are lab scale (i.e. millilitres), a 100 L pilot scale MDC unit achieving partial desalination of seawater has been reported [22]. Thus, MDC technology has been proposed as a suitable pre-treatment for Reverse Osmosis to reduce energy costs for desalination [23].

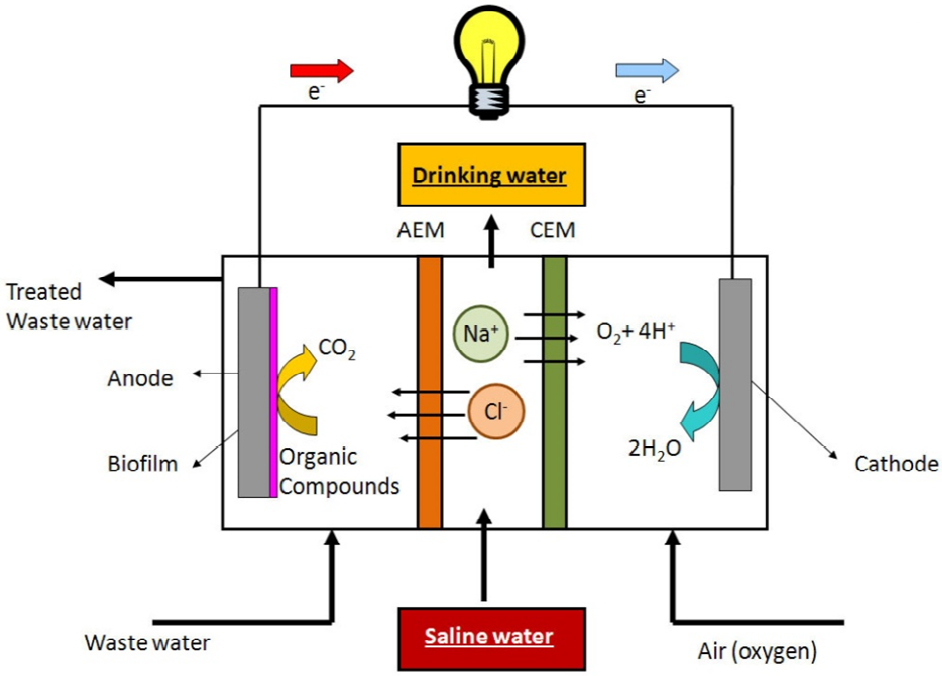


Figure 1. Diagram of MDC unit.

Regarding the performance of the MDC, the main limitations are i) the drastic increase of the internal resistance due to the changes in the conductivity of the medium, and the pH variations in the anodic chamber that can affect the biofilm growth. Related to the efficiency of the desalination process, the main constrains are limited potential to drive ion migration and back diffusion of salts between anodic/cathodic and saline compartment. For the application of MDC technology at a pilot scale, most of the studies use the same start-up protocol: a pre-adaptation of the biological anode under a MFC configuration [7,8], followed by the conversion of MFC into a MDC device by adding a supplementary chamber (saline compartment) that requires the disassembly of the whole system. This strategy for starting-up MDC is a time-consuming procedure of up to 20 days [24] and it does not favour reproducible experimental results at lab scale. Furthermore, most previous studies were conducted on millilitre-scale MDCs (<300 ml) where, to the best of our knowledge, just a few works reported on MDC over one litre [11,25]. So, using a more rationale start-up protocol could enable the scale-up of MDC technology in a realistic way and could help to improve the study of such systems by increasing reproducibility and avoiding time-consuming pre-acclimation strategies.

In the current work, we propose a rational and efficient start-up protocol where desalination up to 90% occurs from the first cycle under the final structural configuration. Once the protocol is performed, the system operates as an autonomous MDC without any energy supply (excluding peristaltic pump energy) and could be used at lab scale for systematic study of MDC performance under different experimental conditions.

2. Experimental Section

2.1 Bacterial strain, culture condition.

A pure culture of *Geobacter sulfurreducens* strain DL1 was used as inoculum for the MDC start-up protocol. The culture was routinely grown at 30 ºC in septum-sealed serum bottles containing freshwater medium (FWM, pH=6.9, EC=12.4 mS cm^{-1}) with the following mineral salts: $NaHCO_3$ 2.5 g L^{-1}; NH_4Cl 0.25 g L^{-1}; $NaH_2PO_4H_2O$ 0.06 g L^{-1}; KCl 0.1 g L^{-1}; $Fe(NH_4)_2(SO_4)_26H_2O$ 0.04 g L^{-1}. The medium was supplemented with a trace mineral and vitamin solutions [26]. Sodium acetate ($NaC_2H_3O_2$ 1.64 g L^{-1} or 20 mM) was used as electron donor and fumarate ($C_4H_2Na_2O_4$, 6.40 g L^{-1} or 40 mM) as sole electron acceptor. Anaerobic conditions and pH buffering (pH 6.8-7.0) were assured by flushing a mixture of N_2/CO_2 (80:20, industrial ALIGAL-12) into the culture media. Traces of oxygen were removed from the gas phase by passing the gas through heated copper fillings. Exponential-phase culture was used for the inoculation into the anode compartment.

