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A Factorial Study of the Effect of Rhamnolipid and Stirring on the Electricity Production, Desalination, and Wastewater Treatment Efficiencies of a Five-Chamber Microbial Desalination Cell

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ABSTRACT

This study was conducted to improve the voltage production, desalination, and COD removal efficiencies of a five-chamber Microbial Desalination Cell (MDC). To do this, rhamnolipid was added to anolytes only and catholytes stirred to determine the effects of these factors on the MDC activity. This was followed by a factorial study to investigate the effects of the interactions of rhamnolipid and stirring on the voltage production, desalination, and COD removal efficiencies of the MDC. Increasing the concentration of rhamnolipid to 240 mg/L improved the peak voltage produced from 164.50 ± 0.11 to 623.70 ± 1.32 mV. Also, the desalination efficiency increased from 20.16 ± 1.97 % when no rhamnolipid was added to 24.89 ± 0.50 % at a rhamnolipid concentration of 240 mg/L, and COD removal efficiency increased from 48.74 ± 8.06 % to 64.17 ± 5.00 % at a rhamnolipid concentration of 400 mg/L. In the stirring experiments, increasing the number of stirring events increased peak voltage from 164.50 ± 0.11 to 567.27 ± 18.06 mV. Similarly, desalination and COD removal efficiencies increased from 20.16 ± 1.97 % and 48.74 ± 8.06 % to 24.26 ± 0.97 % and 50.23 ± 1.60 %, respectively, when the number of stirring events was more than twice a day. In the factorial study, voltage production, desalination, and COD removal efficiencies were 647.07 mV, 25.50 %, and 68.15 %, respectively. However, the effect of the interaction between rhamnolipid and stirring was found to be insignificant ($p>0.05$). Thus, the addition of only rhamnolipid or the stirring of catholytes only can improve the performance of the five-chamber MDC.

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1. INTRODUCTION

The improvement of the electricity generation capacity, desalination, and wastewater treatment efficiencies of the Microbial Desalination Cell (MDC) is necessary for its scale-up [1, 2]. To this end, several interventions including those investigated by Zhang et al. [3] and Morel et al. [4] have been made, in which ion-exchange resins were employed to improve the desalination efficiency of MDCs. Other researchers have considered modifying the conventional three-chamber MDC design to improve the performance and flexibility of the technology [5-7].

Many attempts have been made to use relatively inexpensive catholytes [8, 9] and anolytes [10, 11] to make the technology affordable for scale-up. These attempts encapsulate the use of wastewaters as anolytes [9, 10] and tap water as catholytes [8, 9]. Also, other researchers have used bioathodes [9, 12, 13] as a relatively cheaper and more environmentally friendly option. However, affordability should not compromise electricity generation, desalination, and wastewater treatment efficiencies of MDCs and MFCs. Thus, Wen et al. [14] recommended adding rhamnolipid to anolytes as a measure to increase the electricity generation capacity of MFCs. Biosurfactants including rhamnolipid are microbial products that can reduce surface tension and facilitate microbial mineralization of substrates [15]. Besides, rhamnolipid can reduce the resistance of bacteria cell membranes to electron transfer outside cells, thus supporting electricity generation in BES [14].

In another study, Rismani-Yazdi et al. [16] reported that the stirring/aeration of catholytes of MFCs was a good strategy for reducing mass transport losses in the cathode chamber of this class of bioelectrochemical system. The reduction of mass transport losses is necessary for increasing the quantity of electricity produced by BES. Also, it has been reported that stirring (turbulence in water) increases the concentration of oxygen in water [17] and this is necessary to ensure the efficient operation of MDCs that rely on oxygen as a terminal electron acceptor [9].

Given the possibilities of rhamnolipid and stirring to increase the voltage production, desalination, and COD removal efficiencies of MDCs, we investigated the effect of rhamnolipid and stirring on these parameters associated with a

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five-chamber microbial desalination cell. Also, the effect of the interaction between rhamnolipid and stirring on the electricity generation, desalination, and COD removal efficiencies of the MDC was investigated using a 2^2 factorial design. Factorial designs create protocols that allow for several factors to vary at different levels to produce measurable effects in responses [18]. The presence or absence of significant interaction effects is realized when factors are combined and examined together [18]. Hence, the factorial design was used in this study.

