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Inspection the impact of mixing and external resistance on the Microbial Desalination Cell for electricity generation and desalination efficiency by using Macroalgae as a bio-cathode

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ABSTRACT

Microbial desalination cell (MDC) is a promising and effective desalination method for water treatment and electric power production. Three different external resistances were studied in this research (50, 100 and 150 kΩ) with two concentrations of NaCl (15 and 25 g/L) for each resistor, and the maximum voltages generated were 71, 167 and 202 mV, respectively. The maximum NaCl removal rate from the middle chamber increased from 0.164 g/L/h to 0.226 g/L/h when the external resistance was 150 kΩ for 15 and 25 g/L, respectively. The presence of mixing in the desalting chamber resulted in a maximum voltage of 256 mV with 150 kΩ, a desalting efficiency of 42 % and a removal efficiency of 25 % after 48 h of operation for the bio-cathode. For the chemical catholyte, the maximum voltages obtained were 238, 385 and 442 mV for 50, 100 and 150 kΩ, respectively; the maximum NaCl removal efficiency was 31 % and 33 % for 15 and 25 g/L with 150 kΩ, respectively. This study investigated the possibility of finding a description and equation for COD removal from wastewater in the anode chamber using the Design Experimental® program. The maximum COD removal efficiency obtained was 51 % after 24 h of operation. These findings underscore the potential of MDCs as efficient, sustainable technologies for water treatment and energy production.

1. Introduction

Power electricity is becoming vital to civilisation. The International Energy Agency (IEA) expects that by 2030, worldwide electricity consumption will be 50 % higher than it is today [1]. The huge population increase, high requirement for freshwater resources and global warming will have a negative impact on large regions of the planet, causing a rise in desert regions. According to research, human use of fresh water has risen sevenfold since 1900, with percentages doubling per twenty years [2,3]. Many nations are presently suffering from a scarcity of freshwater resources, with the countries in the Middle East being particularly affected; the quantity of pure water available for human consumption accounts for only 1 % of the world's total freshwater supply [4–6]. By contrast, conventional thermal desalination processes, which use high-pressure membranes, need high amounts of energy, and they require between 3.7 and 650 kW-hours of power per cubic meters of desalinated water [7–9]. Microbial desalination cells (MDCs) are potential bioelectric cells that can generate electricity from wastewater, remove NaCl from brackish water without harming the environment and treat wastewater by lowering COD levels. The biochemical system (BES) requires limitations, expensive stimuli (e.g. platinum) and harmful and toxic chemical oxidants (e.g. permanganate and ferricyanide) in cathode cells. The best alternative to eliminating such restrictions in this system is by incorporating bio-cathodes [10]. Bacteria operate as biological catalysts in the anode chamber, converting organic molecules to create electrons. These electrons then move from the anode chamber to the cathode chamber through an external circuit with external resistance [11–13]. Other options for receiving electrons from the positive electrode were discovered in the cathode chamber, such as oxygen, algae, bacteria or air. In the middle chamber (desalination chamber), the ions in saline water (such as Cl^- and Na^+) move to the anode chamber and cathode chamber through the ionic exchange membrane (IEM) due to the voltage difference between the anode and cathode chambers. The researchers discovered the potential of using bio-cathodes, such as

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algae, instead of harmful materials in the cathode chamber for the creation of electron receptors [14–16]. Microorganisms are employed as a substitute for harmful chemical catalysts in bio-electrochemical systems based on a bio-cathode, which can decrease the cost of operation and construction, preserve the surrounding environment and eliminate pollutants in a safe way [17–19]. The reaction equation in the anolyte solution can be described as follows [20]:

$$\text{Microorganisms} + \text{anaerobic condition} + \text{organics materials} \rightarrow 4ne^{-} + nCO_2 + 4\ nH^{+} \quad (1)$$

The reaction equation in the solution of catholyte can be described as follows [21]:

$$O_2 + 4\ nH^{+} + 4ne^{-} \rightarrow 2H_2O \quad (2)$$

or the reaction equation in the solution of catholyte can be described as [22].