2.2 Microbial Desalination Cell device (MDC)

A commercial multipurpose electrochemical reactor manufactured by ElectroCELL company (Electro MP-1, effective projected electrode area 100 cm^2) was used as MDC device. The MDC prototype (see Supplementary Information, Figure S1) had a compact stack design consisting on several polypropylene compartments and neoprene gaskets for an optimal hermetically seal. The dynamic design of the device allows different cell configurations. In this case, the three compartment configuration was used. A desalination chamber (compartment volume: 70 cm^3) separated the anodic and cathodic chambers (compartment volume of each one: 70 cm^3) by an anionic exchange membrane (AMX Neosepta) and cation exchange membrane (CMX Neosepta),

147 respectively. Both electrodes (i.e. anode and cathode), were composed of carbon felt
148 RVG 4000 (MERSEN Ltd.) and contained graphite plates as electric collectors. The
149 device was closed with stainless steel screws in order to avoid any leakage of the
150 system.
151 The three tanks were connected to the MDC prototype by Pharmed Tubing with an
152 internal diameter 1/4" (SAINT-GOBAIN). A four-channel peristaltic pump (Heidolph
153 Pumpdrive 5201) was used for the recirculation of the streams through the system.
154 Figure S2 (see Supplementary Information) shows a flow diagram of the MDC
155 experimental set-up.
156 The flow rate of all solutions was of 75 mL min^{-1}. The whole system was placed in a
157 temperature controlled room at 30 ºC and kept under anaerobic conditions by flushing a
158 mixture of N_2/CO_2 (80:20, industrial ALIGAL-12) into the tanks. Two reference
159 electrodes (Ag/AgCl KCl 3.5 M, CRISON) were placed into the cell, one in the
160 geometric center of the anodic compartment and the other in the cathodic compartment,
161 in order to measure anode and cathode potential respectively. A power supply (Aim-
162 TTi, 0-15 V, 0-5 A) was connected to the electrochemical reactor to be used during
163 start-up protocol (see Supplementary Information, Figure S1 and S2).
164
165 **2.3. Start-up and operation procedure**
166 **2.3.1 MDC configuration testing**
167 A preliminary process of conventional electrodialysis under abiotic conditions was
168 performed in order to test if the prototype's configuration was feasible for desalination
169 operation. A 2 L solution of FWM supplemented with 20 mM of acetate (1.64 g L^{-1}
170 sodium acetate) as sole electron donor was used as anolyte and a 3.55 g L^{-1} Na_2SO_4
171 solution as catholyte (25 mM Na_2SO_4). A power supply applied a cell potential of 3 V

between anode (positive terminal) and cathode (negative terminal). The saline stream consisted on 2 L of 5 g L^{-1} $NaHCO_3$ (pH=8.70, CE=5.1 mS cm^{-1}). The system was operated in batch mode (streams recirculation) with a multichannel peristaltic pump with flow rate 75 mL $min^{-1}$for all the streams (anolyte, catholyte and saline solution).

2.3.2 Start-up procedure

Prior to inoculation, the cell was sterilized by the recirculation of 70% w/w ethanol/water solution through the whole system (prototype and tubing). A filtered gas mixture of N_2/CO_2 was gassed through MDC for 2 hours in order to guarantee ethanol evaporation and an anoxic environment inside the device. Also, electrolytes and saline solutions were degassed. The anolyte was made of 2 L of FWM with 1.64 g L^{-1} sodium acetate (without any terminal electron acceptor, 20 mM, pH=6.95, CE=5.95 mS cm^{-1}), and the catholyte was 2 L of 3.55 g L^{-1} Na_2SO_4 (0.0025 mM, pH=7.87, CE=4.74 mS cm^{-1}) solution. The saline solution was 2 L of 1.25 g L^{-1} sodium bicarbonate (15 mM , pH=8.70, CE=5.1 mS cm^{-1}).

It is important to note that the main role of using sodium bicarbonate solution as saline stream in this step is to avoid pH and/or conductivity changes in anodic media from continuous migration of anions (i.e. HCO_3^-) from saline compartment, thus preserving optimum anode environment for biofilm colonization of the anode surface.

The volume relation of the electrolytes and saline solution was of 1:1:1 ($V_{an:}$:V_{cat} : V_{desal}). The cell potential was fixed at 1.0 V (i.e. potential between anode and cathode) and then all three solutions were recirculated through the system overnight prior to anode inoculation in order to remove any dead volumes or stagnant zones in the system. After this, pump recirculation is switched off. Then, the anodic chamber was inoculated with 300 ml of exponential-phase *Geobacter sulfurreducens* culture. After inoculation,

the system was allowed to incubate overnight, to ensure cells adhesion to the anode. After incubation, the pumps were activated to recirculate the different solutions through the system. The cell potential (i.e. 1.0 V) is maintained until anode potential and current density are almost constant (45 hours approx., see figure 4A). After this, the cell potential is increased to 1.5V to allow growth of biofilm on anode surface (70 hours approx.). This first cycle was part of the start-up protocol and involved sodium bicarbonate desalination, in a MEC configuration, since a cell potential of 1.0-1.5 V was applied using an external power supply.