2. MATERIALS AND METHOD

2.1. MDC construction

The MDC studied in this research was carved from polyoxymethylene cylinders and supported with cylindrical gaskets (Figure 1). Its compartments were held together with stainless steel bolts and nuts. Anode and cathode had empty bed volumes of 230 cm^3 each, while the desalination chamber had a volume of 77 cm^3. The anode chamber was separated from the desalination chamber by anion exchange membrane (AEM, Membrane International, New Jersey, USA) and the Cathode, Cation Exchange Membrane (CEM, Membrane International, New Jersey, USA). The cathode chamber roof of the MDC had a 4 cm × 6 cm portion cut opened to make room for the aeration and stirring of the water catholyte. Two additional chambers (neutralization chambers), i.e., one adjacent to the anode chamber (N_A) and the other adjacent to the cathode chamber (N_C), were added to create a five-chamber MDC. The neutralization chambers had internal volumes of 150 cm^3 each. The neutralization chamber adjacent to the end of the anode was separated by a CEM, while that to the end of the cathode was separated by an AEM. The anode was a carbon fiber-fill material (0.984" brush part, 400,000 tips per square inch, Mill-Rose, Ohio, USA) and the cathode, a cloth gas diffusion electrode (fuel cell store, New York, USA). The objective of the neutralization chambers is to hold water that will dilute the concentrations of H^+ and OH^- built-up in anolytes and catholytes over the course of experiments. This was the pH control measure employed in this study. In an earlier study, a two-chamber tubular MDC was used for pH control [19].

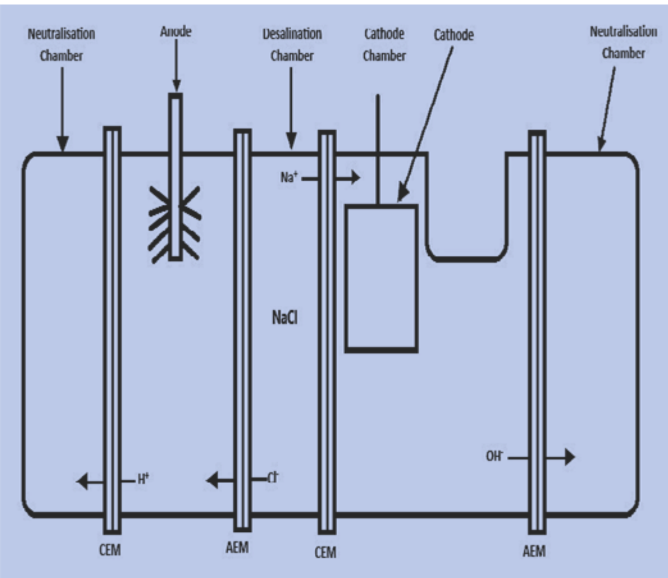

Figure 1. Schematic of the five-chamber microbial desalination cell (FCMDC)

2.2. Medium

The anolyte of the five-chamber MDC contained 3 g/L sodium acetate in 200 ml wastewater (filtrate of cow dung and rumen contents mixed in distilled water) supplemented with 20 ml of mineral solution (5 g/l NH_4Cl and 2.5/l NaCl). The initial COD concentration of the simulated wastewater was 645.00 ± 2.30 mg/L with a pH of 7.08 ± 0.02 and electrical conductivity of $2.37 \times 10^{-3} \pm 0.03$ mS/cm. A 220 ml tap water with a pH of 7.11 ± 0.09 and electrical conductivity of 1.01 ± 0.02 mS/cm was used as catholyte. Tap water was used as catholyte because it is a more environmentally friendly option. The neutralization chambers contained 100 ml of each of distilled water (pH, 7.04), while the desalination chamber contained 75 ml of 35 g/l NaCl solution. Rhamnolipid used was 90 % pure (AGAE Technologies LLC, Oregon, USA) and dissolved in distilled water at concentrations of 80, 160, 240, 320, and 400 mg/L.

