Original Article

J Babol Univ Med Sci
Vol 20, Issu 11; Nov 2018. P:49-57

Investigation of Modified Microbial Desalination Cell performance in Sweetening of saltwater

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J Babol Univ Med Sci; 20(11); Nov 2018; PP: 49-57
Received: Apr 16th 2018, Revised: Aug 7th 2018, Accepted: Sep 4th 2018.

ABSTRACT

BACKGROUND AND OBJECTIVE: Lack of enough fresh water is a global challenge. Water sweetening can be done using thermal or membrane systems that each of them requires significant energy. Microbial desalination cell (MDC) is a new technology which can desalinate water, generate electricity, and simultaneously purify wastewaters in a reactor. However, low current generation and deionization are from main challenges of this process. This study aimed to improve the MDCs efficacy.

METHODS: In this experimental study, a modified three-cell MDC consisted of anode, cathode, and middle chambers, was designed in order to water desalination (20 g/L NaCl). Here, the cathode solution was diffused via ozone (O_3-MDC) and the middle saline solution was pretreated into an ultrasonic bath. Subsequently, the results achieved in O_3-MDC in terms of water desalination and current generation were compared against those of another reactor operated under oxygen diffusion (O_2-MDC), and without ultrasonic pretreatment (control). Biofilm formation on anode surface and dominant bacteria in the O_3-MDC reactor were studied using Scanning Electron Microscopy (SEM), and 16S rRNA gene sequencing, respectively.

FINDINGS: Saltwater pre-treatment caused to increase the electrical conductivity from 28.1 ms/cm to 35.5 ms/cm; and then current generation from 191 to 131 mV after 24 hr operation. O_2-MDC and O_3-MDC were able to remove 74% and 55.58% of NaCl from water, respectively. Proteobacteria, firmicuites and acidobacteria were dominant microbial communities in the anode biofilm based on 16S rRNA sequencing.

CONCLUSION: Based on the results of this study, modified MDC with ozone and ultrasound waves could be an appropriate option for desalinating salt water.

KEY WORDS: *Microbial Desalination Cell, Microbial Fuel Cell, Bioelectric Energy Sources, Saltwater*.

Please cite this article as follows:

Gholizadeh A, Neshat AA, Pakravan F, Miri M, Taghavi M, Nikoonahad A. Investigation of Modified Microbial Desalination Cell performance in Sweetening of Saltwater. J Babol Univ Med Sci. 2018;20(11):49-57.

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Introduction

Lack of enough fresh water is a global challenge because about 97% of the available waters are salty and cannot even be used for irrigation (3-1). So now, in many parts of the world, water desalination and purification of saline waste for reuse are considered as a water supply method for communities (4, 2). In general, sweetening can be done using thermal or membrane systems, each of which requires significant energy (6, 5). The cost of energy accounts for 40% of the total cost of scavenging. For example, a reverse osmosis device consumes about 3 to 7 KW/h of electricity for the production of fresh water per cubic meter of saltwater (7).

One of the new alternative methods is the extraction of energy from microorganisms in the microbial fuel cell (MFC) (9, 8). A MFC can be modified in which de-acidification, flow generation and wastewater treatment are performed simultaneously in a reactor. Such a device is called the Microbial Desalination Cell (MDC) (10-12).

In MDC, bacteria liberate electrons and proton by metabolizing organic matter into the anode. The electrons migrate through the external circuit to the cathode, but protons that cannot pass through the anionic membrane and the external circuit remain in the anode. To compensate for the difference in electrical charge between the two electrodes, the anions and cations in the middle chamber are pulled to the anode chamber and the cathode, respectively. As a result, a water desalination, sewage treatment and electricity generation reactor are carried out (14, 13).

Kokabian et al. obtained a flow rate of 1 W/m3 in a MDC guidance cycle (15). Kalleary et al indicated that MDC can remove green and yellow malachite by 40 to 90% and remove salinity by about 62% (16). However, the process faces the challenge of low flow and low decomposition. The aim of this study was to increase the MDC performance with ultrasonic waves to improve salt ionization and also to introduce an ozone-receiving electron in microbial cells.