2.3.3 Desalination operation

Once the start-up protocol was performed, NaCl desalination was carried out in the MDC. The peristaltic pump was deactivated and the power supply was disconnected from electrode collectors. Then, MDC electrode collectors were connected to an external load of 2.5 Ω. This value of the external load was selected to ensure proper measurement of the electric current in the system by voltage drop measurement. Moreover, it is important to note that in the present study the MDC operates near short circuit (low external resistance value) in order to provide all the energy from organic matter oxidation to the desalination process, as the main objective is to maximize the water production. Anolyte tank was refreshed with new solution of the same composition and volume as above. The catholyte was replaced with 2 L of 3 g L^{-1} NaClO solution (0.3 %, pH=11.14, CE=14.57 mS cm^{-1}). The saline solution was replaced by 0.2 L of 5 g L^{-1} NaCl. The volume relation was 10:10:1 ($V_{an:}$:V_{cat} : V_{desal}). Once all the solutions were replaced, the recirculation pump was activated to start desalination.

2.4 Electrochemical equipment

The data acquisition of the anode/cathode potential and electric current was performed using a custom Visual Basic Program and ModBus modules (ICP-DAS). Reference electrodes (Ag/AgCl 3.5 M, CRISON) were placed in anode and cathode compartment, using lugging capillary (Teflon).

2.5 Analytical methods

Electric conductivity measurements were carried out using GLP 31 conductivity meter (CRISON). A GLP 21 pH-meter (CRISON) was used to measure pH. Both measurements were recorded at 25 ºC. For conductivity readings, solution samples were analyzed throughout the experiments. Carrying out a set of conductivity calibration tests at 25.0 ± 0.4 ^{o}C, determined a conversion factor of κ = 0.55 for converting electric conductivity (EC, mS cm^{-1}) into NaCl concentration (g L^{-1}) (see Supplementary Information).

For total COD determination, 15 mL of sample were collected and kept at 4ºC until analysis by APHA method 5520. The content of acetate in the anolyte was measured with HPLC with a ZORBAX PL Hi-Plex H Guard Column (50 mm × 7.7 mm, Agilent Technologies, Madrid, Spain) and mobile phase of 0.1% H_3PO_4. The sample volume was 50 μL, mobilized at a flow rate of 0.5 mL·min^{-1}. Acetate was detected by using UV detector at 210 nm.

2.6 Process parameters

Current density (j, mA cm^{-2}), and power (P, W) in all experiments were calculated using Eqs. 1, and 2, respectively.

$$j = \frac{I}{A_m} \quad (1)$$

$$P = E_{cell} \times I \quad (2)$$

where E_{cell} is the voltage applied across the MDC system (V), I is the operating current (A) and A_m is the effective electrode surface area (cm^2). Cell potential, anode and cathode potential, as well as electric current data were measured throughout the process. Specific energy consumption (SEC, kWh m^{-3}) defines the energy required for producing one cubic meter of fresh drinking water by the MDC system (Eq. 3).

$$SEC = \frac{1}{3.6 \times 10^6 \times Q_t} \int E_{cell} I(t) dt \quad (3)$$

In Eq. 3, Q_t is the volume of the treated water (m^3). In desalination experiments, water production (WP, m^3 $m^{-2}h$), defining the cubic meter of drinking water produced by the MDC system per available membrane surface area per hour, was calculated using Eq. 4;

$$WP = \frac{Q_t}{A_m \times t_d} \quad (4)$$

where, t_d is the total time taken for the completion of the desalination process (h). Current efficiency (η_c, %) defines the ratio of total mass of salt removed from the saline channel to the amount of electric charge transferred across the membranes (ECT, C m^{-3}) over a complete process of desalination. η_c and ECT were calculated using Eqs. 5 and 6, respectively.

$$\eta_c = \frac{\upsilon \times z \times F \times \left(c_s^f - c_s^p\right)}{ECT} \quad (5)$$

$$ECT = \frac{1}{Q} \int I(t) dt \quad (6)$$

where, υ and z represent the stoichiometric coefficient and the valence of the salt ions, respectively and F is the Faraday constant (96,485 C mol^{-1}). c_s^f and c_s^p represent the initial and final molar concentrations of salt in the saline tank (mol m^{-3}), respectively.

3. Results and Discussion

In this section we have explored a rational and efficient start-up protocol where desalination occurs (Figure 2) while current efficiency was compared to conventional electrodialysis.

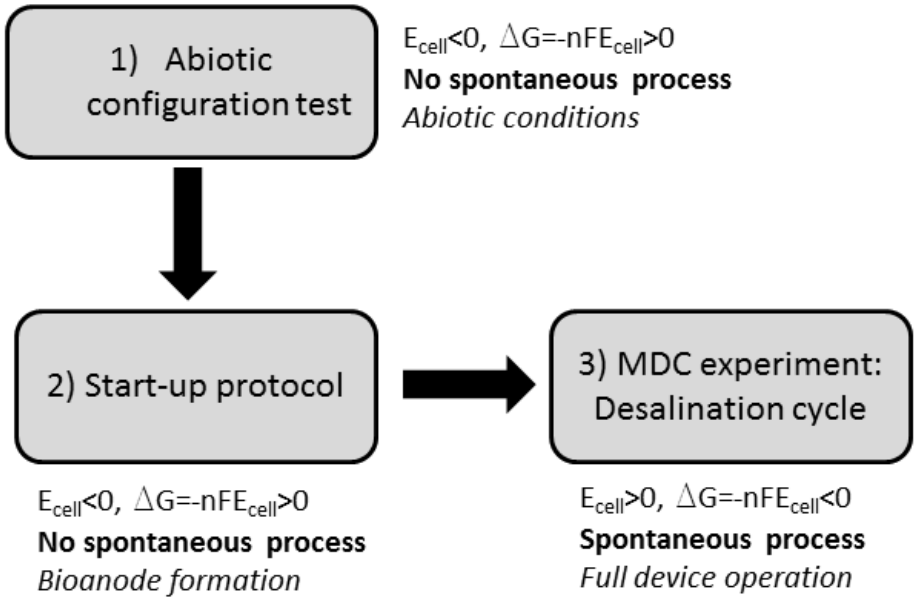


Figure 2. Schematic diagram: start-up protocol for MDC study at lab scale.