2.3. Experimentation, analysis, and calculations

The anolyte of the MDC was inoculated with pre-acclimated bacteria, as described by Cao et a. [20]. Experiments were carried out in two stages. Stage 1 determined the concentrations of rhamnolipid and stirring speeds that would produce the best voltages, desalination, and wastewater treatment results. Stage 2 involved conducting a factorial study in which the selected rhamnolipid concentrations and stirring speeds (factors) were randomized at different levels to obtain the best combination of these factors which would yield the best outcomes. The concentrations of rhamnolipids studied were 80, 160, 240, 320, and 400 mg/l. This range of concentrations was chosen based on the work of Wen et al. [14]. The stirring regimes studied were Regime 1 (10 seconds of stirring, once a day), Regime 2 (10 seconds of stirring, 12-hour interval, twice a day), Regime 3 (10 seconds of stirring, 8-hour interval, three times a day), Regime 4 (10 seconds of stirring, 6-hour interval, four times a day), and Regime 5 (10 seconds of stirring, 4-hour interval, six times a day). These stirring regimes with a speed of 60 rpm were selected in terms of cathode chamber volume and effect of stirring on water flux from the cathode chamber into adjacent chambers. In the control experiment, neither rhamnolipid nor stirring was applied. For the factorial study, rhamnolipid varied at concentrations of 240 and 160 mg/l that represented high (1) and low (-1) levels of the factor (rhamnolipid), respectively. Stirring regimes varied were Regime 3 and Regime 1 representing high (1) and low (-1) levels, respectively. These factor levels (240 and 160 mg/l, and Regime 3 and Regime 1) were selected because they facilitated the highest and moderate electricity generation rates, desalination, and COD removal performances of the MDC. Regime 3 was particularly chosen because it not only produced large outputs of the measured parameters but also required a comparatively fewer stirring events. The factorial study was designed using DOE in Minitab16. The voltages were measured every five minutes across a 1000 Ω external resistor with a digital multimeter (Keithley 2700, Ohio, USA). A voltage drop below 45 mV indicated the end of the desalination cycle. Cyberscan Waterproof pH/conductivity/TDS/°C/°F PC 300 series multi-parameter (Eutech instruments-Thermo scientific, Massachusetts, USA) was used for measuring conductivity and pH changes. The dissolved oxygen levels were monitored using Hach HQ30d Flexi DO/Temp-meter (Loveland, Colorado, USA) and COD levels measured using the reactor digestion method. Desalination and removal percentage of COD was calculated through Equations 1 and 2.

$$\% \text{ Desalination} = \frac{\text{Initial saltwater conductivity} - \text{final saltwater conductivity}}{\text{Initial salt conductivity}} \times 100\ \% \quad (1)$$

$$\% \text{ COD removed} = \frac{\text{Initial COD} - \text{Final COD}}{\text{Initial COD}} \times 100\ \% \quad (2)$$

3. RESULTS AND DISCUSSION

3.1. Effect of rhamnolipid on voltage production

The average peak voltage produced by the MDC in the control experiment was 164.50 ± 0.11 mV (Figure 2). Upon the addition of rhamnolipid to anolytes, the peak voltage production increased from 164.5 ± 0.11 to 623.70 ± 1.32 mV at a rhamnolipid concentration of 240 mg/L (Figure 2). Thereafter, voltage production declined as the concentration of the rhamnolipid increased (Figure 2). The initial increments in voltage productions were associated with the ability of rhamnolipid to make substrate bioavailable to exoelectrogens so that it can be used in electricity production. This is possible through the ability of rhamnolipid to reduce surface tension and increase micelle formation which facilitate biodegradation of substrates [15, 21]. Also, the addition of rhamnolipid increased the electrical conductivity of the anolyte, thereby enhancing electricity production. For instance, the mere addition of rhamnolipid at a concentration of 240 mg/L increased the electrical conductivity of anolyte from $2.37 \times 10^{-3} \pm 0.03$ to 5.33 ± 0.17 mS/cm. Increment in the electrical conductivity of anolytes through rhamnolipid addition was also reported by Wen et al. [14]. On the other hand, a decrease in voltage production after a rhamnolipid concentration of 240 mg/L was attributed to the possibility that the higher concentrations (>240 mg/L) of the rhamnolipid inhibited the metabolic activities of exoelectrogens. According to Nickzad and Deziel [22], bacteria are intolerant of high concentrations of rhamnolipid. *Staphylococcus aureus*, for instance, is very intolerant of rhamnolipids, even at lower concentrations [23].