$$1e^{-} + [Fe\ (CN)_6]^{-3} \rightarrow [Fe(CN)_6]^{-4} \quad (3)$$

The first bio-cathode study for nitrogen extraction in a cathode chamber was conducted in an MFC, in which nitrate was cycled from anode-treated wastewater via a bio-cathode chamber [23]. Cao et al. recently transformed MFCs to MDCs to achieve 90 % of NaCl removal. Wen et al. [23] studied the idea of infusing air into the MDC system, as they offered external ventilation to the cathode chamber to maintain an active environment. For the treatment of residential sewage, Choi and Ahn [20] examined five distinct kinds of stacking contacts: parallel within parallel flow, series within parallel flow, series within series flow and single in series flow [24]. In the study of Fanyu et al., they simultaneously evaluated the performance of bio-cathode (algae) in treating sewage and saline water and electrical energy yield [25]. The way that photosynthesis MDCs (PMDCs) function is analogous to a biological procedure that occurs in a sea water ecosystem. For instance, every day, macroalgae absorb CO_2 from the atmosphere to create oxygen and organic matter, which serve as a source of material and oxygen supply for bottom-dwelling heterotrophic bacteria, which can produce carbon dioxide [26–28]. Macroalgae cells release oxygen, which acts like a final electron acceptor in PMDCs, performing the same function [29]. Algae assist in reducing the amount of carbon dioxide that remains in water bodies after treatment inside a closed system. They also assist in the extraction of nutrients from sewage during photosynthetic processes and oxygen production, in which O_2 serves as an acceptor to generate electric power [30–32]. The algae are additionally utilised in a bio-cathode, which is a device that aids the cathode compartment of the MDC generate oxygen through photosynthesis. It is referred to as a PMDC while in use. Its numerous benefits, such as quick growth, growth in aqueous media, carbon dioxide, solar energy conversion rates, capacity to absorb undesirable components, production of oxygen gas and lack of need for costly catalysts, make it a popular option [33]. Additional benefits of algae include their ability to break down contaminants into biological elements like starch, carbohydrates, lipids and proteins. This feature allows algae to supply treatment that is less harmful and more friendly to the natural world compared with other materials. Additionally, algae contain chlorophyll organelles, which assist in absorbing sunlight for photosynthesis and separate carbon from the gas and liquid states [34]. Macroalgae's rapid growth negatively impacts the ecosystem and recreational qualities of inhabited coastal regions worldwide [35]. The bioremediation of industrial wastewaters using microalgae *Chlorella vulgaris* was studied by Madadi et al. (2016). According to the study's findings, a surfactant–*C. vulgaris* combination is a preferred method of wastewater treatment and may be recommended for the removal of nutrients from petrochemical effluent. The study conducted by El-Kassas and Mohamed (2014) examined the development of microalgae biomass on textile waste effluent as a possible solution to mitigate the environmental effects of effluent discharge into water sources. The possibility of using *C. vulgaris* to remediate textile waste water effluent was examined by adopting a central composite design (CCD). This study analysed how the microalga *C. vulgaris* adapts to textile waste effluent and calculated the ideal dilution percentage for the waste effluent to produce the most biomass and to eliminate colour and COD. The findings showed that the largest removal of colour and COD from textile waste effluent was 17.5 %. According to Zhao et al. (2019) and Shi et al. (2020), algae and electroactive bacteria have a symbiotic connection in which the former supply oxygen and organic substrates, while the latter aid in electron transfer mechanisms that are essential for MDC operation. Improving comprehension on this interaction is crucial to raise algae's total productivity in MDCs.

Box–Behnken designs (BBD) need fewer goals than the traditional experimental approach to generate a high level of development. This approach and the full factorial method can effectively eliminate certain runs to maintain an advanced interface description. The BBD employs 12 centre edge nodes and three main nodes to accommodate a second-order equation [36]. With the addition of three extra elements in the centre, the central composite plus BBD becomes a full factorial design [37]. BBDs additionally include marks at the centre points of the borders of the simple cubic design area, in addition to the centre [38].

Understanding the efficiency of algae, which depend on a number of elements like the presence of light, the presence of carbon dioxide, the creation of a growth medium and other suitable environmental conditions, is essential before using algae inside the cathode compartment [16]. The amount and type of light have a remarkable impact on photosynthetic processes, with clear light promoting optimal development. Long-term exposure to bright light has a detrimental impact on the quantity of electricity generated, which in turn impacts how quickly algae grow inside the systems [18,22,39]. This study aimed to determine the impact of the organic matter content in combined sewage water on the effectiveness of PMDCs, as well as analyse the role of microalgae in the bio-cathode of these cells. The formation of biofilms and evaluation of contamination are two major topics covered in detail in this paper.