3.1 Testing desalination under an abiotic MDC configuration

In order to characterize the performance of the cell stack under non-limiting conditions an abiotic electrochemical desalination was carried out. An electric potential of 3.0 V was applied between anode (positive terminal) and cathode (negative terminal). Under this condition, water was oxidised on the anode ($2H_2O$ (l) $\rightarrow O_2$ (g) + $4H^+$ + $4e^-$, E^0= -1.23 V) while water was reduced on the cathode ($4H_2O$ (l) +$4e^-$ $\rightarrow$ $2H_2$ (g) + $4OH^-$, E^0= -0.42 V) and a solution of 5 g L^{-1} $NaHCO_3$ was desalinized in the middle compartment (Figure 3C).

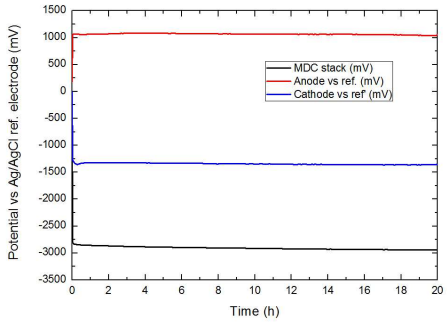
The electrodes potential remained constant throughout the desalinization process (Figure 3A). It is known that the potential of the whole MDC should correspond to the following equation:

$E_{cell}=E_{cathode}-E_{anode}-R_{cell}\,I$ [Ecell<0, $\Delta G=-nFE_{cell}>0$, No spontaneous process] (7)

where E_{cell} is the applied potential (mV), $E_{cathode}$ is the cathode potential (mV), E_{anode} is the anode potential (mV), R_{cell} is the overall resistance of the cell (Ω), and I is the

electric current that circulates through the system (mA). The electric current started at a maximum current density of j= 0.72 mA cm^{-2} and decreased to values around 0.32 mA cm^{-2} after 20 hours of polarization (figure 3B). This behaviour of the current output is typical of conventional electrodialysis systems [27] operating at a constant applied potential in batch mode. This is due to the electric conductivity of the saline compartment (i.e. saline stream) decreasing during the desalination process, and in turn increasing the overall resistance of the cell. Indeed, after 24 hours of cell operation, a 68.6% desalination was correlated to a 75% decrease of the electric current (Figure 3C).

A)



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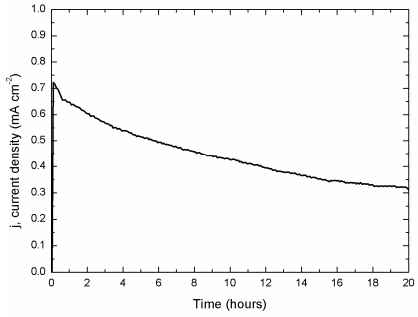

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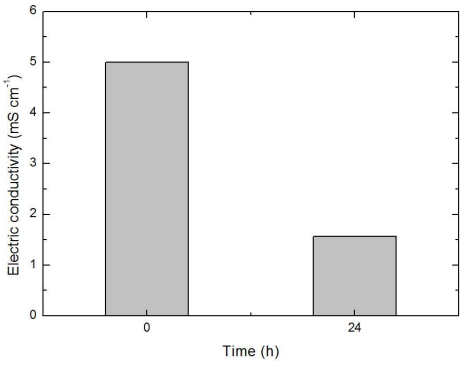

D)

Experimental Conditions	
Anolyte	2 L / FWM + 1.64 g L^{-1} acetate + 2.5 g L^{-1} sodium bicarbonate
Catholyte	2 L / Na_2SO_4 3.55 g L^{-1}
Saline Stream	2 L / 2.5 g L^{-1} sodium bicarbonate
Flow rate (3 chambers)	75 mL min^{-1}

Figure 3. A) Potential for the anode, the cathode and the whole cell; B) Electric current produced during the desalination process; C) Electric conductivity drop in the desalination compartment; D) Chemical composition of anolyte, catholyte and saline stream employed in the MDC configuration test.

In this abiotic experiment desalination process was completed after 24 h, thus specific energy consumption (SEC) was 1.46 kWh m^{-3}, water production (WP) 10 L m^{-2} h (0.01 m^3 m^{-2} h) for the initial conductivity of 5 mS cm^{-1} (i.e. brackish water), and the current efficiency of the process was η_c=94%. For similar water conditions in electrodialysis experiments (analogous ion exchange membranes system), the SEC is in the range of 0.6-0.8 kWh m^{-3} [27]. Even if the systems are not comparable from construction point of view (i.e. different configuration of the unit cell, higher thickness for anode/cathode and saline compartments, low conductivity in the anode compartment to avoid microorganisms saline stress), abiotic desalination experiments could be useful to compare the energy consumption in different MDC prototypes at lab scale. Also, it is important to note that efficiency in the desalination process is similar to conventional electrodialysis stacks, suggesting proper assembly and a correct operation in abiotic conditions for the MDC system. The current efficiency is lower than 100%, due to the non-ideal membranes and the existence of shunt currents.