Methods

This experimental study after approval by the Ethics Committee of Yazd University of Medical Sciences with registration code of IR.SSU.SPH.REC.1395.35 was conducted. The MDC is designed with three anode, middle and cathode chambers with a diameter of 5 cm and a volume of 75, 38 and 75 mL (Fig. 1). First, the reactor was used as MFC (17) for one month, then as an MDC, an anionic membrane (AEM, AR204SXR412, Ionic, USA) between the anterior and middle chamber and the cationic membrane (CEM, CR67, MK111, Ionic, USA) between the middle chamber and a cathode.

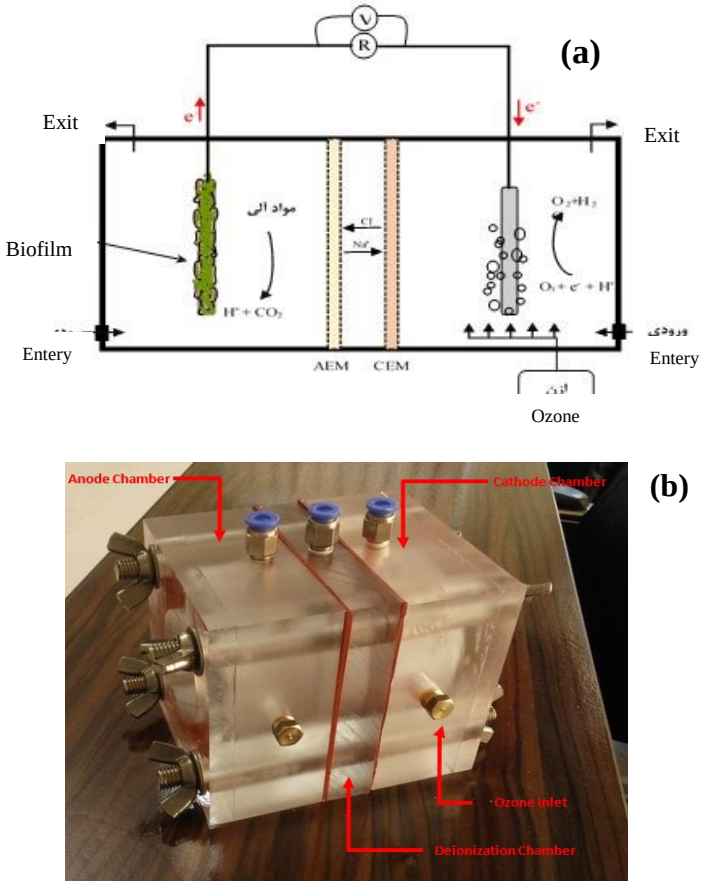

Figure 1. Schematic view (a) and real (b) Modified MDC Reactor

Before testing, the membranes were submerged in a 1 molar solution of NaOH and HCl for 2 hours, then washed with deionized water. Anode electrode (30×25 mm), porous graphite and electrodes of carbon fiber clad with 0.5 g/cm2 of platinum were selected and connected to one another by titanium and 100 ohm resistors (18). Anode chamber with sewage anaerobic sludge as well as Peptone Water (25g/L) and 1.6 g C6H12O6, 4.4 g KH2PO4, 3.4 g K2HPO4 • 3H2O, 1.5 g NH4Cl, 0.1 g MgCl2 • 6H2O, 0.1 g CaCl2 • 2H2O and g 0.1 KCl (in liter of distilled water). Also, this solution (enolite) was circulated with external

peripheral feeding solution (100 mL) at a rate of 0.05 mL/min (24 hours of hydraulic time) with two peristaltic pumps. The enolite was subjected to N2 gas injection (40 mL/min) for 10 minutes (17 mL/min) and replaced every 48 hours. The middle chamber was filled with NaCl/g/L solution.