This work is novel in that it goes beyond previous research on MDCs in several ways. MDCs are presented in the paper as a sustainable and energy-efficient substitute for traditional high-energy desalination methods, addressing the urgent worldwide challenges of rising power use and freshwater needs. Eco-friendly and economical MDCs are a step closer with the use of bio-cathodes instead of hazardous chemical catalysts, which can lower operating costs, preserve environmental safety and remove pollutants in a sustainable manner. Combining chemical catholyte, mixing and high resistance can result in a comprehensive performance boost that is far better than traditional MDC configurations. Furthermore, by investigating the effects of light, carbon dioxide and environmental factors on photosynthetic processes, the study investigated the efficiency of algae in bio-cathodes and helped optimise their performance in practical applications. These discoveries improve our knowledge of and ability to use MDCs in wastewater treatment, power generation and water desalination, making them applicable and useful in tackling today's pressing global issues. The primary goal of this project was to use MDCs to develop a sustainable and effective approach for treating wastewater, producing electricity and desalinating water all at the same time. To minimise operating expenses and their negative effects on the environment, optimised the system to increase voltage generation, enhanced the removal rates of NaCl and chemical oxygen demand (COD) and integrated bio-cathodes to substitute hazardous chemical catalysts. The study aimed to satisfy the growing worldwide need for fresh water and renewable energy sources while offering a workable, low-energy substitute for traditional, high-energy desalination techniques and creating a sustainable and effective MDC system that can effectively treat wastewater, produce electricity and desalinate water at the same time. This system will also address the challenges of optimising voltage generation, enhancing the removal rates of COD and NaCl and lowering operational costs and environmental impact by utilising bio-cathodes.

2. Materials and methods

2.1. Microorganisms and electrolyte

In all MDC experiments, 45 mL of adjusted anaerobic sludge from the anaerobic sludge of the first gas power plant in southern Baghdad, Iraq was added. In the anode chamber, a synthetic wastewater solution with 2.778 g/L sodium acetate was used. The types of bacteria available in the activated sludge were studied. Many types of bacteria were found, but the dominant bacteria in the activated sludge were *Pseudomonas fluorescens*. In the bio-cathode chamber, macroalgae were obtained from the Tigris River; the algae in the cell were as follows: 60 % *Chlamydomonas*, 30 % *Cladophora glomerata* and 10 % *Lyngbya limnetica*. The electrodes used in the anode and cathode chambers were pure graphite plate (8 × 0.5 × 5 cm).

2.2. SEM and EDS analysis

X-ray spectroscopy was combined with scanning electron microscopy (SEM). The surface form and topographical features were investigated by SEM. The resulting 3D photos precisely portrayed the surface contour. Energy-dispersive X-ray spectroscopy (EDS) was used to analyse the elements that make up the precursors. EDS-SEM analysis was conducted using Vega III 9TESCAN, Czech Republic.

2.3. Medium preparation and culturing of algae

For the chemical cathode, a catholyte solution was prepared in the experiments that relied on ferricyanide solution as an electron acceptor with a concentration of 16.5 g/L (ferricyanide solution is considered one of the ideal chemical solutions for receiving electrons). The initial pH of the solution was pH 6.5, and the initial concentration of the salts was 4400 mg/L. Solution temperatures were kept within 25 °C. Ferricyanide solution was prepared from Bab al-Moadham markets in Baghdad, Iraq.

For the bio-cathode chamber, macroalgae were utilised as a catalyst in the cathode chamber to produce oxygen in the PMDC system. In November, a sample was obtained from the Tigris River's sources in Baghdad. It was washed with distilled water from the sand and mud around the algae. The algae were maintained at room temperature in distilled water with BG11 nutrient medium added (BG11 contained the following: $NaNO_3$ (1.5 g/L), K_2HPO_4 (0.04 g/L), $MgSO_4 \cdot 7H_2O$ (0.075 g/L), $CaCl_2 \cdot 2H_2O$ (0.036 g/L), $(NH_4)_5[Fe(C_6H_4O_7)_2]$ (0.006 g/L), $C_6H_8O_7$ (0.006 g/L), $C_{10}H_{16}N_2O_8$ (0.001 g/L) and Na_2CO_3 (0.02 g/L) with trace minerals (1 mL with pH adjusted to 7) with a 28-Watt white light.