3.2 Start-up procedure for the Microbial Desalination Cell (MDC).

Once the system was abiotically tested, the MDC was ready to host a microbial anode. So, maintaining the previous setup and electrolytes composition, a cell potential of 1.0 V was set between electrodes and sterile solutions were recirculated overnight through the two chambers. The anode was inoculated without recirculation (recirculation pump switched OFF) and incubated for 20 hours, as previously noted in section 2.3.2. So, the complete acetate oxidation to CO_2 by *G.sulfurreducens* ($C_2H_4O_2 + 2\ H_2O \rightarrow 2\ CO_2 + 8\ e^- + 8\ H^+$, E^o=-0.290 V) and the reduction of water ($2\ H_2O + 2e^- \rightarrow H_2 + 2\ OH^-$, E^o=-0.8277 V) were the main reactions on anode and cathode respectively, being the process no spontaneous ($E_{cell}<0$, $\Delta G=-nFE_{cell}>0$).

328 During this period (Figure 4A) the anode potential dropped from 500 mV to values near
329 -300 mV (vs. Ag/AgCl KCl 3.5 M) while the current density increased from the original
330 values to j=0.048 mA cm^{-2}. Both phenomena are characteristic of *G. sulfurreducens*
331 electroactive behaviour [28–30] when the electric current is obtained from acetate
332 oxidation. On the other hand, the cathode potential decreased following the anode
333 potential in order to maintain cell potential (water reduction was the main reaction in
334 the cathode).
335 After 20 hours of operation, the electric current decreased 33% (Figure 4B). Local
336 starvation of electron donors (i.e. acetate) as a consequence of an insufficient mixing of
337 the solution may have caused this bacterial metabolic limitation. Moreover, local pH
338 decrease could also be a factor that affects bacterial cells performance. Even if
339 bicarbonate ions migrate from the desalinization chamber, the lack of flow through the
340 anode chamber could produce internal biofilm acidification as a result of microbial
341 metabolism. Those physiological limitations were confirmed once electrolyte
342 recirculation was re-started according to the sudden raise of 375% in current generation
343 (Figure 4B).
344 After 24 hours operating with continuous media flow, a stable current output of 11.2 mA
345 was reached (j=0.112 mA cm^{-2}). At the same time, the anode potential decreased
346 indicating an insufficient anode polarization. This is consistent with the maximal
347 electroactive performance of *G. sulfurreducens* (-0.2 V (vs. Ag/AgCl) reported
348 elsewhere [31,32]. When the electrode polarization was below this potential, the biofilm
349 cells became reduced due to the charge accumulation in a network of redox proteins
350 made of multiheme c-type cytochromes [30,33,34]. The outstanding nature of this
351 network allows *Geobacter* strains to exhibit a capacitive property unique in microbes
352 [35,36]. So, in order to increase current production and biofilm discharge, cell voltage

353 was extended from 1 V to 1.5 V, leading to the expected rise in current output of 330%
354 (i.e. from 9.6 to 32.0 mA) in just 8 h. Moreover, upon repolarization after inoculation,
355 there was a discharge peak confirming a charge accumulation in the cytochrome
356 network due to the capacitor effect of the biofilm. Then, current increased from 24.0 to
357 32.0 mA suggesting the presence of a biofilm colonizing the electrode surface with
358 enough electroactive capacity to produce an electric field in the system able to drive the
359 migration of ions from the saline compartments to anode/cathode compartments.
360 Indeed, electric conductivity of the saline chamber significantly decreased until it had
361 removed 94% of the initial bicarbonate ion at the end of the desalination cycle (110 h)
362 (Figure 4C).
363 It is important to note that the main role of using sodium bicarbonate solution as saline
364 stream is to avoid pH and/or conductivity changes in anodic media from continuous
365 migration of anions (i.e. HCO_3^-) from saline compartment, thus preserving optimum
366 anode environment for biofilm colonization of the anode surface. In this sense, also
367 other experimental conditions as high solution flow rate (75 mL min^{-1}) ensured adequate
368 substrate concentration inside the carbon felt anode, minimizing local pH variations
369 (Figure S3) or substrate depletion (removal of COD < 49% of the original COD during
370 the start-up procedure).
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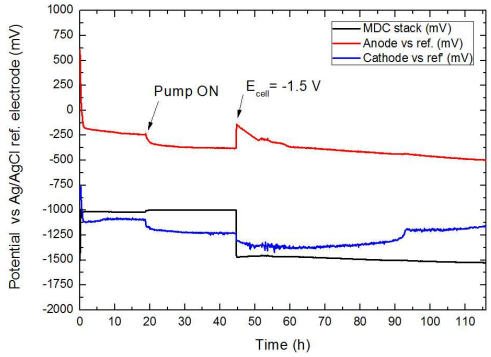
A)



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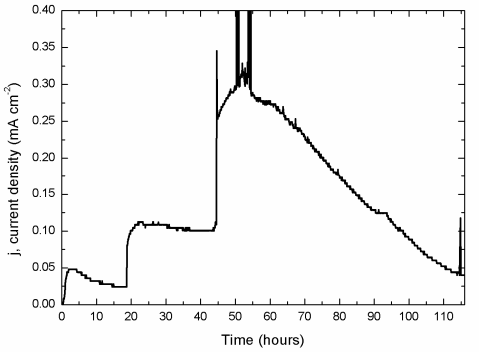