To investigate the effect of applying ultrasound on the efficiency of the reactor, experiments were carried out parallel to the reactor containing oxygen instead of ozone (O2-MDC) under the conditions of processing and non-processing of ultrasound saline at a frequency of 22 kHz and 15 minutes (19), and the results with the reactor Containing ozone (O3-MDC) were compared. When the salt concentration in the middle chamber was less than 1 g/L, this solution was replaced (a driving cycle). The cathode chamber was filled with phosphate buffer and replaced every 48 hours. Ozone was used as an electron receptor in the cathode chamber. Ozone was produced by ozone generator using oxygen intake gas (95% purity) and continuously injected (8.26 mg/min) into catholyte (cathode chamber solution).

The amount of desalting and flow generation in O3-MDC and the ultrasonic sonication solution were compared with the values obtained in the control reactors (O2-MDC and non-ultrasonic, non-ultrasonic). Measurement of salinity of water was carried out by removing the sample from the middle solution every 24 hours and measuring its electrical conductivity (EC) using a conductivity meter (HQ40d). After measurement, the solution was returned to the chamber. Productive voltage (E, V) was recorded every 5 minutes by multimeter. Flow (I, A) was determined according to Ohm's law. The concentration of ozone in the inlet and outlet gas was measured by iodometric titration (20). The power density (PAn, W/m2) was measured based on the anode cross section (AAn, m2).

$$P_{An} = \frac{E^2}{A_{An} R_{ex}}$$

The polarity curve was plotted by measuring the reactor voltage at Ω 10 to 1 MΩ (min 10 for each resistor). The total desalination rate (TDR, mg/h) was calculated as follows:

$$TDR = \frac{(C_0 - C_t)V_d}{t}$$

Where C0 and Ct are the initial and final concentrations of NaCl; Vd (L) are the volume of the middle fluid; and (h) t is the time of desalting. Biofilm formation at anode level was monitored using an electron scanning microscope, and the growth of the bacterium in O3-MDC was monitored by PCR of the S rRNA16 gene. Samples were prepared from enolite and middle anode. Enolite samples were taken without any preprocessing.

The method of DNA extraction and gene amplification is detailed in previous studies (13). In total, about 150 samples from O3-MDC and O2-MDC reactors were collected in a double-repeat steady run (each about 75 samples). Data were analyzed by using SPSS Ver. 20 and by Wilcoxon and paired T-tests and $p<0.05$ was considered significant.

Results

Effect of ultrasound waves and bioelectricity production: EC of saline solution was 35.7±1.2 mS/cm, which was 39.5±2.4 mS/cm after preprocessing with ultrasound. The 24-hour operating time of O2-MDC, Voltage and EC pre-dispensed anchorage was 131±7.4, 27.6±2.6 mS/cm, respectively. These values were 119±11.9 mV and 28.1±1.5 mS/cm in the crude solution. Therefore, in previous experiments, its preprocessing was performed. The maximum OCV in O2-MDC and O3-MDC was 628.3±3 and 1331±11.6 mmol/L, respectively.

This value was 178.8±1.8 mV and 793±7.7 mV, respectively (Fig. 2 (a) and b) respectively. Also, the maximum current density of O2-MDC and O3-MDC was 1.16±0.57 A/m2 and 5.27±0.55 A/m2 respectively; the current density in O2-MDC and O3-MDC in the lag phase was initially The increase and then decreased, but the slope of the decrease was higher in O3-MDC, however, the O3-MDC voltage was higher than O2-MDC at all steady-state times.

Due to the abnormalities of the data, Wilcoxon test was used to analyze the data. Significant differences in the results of two reactors were confirmed in the crossover mode ($p<0.001$ and $Z=-27.84$). Polar curve: The maximum power density of O3-MDC was 4.06±0.63 W/m2, which is about 11 times its value in O2-MDC (W/m2, 369.0±0.04 W/m2).

The use of O3-MDC for irrigation desalination reduced internal resistance to O2-MDC, so that based on the polar curve, the internal resistance of 305±4 and 71±2/5 mm were estimated in O2-MDC and O3-MDC, respectively. These results show higher ozone yields relative to oxygen as electron receptors in the reactor. Data analysis showed significant difference between two O2-MDC and O3-MDC reactors ($p<0.05$).