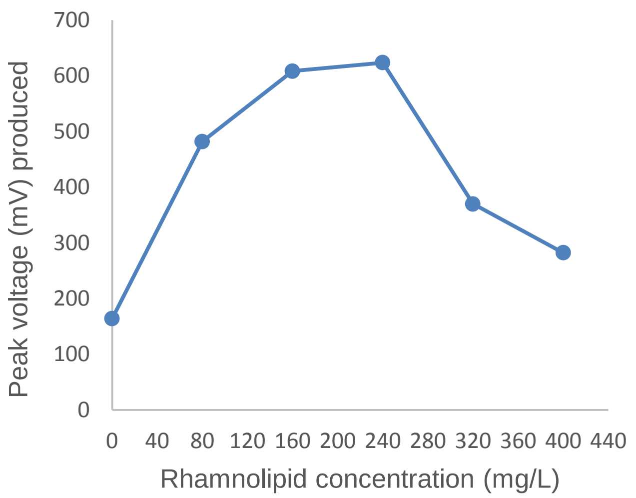

Figure 2. Peak voltages produced under rhamnolipid addition. [Zero (0) on the horizontal axis represents control experiment]

3.2. Effect of rhamnolipid on desalination

The addition of rhamnolipid improved the desalination performance of the MDC under study. Percentage desalination increased from 20.16 ± 1.97 % in the control experiment to 24.89 ± 0.50 % at a rhamnolipid concentration of 240 mg/L. The percentage of salt removal was in the order of voltage production. That is, increasing the concentration of rhamnolipid increased the desalination percentage up to a rhamnolipid concentration of 240 mg/L and thereafter, decreased to 21.83 ± 3.06 % at a rhamnolipid concentration of 400 mg/L (Figure 3). Since desalination in the MDC technology relies on electricity production in principle [24], a similarity in trend observed between voltage production and percentage desalination was a reflection of this fact.

It was also observed that the concentration gradient in the less concentrated water in the neutralization chambers, anolyte, and catholyte facilitated water osmosis into the desalination chamber, thereby diluting the concentrated saltwater in the desalination chamber. The result was an increase in the volume of water in the desalination chamber to ~ 77 cm^3. Ping et al. [8] and Mehanna et al. [25] also reported dilution as a contributing factor in desalination in their studies. In the study of Mehanna et al. [25], for example, the concentration gradient (dilution effect) could contribute to conductivity reduction by as high as 43 ± 6 % in a 20 g/L salt solution.

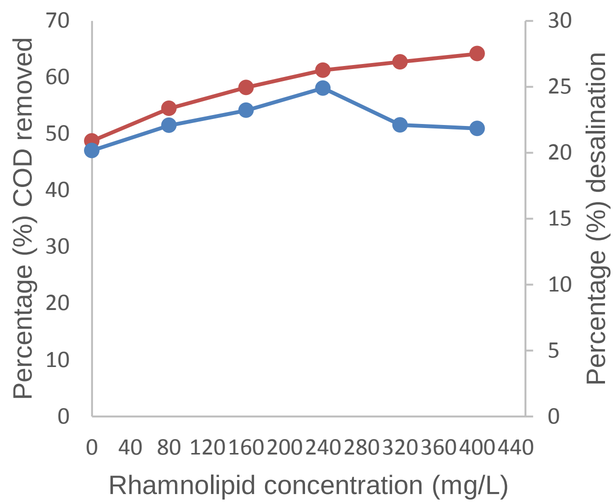

Figure 3. Desalination and COD removal efficiencies under rhamnolipid addition. [Zero (0) on the horizontal axis represents control experiment, the blue line represents percentage desalination and the red line represents percentage COD removed]

3.3. Effect of rhamnolipid on COD removal

A COD removal efficiency of 48.74 ± 8.06 % was produced from the control experiment and this was improved when rhamnolipid was added to anolytes (Figure 3). At a rhamnolipid concentration of 80 mg/L, the COD removal efficiency of the MDC was 54.48 ± 3.43 %. Further addition of rhamnolipid resulted in a COD removal efficiency of 64.17 ± 5.00 at 400 mg/L (Figure 3). The positive effect of rhamnolipid on COD reduction was probably due to its ability to make substrates bioavailability to microbes. Whang et al.

[21] articulated this by reporting that rhamnolipids lower surface tension and thereby, increase the bioavailability of substrates to microbes for biodegradation. The higher COD removal at a rhamnolipid concentration of 400 mg/L was attributed to the degradation of substrates by other bacteria that coexisted with exoelectrogens in the anode chamber. This was possibly the case because beyond 240 mg/L rhamnolipid concentration, the voltage production declined (Figure 2), implying that exoelectrogens were negatively affected by higher concentrations of rhamnolipd.