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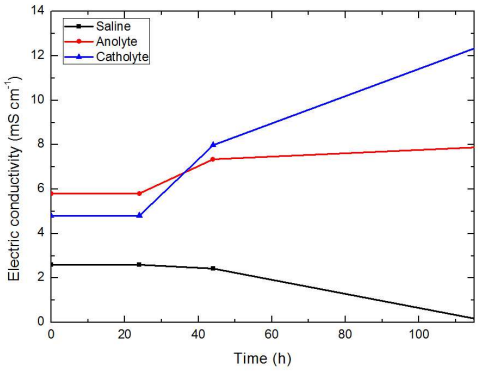

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Saline Stream	2 L / 1.25 g L^{-1} sodium bicarbonate
Flow rate (3 chambers)	75 mL min^{-1}

Figure 4. A) Potential for anode, cathode and whole cell. Black arrow indicate time for starting up the recirculation; B) Electric current obtained during the desalination process; C) Electric conductivity in the three chambers (anodic, cathodic and saline chamber) D) Characteristics of anolyte, catholyte and saline solution used during the MDC start-up procedure.

3.3 Microbial Desalination Process

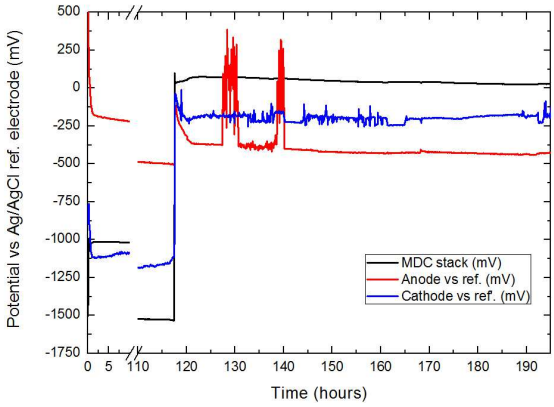
The start-up period was considered to be completed after 100 hours of operation, when an electroactive *G.sulfurreducens* biofilm had developed. At this point, the catholyte was replaced with 2 L of NaClO 3 g L^{-1} (0.3%, pH=12), the anolyte was refreshed (2 L, FWM, pH=6.9, 1.64 g L^{-1} / 20 mM sodium acetate) and saline solution was changed to 0.2 L solution of NaCl 5g L^{-1}. Finally, the circuit was closed using a shunt resistance

(external load) of 2.5 Ω that allowed monitoring of the electric current through the MDC unit. In this condition, the oxidation of acetate to CO_2 by *G.sulfurreducens* and the reduction of NaClO to chloride ion ($ClO^- + H_2O + 2\ e^- \longrightarrow Cl^- + 2\ OH^-$, E^o=0.89 V) are the main reactions on anode and cathode respectively, enabling the spontaneous process of desalination (migration of ions from saline compartment, $E_{cell}=E_{cathode}-E_{anode}>0$, $\Delta G=-nFE_{cell}<0$).

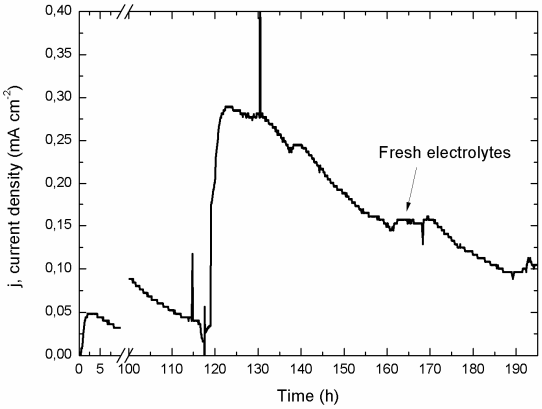
During the initial desalination period, the electric current production dropped from 28 mA to 15 mA while the anode potential remained stable (Figure 5A). The reduction of electric conductivity of the saline stream –and the increased internal resistance due to this fact - is the main reason for this current shift. Although more than 20% of the total acetate (enough for microbial growth) was detected in the anolyte after this current drop, anolyte was replaced by new fresh anolyte (2 L, FWM, 1.64 g L^{-1} / 20 mM sodium acetate) in order to assure bioanode performance (i.e. no influence by changes concentration or metabolite accumulation). In the same context, catholyte was also replaced by new media (2 L, NaClO 3 g L^{-1}, pH=12).

The MDC device was able to desalinate 65% of the NaCl content in the first 18 hours, and 90% in 65 hours (end of desalination cycle, 1mS cm^{-1}, drinking water limit) (Figure 5C). At the same time, both the anolyte and the catholyte conductivity remained stable, mainly due to the high ratio anolyte/saline volume and/or catholyte/saline volume. Electric resistance of MDC system was constant (2.5 Ω, value of external load).