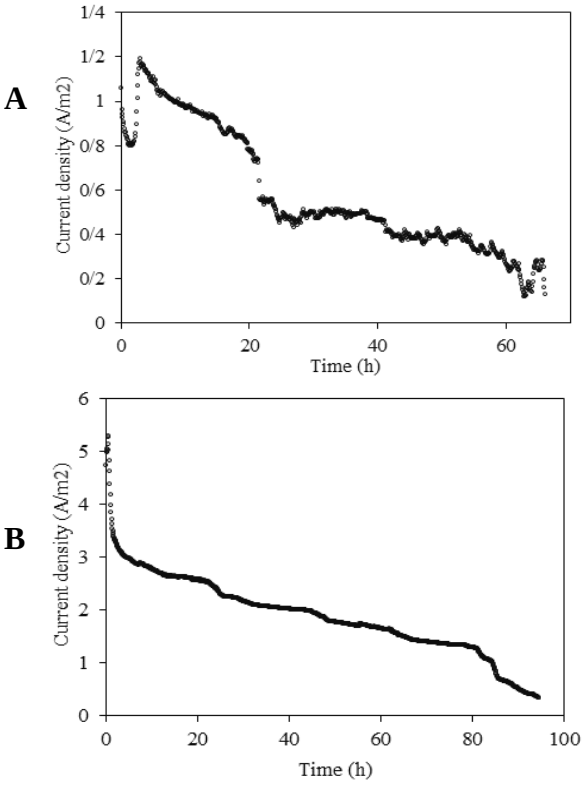

Figure. 2. Electrical conductivity A: O2-MDC and B: O3-MDC. Compact Voltage, NaCl solution g/L20

Desalination: Desalination was found to be faster in producing higher flow (Fig. 3). The mean TDR of O2-MDC and O3-MDC was 2.02±0.20 mg/h and 2.58±0.38 mg/h respectively. In the first 24 hours, O2-MDC and O3-MDC eliminated more than 18.9%±1.2 and 35.8%±3.1.3 salt. O2-MDC produced a mean flow rate of 0.42±0.2 mA and eliminated 55.58%±0.25% of the solution from the middle solution, while in O3-MDC, the mean flow was 1.41±0.31 mA, and 74.8±1.3% salt was removed from the solution. This represents an 18 percent increase in desalination performance. In O2-MDC and O3-MDC control samples, 19% and 21% of salt were eliminated, respectively.

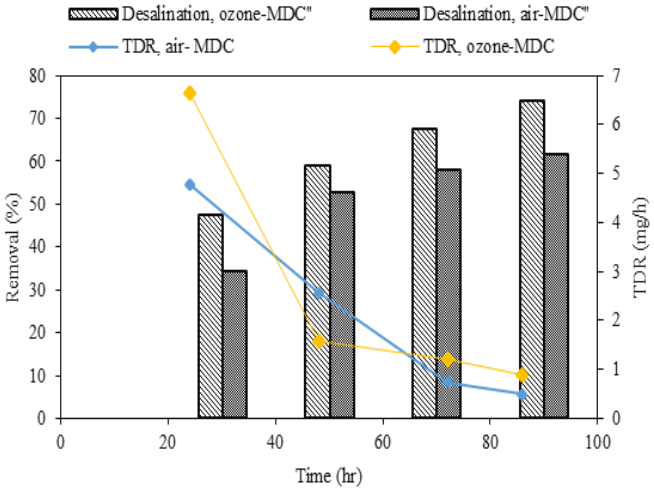

Figure 3. TDR in O2-MDC and O3-MDC (in% NaCl/h)

Morphology of the anode level: An examination of the SEM images of the anode level before and after the experiment (Fig 4) shows a significant change in the morphology of the anode, in which the new electrode has a smooth surface without microorganisms, while the anode image used to indicate surface occupation by germs.