3.4. Effect of stirring on voltage production

The control experiment recorded the least voltage (164.50 ± 0.11 mV) produced, while the experiments operating with the stirring regimes 3, 4, and 5 produced the highest peak voltage of ~ 567.27 ± 18.06 mV, on average (Figure 4). The higher peak voltage production of the higher stirring regimes (3, 4, and 5) was attributed to their support of higher oxygen concentration in the water catholyte (Table 1). Oxygen is required for reduction reactions in the cathode chamber. In an earlier work, Kokabien and Gude [26] also observed that voltage production was high when the oxygen concentration of catholyte was high. This positive relationship between electricity production and oxygen concentration was also reported by Rismani-Yazdi et al. [16] and Oh et al. [27]. These earlier works concluded that power productions were proportional to dissolved oxygen concentrations in bioelectrochemical systems. The production of similar voltage outputs (Figure 4) among the stirring regimes 3, 4, and 5 was attributed to their provision of similar oxygen concentrations (Table 1) in the water catholyte.

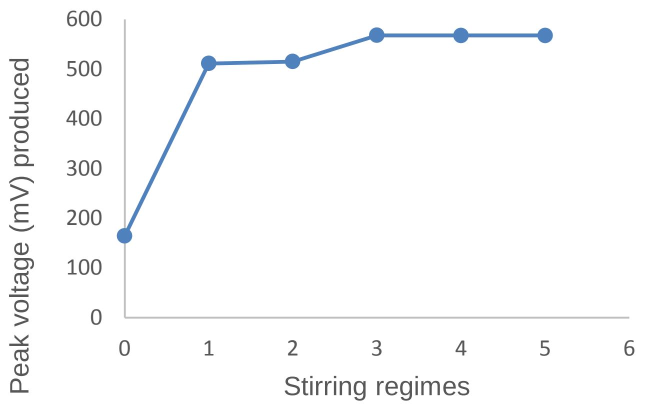

Figure 4. Effect of stirring regimes on peak voltage production. [Zero (0) on the horizontal axis represents control experiment]

Table 1. Dissolved oxygen (DO) concentrations of catholyte

Stirring regime	DO (mg/L)	Temperature (°C)
1	7.60 ± 10	28 ± 1.10
2	7.70 ± 1.51	28 ± 1.19
3	8.70 ± 20	28 ± 2.12
4	8.70 ± 1.11	28 ± 0.07
5	8.64 ± 0.60	28 ± 0.42

3.5. The effect of stirring on desalination

The highest percentage of desalination (~24.26 ± 0.97 %) was achieved when either of Stirring Regimes 3, 4, or 5 was applied and the least produced in the control experiment (Figure 5). Percentage of desalination increased in the same order as voltage production. This observation was attributed to the fact that desalination in MDCs principally relies on voltage production and this creates a positive correlation between voltage production and desalination. Voltage production causes the separation and movement of Cl^- and Na^+ ions out of the desalination chamber into the anode and cathode chambers, respectively, thereby causing desalination in the middle chamber [20].

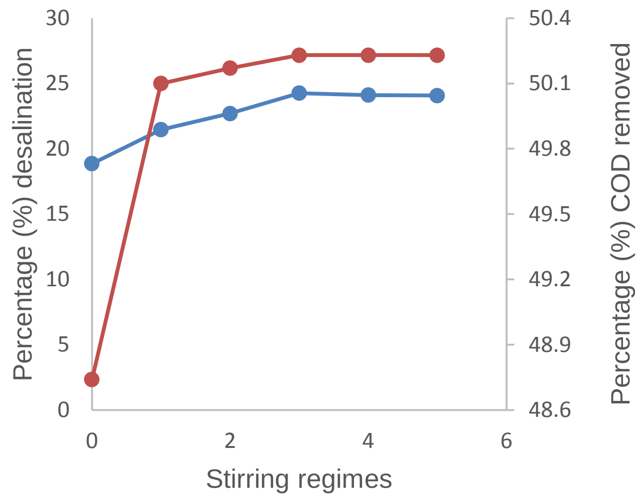

Figure 5. Effect of stirring regimes on desalination and COD removal efficiencies. [Zero (0) on the horizontal axis represents control experiment, the blue line represents percentage desalination and the red line represents percentage COD removed]