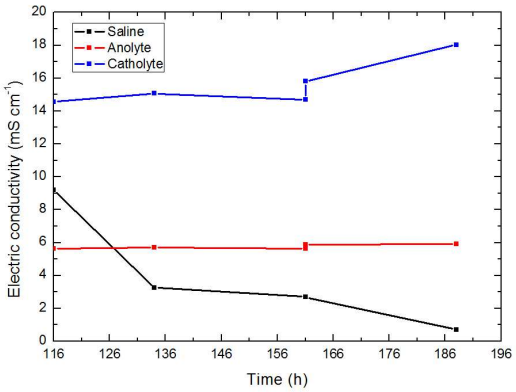
A)



B)



C)



D)

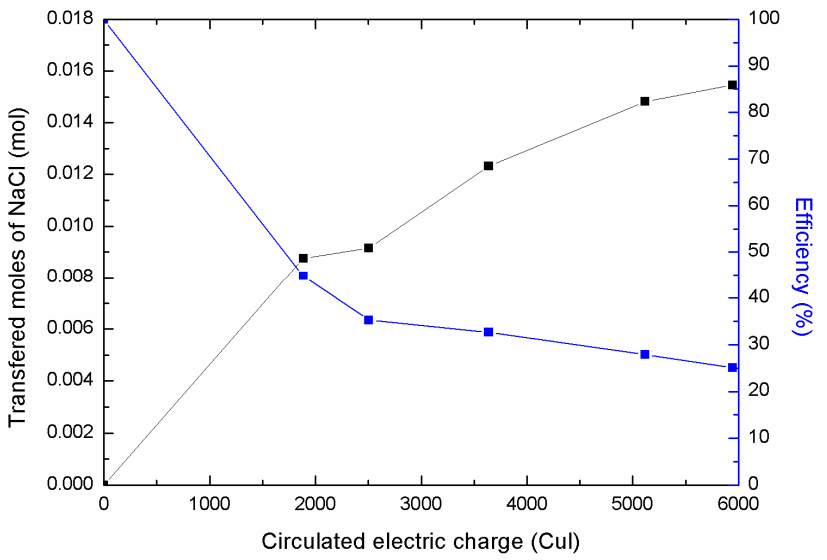
Experimental Conditions	
Anolyte	2 L / FWM + 1.64 g L^{-1} acetate + 2.5 g L^{-1} sodium bicarbonate
Catholyte	2 L / NaClO 3 g L^{-1}, pH=12
Saline Stream	0.2 L / NaCl 5 g L^{-1}
Flow rate (3 chambers)	75 mL min^{-1}
Shunt Load	2.5 Ω

Figure 5. A) Potential for anode, cathode and whole cell; B) Electric current produced during the desalination process. Black arrow indicates the replacement of anolyte and catholyte; C) Electric conductivity of the anodic, cathodic and saline streams; D) Chemical composition for anolyte, catholyte and saline stream employed in the desalination process

Another key aspect for the proper performance of the microbial biofilm on the anode is the pH. In that sense, both anolyte and catholyte pH slightly increase in less than one unit allowing the maintenance of the anodic biofilm (Figure S4). Actually, COD removal was incremented from 49% under power supply conditions to 53% under MDC mode operation.

At the end of the process, water production was 0.308 L m^{-2} h (initial concentration

426 NaCl 5 g L^{-1}) while efficiency vs circulated electric charge was η_c=25% (Figure 6). The
427 decrease of the efficiency could be attributed to back-diffusion processes [37] and the
428 low available potential (compared to the abiotic desalination). Also, it is assumable that
429 initial conductivity of saline stream and ratio among anolyte-catholyte-saline tank
430 volumes would have and strong effect on the efficiency of the process, and this effect
431 should be studied before scale-up and optimization of MDC technology.
432 In relation to the specific energy consumption (SEC), it is important to note that energy
433 consumed associated to MDC-based desalination was equal to 0 (excluding pumping
434 energy). We indeed considered negligible the energy produced by the external load used
435 for voltage/current monitoring (i.e. energy produced 0.082 Wh, 412 Wh per m^{-3} of
436 desalted water, or 20.63 Wh per m^{-3} of treated water),.
437 Conventional reverse osmosis system or electrodialysis system typically require an
438 energy value of 0.5-0.8 kWh m^{-3} to drive the same desalination process (from of 5 mS
439 cm^{-1} to 1 mS cm^{-1}) exhibited by our MDC [38]. On top of that, the associated emissions
440 of 0.3-0.48 kg $m^{-3}$of CO_2 would be avoided (using the value 600 g CO_2 kWh^{-1}in the
441 average European Union energy mix [39]). Moreover, if we consider that the energy for
442 waste water treatment is in the range of 0.2-0.4 kWh m^{-3}, and assuming a ratio waste
443 water/desalted water 10:1, we could avoid the emissions of 1.2-2.4 kg of CO_2 per m^{-3} of
444 desalted water. Thus, the challenge is to develop MDC technology and scale up to a
445 reasonable production of desalinated water (i.e. small and medium size desalination
446 plants are in the range of 2,000-10,000 m^3 day^{-1}).
447



448

449 **Figure 6**. NaCl transferred (mol) and Efficiency (%) of the process vs circulated electric
450 charge (Cul).

451 The MDC system presented in this paper was able to produce desalinated water with a
452 simplified start-up procedure compared to other experimental systems reported in the
453 literature adapting the microorganism before starting the MDC operation [24,25], thus
454 significantly reducing the start-up period. Also, the start-up strategy presented in this
455 study avoids the need of pre-running as a MFC and disassembly for incorporating the
456 saline compartment, as presented in some papers for MDC operation at lab scale
457 [7,8,13]. So, the start-up protocol presented is not only optimized for time but also
458 simplified in operation, making it a more feasible strategy for future scaling-up. Also,
459 the start-up protocol presented could be used at lab scale to systematically study MDC
460 performance. It is useful tool to design and optimize MDC experimental set-ups (i.e.
461 study of energy consumption in abiotic conditions).