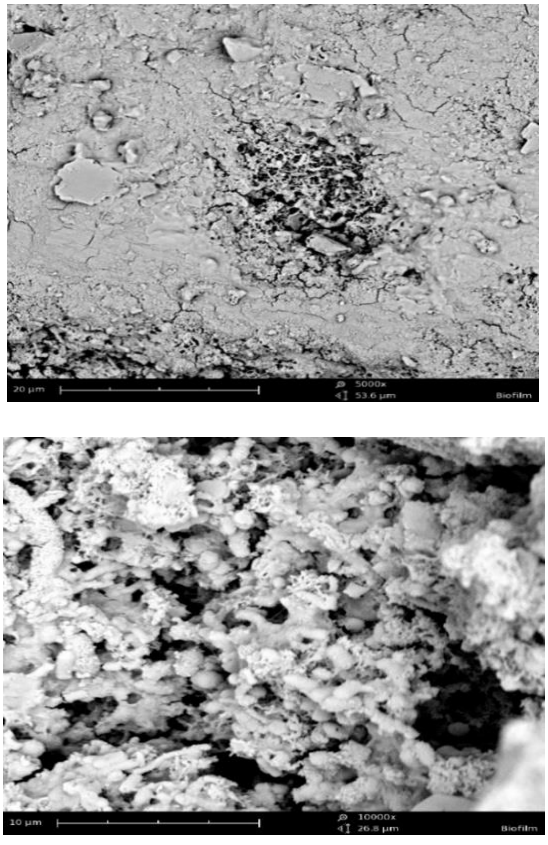

Figure 4. SEM Images A: Before, and B: After completing the tests

Dominant bacteria: The sequencing of s rRNA 16 was used to represent bacterial communities (22, 21). Figure 5 shows the samples of this study.

The samples taken from the enolite and anode (bands 2 to 5) show the presence of bacteria, while there was no spectrum in the control sample (band 6), so the bacterium was not present in the band. Also, bacteria in biofilm anode and enolite are listed in Table 1. The anode biofilm has a more diverse microbial community than enolite. Proteobacteria (Proteobacteria) were dominant microbial communities in anode and enolite biofilm. Firmicutes and Acidobacteria were also identified in biofilm samples, while they were not present in enolite. However, Epsilon proteobacteria and bacterioides (Bacteroidia) were present only in the enolite.

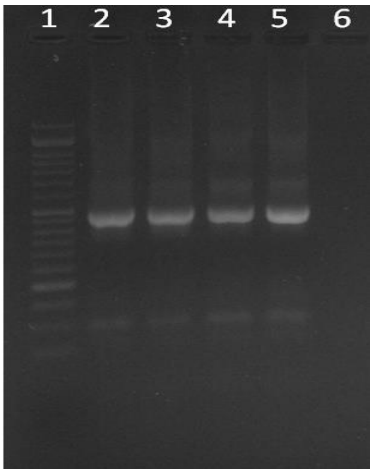

Figure 5. amplification product using general primer

Product size: 468 bp, arrange samples from left to right: column 1: bp50 DNA ladder, column 2: sample isolated from the electrode, column 3: sample isolated from the electrode, column 4: enolite sample, column 5: Example of enolite, column 6: control sample

Table 1. Identified bacteria in enolite and anode microbial desalting reactors

Sequence ID	Family	Class	Category	similirity (%)	Gene bank number
Anode biofilm					
MA1	Acidobacteriace	Acidobacteria	Acidobacteria	88	HG763957.1
MA2	(Chromatiaceae)	Gamma perothebacteria	Proteobacteria	91	HQ877094.1
MA3	(Ectothiorhodospiraceae)	Gamma perothebacteria	Proteobacteria	87	KC009941.1
MA4	(Burkholderiaceae)	Beta Proteobacteria	Proteobacteria	87	KP772724.1
MA5	Clostridiace	Clostridia	Firmicute	89	KU045501.1
Enolite					
MS1	Compilabacticase	Epsilon Proteeobacteria	Proteobacteria	89	KF721645.1
MS2	Proletase	Bacterioid	Bacteriotite	86	GU955392.1

Discussion

In this study, ultrasonic waves have a positive effect on electricity production and salinity removal. This finding is due to the fact that, in very high-salt waters, chlorine and sodium ions are close together, stick together and form crystals, so that part of the ions are inactive in water (13); applying ultrasound to the salt

solution increases ionic mobility and their deionization becomes better. The positive effects of ultrasound have been proven in many environmental studies (23-25). The production voltages in O3-MDC were much higher than those obtained in O2-MDC. The molecular ozone is highly reactive and has a higher oxidation potential (V 2.07) relative to oxygen (23.1 V) (26).