3.6. Effect of stirring on COD removal

The percentage of COD removal from anolytes increased from 48.74 ± 8.06 % in the control experiment to ~ 50.03 % ± 1.60 in the experiments operating with the stirring regimes 3, 4, and 5 (Figure 5). This was a rather marginal increase. It was also observed that the effect of stirring on COD removal assumed a similar trend as the effect of stirring on voltage production (Figure 4). This similarity in trend was probably because of the relationship between voltage production and COD removal. As exoelectrogens biodegrade substrates (COD removal), electrons are released and then, transferred to the cathode through the anode for voltage production [28]. Thus, when exoelectrogens find the use of the anode more favorable for energy gain, more substrates are biodegraded for voltage production and consequently, much more COD is removed. Also voltage production increases when the cathode chamber conditions are favorable (adequate supply of oxygen) and since stirring increases the concentration of dissolved oxygen in the catholyte, it thus supports COD removal through voltage production.

3.7. Effect of factor interaction (rhamnolipid and stirring) on voltage production, desalination, and COD removal

3.7.1. Effect of factor interaction (rhamnolipid and stirring) on peak voltage production

The highest peak voltage produced in the factorial study was 647.07 mV (Table 2). This was achieved with a rhamnolipid concentration of 240 mg/l and the stirring regime 3. This voltage (647.07 mV) was higher than the highest voltage obtained (567.77 mV; Figure 4) when only stirring was the factor being investigated and the 623.70 mV (Figure 2) obtained when rhamnolipid was the only factor studied in the preliminary experiments.

The higher voltage production performance in the factorial experiment was possibly due to the simultaneous improvement in both anolyte and catholyte conditions. The addition of rhamnolipid accelerates electron transfer out of bacteria cells [14] while stirring of catholyte provides adequate dissolved oxygen concentration in the cathode chamber (Table 1). Under adequate oxygen concentrations, mass transport losses (voltage loss) can be reduced in oxygen-depended cathodes, thereby improving voltage production [16].

An ANOVA output (Appendix A) showed that although the interaction of stirring and rhamnolipid improved voltage production, such interaction effect was not significant ($p = 0.337$). However, the main effects of stirring and rhamnolipid individually were significant ($F = 33.40$, $p = 0.000$) on voltages produced (Appendix A). The significance of the main effects of rhamnolipid and stirring was attributed to the ability of rhamnolipid to reduce internal resistance [14] and stirring (water turbulence) to increase oxygen concentration in water [17].

The insignificant effect of the factor interaction on voltage production was attributed to the possibility that the factor combinations at all levels (whether high, low, or both) produced near-optimal voltages attainable with the MDC. Thus, operating the MDC with any factor combination would not lead to marked changes in peak voltages as observed (Table 2).

The factorial analysis yielded the model equation:

$$\text{Voltage} = 639.732 + 4.185\ (\text{Rhamnolipid concentration}) + 1.997\ (\text{Stirring regime}) \quad (3)$$

R^2 for the model was 89.45 %.

3.7.2. Effect of factor interaction (rhamnolipid and stirring) on desalination

The highest percentage of desalination recorded in the factorial study was 25.50 %. This was produced when both rhamnolipid concentration and stirring were high (Table 2). This desalination percentage was higher than the highest desalination efficiency (24.89 %) achieved when rhamnolipid was the only factor studied (Figure 3) and 24.26 % achieved (Figure 5) when stirring was the only factor studied in the preceding experiment. The improved desalination performance of the MDC under considerable stirring regime and a high rhamnolipid concentration was attributed to the improved voltage production (Table 2) under these conditions (240 mg/l rhamnolipid and regime 3). In MDCs, desalination results from electricity production and thus improved voltage productions leads to improved percentage desalination and, in turn, promoted the desalination percentage [24].

The factorial analysis also revealed that the interactive effect of rhamnolipid and stirring did not produce a significant ($p = 0.073$, Appendix B) effect on desalination percentage. This was evident in the marginal difference (~ 1%) between the desalination percentage in the preceding experiments and that recorded in the factorial study. This insignificant effect of factor interaction on desalination was traceable to the insignificant effect of the factor combinations of rhamnolipid and stirring on voltage production. An effect on voltage production directly affects desalination because of the dependence of desalination on voltage production.

However, the ANOVA from the factorial study showed that the main effects of rhamnolipid and stirring individually were significant ($p = 0.000$, Appendix B).