462 The desalination performances of several MDC studies (including the current one) are
463 shown in Table 1. Even the experimental set-ups presented in the literature are different
464 in configuration (i.e. compartments volumes, cathode reactions, operation mode in
465 continuous or batch) and it is not possible direct comparison among them, the efficiency

of our treatment was remarkable considering the low ratio of volumes for anolyte and catholyte compared to the ones reported in the literature with similar removal rate. In some cases, those volumes ratios were quadrupled or even 100 times larger than the volumes employed in our system [8,13]. Those studies using similar volume ratio reached less than 50% of the desalination obtained in the MDC presented in this study [9,40,41].

Table 1. MDC system comparison ([a] V_{an}: anolyte volume, V_{cat}: catholyte volume, V_{desal}: desalinated water volume; [b] With additional voltage of 0.55 V; Removal (%): desalination percentage; COD: percentage of Chemical Oxygen Demand removal.

V_{an}: V_{cat}: V_{desal} [a]	Start-up protocol	Initial concentration of Salt	Salt Removal (%)	COD Removal(%)	Ref.
100:33:1	Transfer of pre-adapted MFC anode (10 days)	5 $g.l^{-1}$ NaCl	88	-	[7]
36:11:1	Transfer of pre- adapted MFC anode (10 days)	20 $g.l^{-1}$ NaCl	80	-	[13]
10:10:1	Bicarbonate desalination	5 $g.l^{-1}$ NaCl	90	53	This study
4:4:1	1 week anode in-sit stabilization	0.5 $g.l^{-1}$ NaCl	95.8	-	[25]
4:2:1	Inoculation with a mixture sludge	35 $g.l^{-1}$NaCl	42	74-77	[22]
3:3:1	Transfer of pre- adapted MFC anode	5 $g.l^{-1}$ NaCl	46	25	[24]
1.5:1.8:1	Transfer of pre- adapted MFC anode	20 $g.l^{-1}$ NaCl	<84	72-94	[8]
1:2:1	Inoculation of a pre-a adapted biofilm	5 $g.l^{-1}$NaCl	43	-	[9]
1:2:1	40 days anode *in situ* stabilization	20 $g.l^{-1}$ NaCl	37[c b]	-	[40]

Related to volume ratios, in the present MDC system 10:10:1 ratio was key to avoid the generation of brine, one of the main environmental problems associated to desalination technologies. However, more research should be performed to optimize a) the ratios for different applications (i.e. desalination of brackish water for irrigation, seawater for drinking water) and b) the nature and organic load of the wastewater (i.e. municipal or industrial wastewater). Other important parameter to explore in order to maximize water production would be the thickness of the saline compartment that would decrease internal resistance of the MDC system [41].

From the point of view of energy production in MDC systems, an increase in the energy production is related to a drastic decrease in desalination rate or production of water, as a great amount of available energy from water oxidation is diverted towards energy production (rather than desalination process). Even whether the production of energy is an interesting topic itself, increasing desalinated water production is a key point to develop MDCs as sustainable and low-energy desalination technology. For this reason, in the present study the MDC operates near short circuit conditions, thus providing all the energy from organic matter oxidation to the desalination process, maximizing the water production.

It is important to note here that in the case of MDC, to operate in such conditions of low external load, it is not producing a degradation of the biofilm performance due to acidification of the media. The reason is that even the operation conditions are near the short circuit conditions from the point of view of the electrochemical device, the anode potential is similar to the equilibrium (i.e. open circuit potential) of the bioanode (see figure 5A, red line). So, short circuit condition for the MDC is somehow different from MFC short circuit conditions, as in the latter the anode potential is decreased due to high overpotential, thus providing higher density current (i.e. higher rate of organic

matter oxidation) and consequently problems of biofilm acidification, producing decrease in the performance of the system. In the case of MDC system, a part of the available electrochemical potential in the whole device is dropped in the saline compartment, and this effect is more noticeable when desalination process is progressing.

Finally, the use of NaClO reduction as the cathodic reaction (used in this study) has been tested in similar bioelectrochemical systems for waste water treatment [42] as a suitable alternative for oxygen reduction with increased performance (due to faster kinetics). So far, the present study is the first using a MDC with this cathodic reaction for desalination. However, it is important to indicate that the use of NaClO as catholyte could produce both damage on membrane surface in the long term and environmental issues related to chlorinated by-products formation (i.e. chlorinated organic matter). Consequently, more strategies for cathodic reactions and catholyte regeneration should be studied in the future to enable the development of MDC technology as sustainable desalination process, as well as operation with real waste water and brackish/sea water.

4. Conclusions

Microbial Desalination Cells constitute an innovative technology where microbial fuel cells and electrodialysis merge in the same device for obtaining fresh water from saline water with no energy-associated cost and greenhouse gas emissions. However, a number of factors should be further explored in order to optimized the methodology and make it profitable. The start-up procedure as well as the modular nature of our MDC prototype was demonstrated a viable alternative to the conventional procedures used. The start-up protocol presented here, could allow our MDC prototype to be a real possibility for scaling-up as either a stand-alone process or as a pre-treatment method combined with other well established desalination technologies such as reverse osmosis (RO) [9,23] or reverse electrodialysis.

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