In this case, almost all of the electrons that are oxidized into the cathode through the orbital circuit increase between the anode and the cathode of the potential difference, which is a higher ozone efficiency factor. Ozone injection into a catholic solution does not produce hazardous side-effects and its performance is maintained at a wide range of pH levels. In a bio-electrochemical reactor, ozone is converted to oxygen for a short time; this oxygen will re-enter the reaction (26). The flow density generated in O2-MDC and O3-MDC increased at initial guidance, then decreased. This electrical trend is also seen in other similar studies, as the internal resistance increases due to the gradual decrease of the electrical conductivity of the salt solution and the use of substrate (27, 28).

In addition, the gradual increase in salt concentration in the enolite can inhibit microbial activity (29, 13). The internal resistivity (polarity curve slope) of O2-MDC and O3-MDC was 305 and 71 ohm, respectively. In practice, the internal resistance of the bioelectrochemical system strongly affects the electrical outflow and depends on factors such as the material of the anode and cathode, the reactor size, chemical properties of the solvent, temperature, electrical conductivity, ion mobility, and the electrode surface and the type of electron receptor (29,30).

Liang et al. used carbon nanotubes, flexible graphite and activated carbon as an anodic MFC material and obtained internal resistance of 263, 301, and 381 ohms (31). Kim et al. found that the type of membrane could affect maximum power density, and the internal resistance in air cubic MFCs (91-84 ohm) was lower than that of water bottle MFCs (1272 -1230 ohms) (32). The use of thinner desalting rooms can reduce internal resistance and improve ion removal (33).

It was found that desalting efficiency is a function of flow generation and desalination is faster in higher flows. However, with the increase in time, TDR reduces the activity of the active ion in the middle room. In addition, the gradual increase in electrical conductivity in enolite solution is harmful to microorganisms and disrupts microbial activity (34). The performance of O2-MDC in biomass production is similar to that of other MDC systems with electron airborne receptors or ferricyanides.

In a study by Luo et al., which used ferricyanide, MDC removed about 66% of salt in 400 hours of lead, and in the long run, the flow density and desalting rate were 47% and 27%, respectively (34). In the study of Sevda et al., the phosphate buffer as catholyte and real sea water as the middle solution, the maximum desalting rate was 19.9%, which is lower than the present study (29).

When ozone was used in the MDC, the average desalting recovery was remarkable (from 55.8% to about 74%), the reason for such findings was previously mentioned. The O2-MDC and O3-MDC control system eliminated only 19% and 21% salt, respectively, indicating that the desalination of water was mainly due to the production of electric current and other factors such as normal osmotic ion exchange played a minor role. Proteobacteria were dominant microbial communities in anode and enolite biofilm. Proteobacteria have been reported extensively in previous studies as electrons, and this is probably due to competition in the transmission of extracellular electrons (27,35,36).

Epsilon proteobacteria and bacterioides were found only in enolite specimens. Bacteria are also gram-negative bacteria that have been reported in bioelectrochemical and saline water systems (37, 38). The presence of acidobacteria may be due to the low pH of the O3-MDC enolite, because they can withstand acids, metal contamination and hardened environments. The presence of fermiquits confirms the existence of anaerobic conditions because these bacteria are anaesthetized (39,40).

Rabaey et al. concluded that gamma pertobacteria and pyramicovites have the capacity to generate electricity (9). The limitations of the present study include the failure to identify the contribution of each bacterium in the reactor due to lack of facilities, the cost of ozone production, long-term non-examination, and the lack of evaluation of the amount of organic matter removed.

So further studies are recommended. In general, the sonication of ultrasound salts and the application of

ozone-receiving electrons in the cathode chamber instead of oxygen can improve MDC performance as a process of water desalination or an undertone for lower scavenging processes, however, the operation of the process requires more extensive research.

Acknowledgment

Hereby, we would like to thank the Vice-chancellor for Research and Technology of Yazd University of Medical Sciences for the financial and spiritual support of this research.

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