The model produced from the factorial analysis for percentage desalination had a high R^2 value of 90.49 % and was defined by:

$$\%\ \text{Desalination} = 24.3917 + 0.6250\ (\text{Rhamnolipid concentration}) + 0.3583\ (\text{Stirring regime}) \quad (4)$$

Table 2. Performances of the FCMDC in the factorial study

Standard order	Run order	Rhamnolipid concentration (Coded units)	Stirring regime (Coded units)	Responses		
				% Desalination	Peak voltage (mV)	% COD
7	1	-1	1	24.00	640.50	65.47
4	2	1	1	25.50	645.71	68.15
6	3	1	-1	24.50	642.53	67.43
11	4	-1	1	24.30	638.00	65.92
9	5	-1	-1	23.50	630.92	65.00
5	6	-1	-1	23.20	633.09	64.88
8	7	1	1	25.10	643.22	67.91
3	8	-1	1	24.60	635.87	66.24
12	9	1	1	25.00	647.07	67.6
10	10	1	-1	24.80	640.97	67.89
1	11	-1	-1	23.00	634.90	65.32
2	12	1	-1	25.20	644.00	67.31

Where; -1 represents rhamnolipid concentration at 160 mg/L (Low) and 1 represents 240 mg/L (High)
-1 represents stirring regime 1 (Low) and 1 represents stirring regime 3 (High)

3.7.3. Effect of rhamnolipid and stirring on COD reduction

With the factor combination of high rhamnolipid concentration (240 mg/L) and high stirring regime (stirring regime 3), a high percentage of COD reduction of 68.15 % was achieved. This was higher than the 61.26 % produced when only rhamnolipid (240 mg/L) was the factor investigated (Figure 3) and the 50.23 % achieved when stirring was the factor investigated (Figure 5) in the preliminary experiments. An analysis of variance (Appendix C) showed that the interaction between rhamnolipid and stirring had no significant ($p = 0.221$) effect on COD reduction. This possibly resulted from the fact that COD reduction was primarily affected by anode chamber conditions, where COD removal took place. Thus, an improvement in the cathode chamber conditions might not ensure an improved efficiency in COD removal.

The predictive model derived from the factorial study for % COD removal was:

$$\% COD = 66.5933 + 1.1217 \text{ (Rhamnolipid concentration)} + 0.02883 \text{ (Stirring regime)} \quad (5)$$

R^2 value of the model was 95.63 %

4. CONCLUSIONS

The addition of rhamnolipid to the anolyte and the stirring of the catholyte of the MDC studied improved the MDC's voltage production, desalination, and COD removal efficiencies. An appropriate increase in the concentration of the rhamnolipid and an increase in the number of stirring events increased the voltage production, desalination, and COD removal efficiencies of the MDCs. The positive effect of rhamnolipid on the MDC peaked at a concentration of 240 mg/L for voltage production and desalination, whereas for COD removal, it peaked at the rhamnolipid concentration of 400 mg/L. In the experiment involving stirring, the positive effect of stirring peaked at regime 3 (three stirring events a day). It was also found that the main effects of the rhamnolipid and stirring were significant in the factorial study. However, their interaction effects were insignificant on the voltage production, desalination, and COD removal of the MDC.

5. ACKNOWLEDGEMENT

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NOMENCLATURE

MDC	Microbial Desalination Cell
MFC	Microbial Fuel Cell
FCMDC	Five Chamber Microbial Desalination Cell
BES	Bioelectrochemical System
COD	Chemical Oxygen Demand
DO	Dissolve Oxygen

APPENDICES

APPENDIX A. Analysis of variance for voltages (coded units)

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Main effects	2	258.011	258.011	129.005	33.40	0.000
2-Way interactions	1	4.037	4.037	4.037	1.05	0.337
Residual error	8	30.899	30.899	3.862		
Total	11	292.947				

Appendix B. Analysis of variance for desalination (coded units)

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Main effects	2	6.2283	6.2283	3.11417	35.93	0.000
2-Way interactions	1	0.3675	0.3675	0.36750	4.24	0.073
Residual error	8	0.6933	0.6933	0.08667		
Total	11	7.2892				

Appendix C. Analysis of variance for COD (coded units)

Source	DF	Seq. SS	Adj. SS	Adj. MS	F	P
Main effects	2	16.0953	16.0953	8.04763	86.74	0.000
2-Way interactions	1	0.1633	0.1633	0.16333	1.76	0.221
Residual error	8	0.7423	0.7423	0.09278		
Total	11	17.0009				

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