2.4. MDC operation

The anode chamber was filled with sodium acetate at a concentration of 2.778 g/L (1250 mg/L COD concentration) with the addition of 40 mL of sludge, which increased the COD concentration to 1350 mg/L, whereas the cathode chamber was filled with macroalgae. In the middle chamber, two concentrations of salt water (15 and 25 g/L) were employed to investigate the impact of the system's desalination rate. pH had no significant effect on the system, as the pH of the anode chamber was 8.61, which declined to 7.01 after 48 h, but it did not reach acidic levels. The pH may have affected bacterial growth in the anode chamber but not in the cathode chamber. The use of a buffer may have yielded more favourable results, but the pH rose from 6.61 to 7.05 after 48 h of operation. All studies were conducted under standard pressure and temperature (25 °C and 1 atm), and each experiment was repeated twice to obtain consistent findings. This paper included a comparative analysis of the production of electric power, water desalination and the removal of organic matter from wastewater by using bacteria extracted from the wastewater of oil stations. An anaerobic environment was created for the production of electric energy when grown on organic matter using the batch system. The efficiency of the system was compared when using chemical cathodes (ferricyanide) and biological cathodes (macroalgae).

2.5. Analyses and calculations

The voltage and current across 150 kΩ external resistance were recorded every 180 min by using a digital multi-meter (Fluke, 287/FVF). The power density was calculated by (p=(V*I)/(volume of anode/cathode electrode)). The Coulombic efficiency (CE; which is the proportion of entire electrons delivered from the anode to total potential electrons produced by organic component reduction) was calculated using Eq. (3). COD tests were carried out using COD Set-Up 200 LOVIBOND. TDS removal, electrical conductivity and salinity removal were verified by using a conductivity meter (Extech EC400 ExStik Waterproof Conductivity, Temperature, Salinity and TDS Meter). The pH was measured by pH meter (ISOLAB density & sedimentation & temperature & time & pH & weight measurement meter). The efficiency of charge transfer was evaluated using Eq. (4) [11,40].

$$CE = \frac{\Sigma[I(A) \times t(s)]}{96485\left(\frac{C}{mole(e^-)}\right) \times COD_{removal}(mole) \times 4\left(\frac{mole(e^-)}{mole(O_2)}\right)} \quad (4)$$

Where I is the electric current (A), and t is time (s). COD removed refers to the amount of COD removed within time t.

$$\eta = \frac{Q_{th}}{Q} = \frac{F \times (C_{in} - C_{out}) \times V_D}{\Sigma[I(A) \times t(s)]} \quad (5)$$

Where F is the Faraday constant, Q is the total gathered Coulomb, Q_{th} is the theoretical transfer charge, C_{in} is the beginning molar concentration of salt solution; C_f is the ending molar concentration of salt solution; V_D is volume of desalination; I is the electric current; and t is time.

2.6. MDC operation

Three cubic chamber MDCs (10 × 7 × 10 cm) were made using plexiglass. To isolate between chambers, anion exchange (6 × 6 cm, AMI-7000, Membranes international) and cation exchange membranes (6 × 6 cm, CMI-7001, Membranes international) were used. Before use, the membranes were immersed in 5 % of NaCl solution for 24 h and rinsed with distilled water. This process facilitated hydration and expansion. A graphite plate (8 × 0.5 × 5 cm) was used as cathode and anode electrodes. The working volume of the anode chamber was 350 mL, that of the desalination chamber was 250 mL and that of the cathode chamber was 350 mL.

3. Results and discussion

3.1. Electricity performance for MDC

To determine the optimum external resistance for the MDC, several values of external resistance were evaluated to identify the one that would result in the maximum power generation (Fig. 5). The maximum external resistance that would yield high power generation was 150 kΩ.

Chemical catholyte experiments were conducted using an outer closed loop and an external load of 50, 100 and 150 kΩ under two different NaCl concentrations inside the desalination compartment (15 and 25 g/L). Two techniques were employed: one involved thorough mixing inside the desalination compartment, and the other did not use any mixing process. All experiments ran for 24 h with 1350 mg/L COD for simulated wastewater in the anode compartment. For the bio-cathode, experiments were conducted using an outer closed loop with an external load of 50, 100 and 150 kΩ under two different NaCl concentrations inside the desalination compartment (15 and 25 g/L). Two techniques were also employed: one involved thorough mixing inside the desalination compartment, and the other did not use any mixing process in the desalination compartment. All experiments ran for 24 h

with 1350 mg/L COD for simulated wastewater in the anode compartment. During the initial few minutes of operation, the electric energy generated by the chemical solution cathode increased rapidly until it reached its peak, followed by a brief period of stability and growth and then gradually declined for the remainder of the operating duration. As shown in Fig. 1, a distinct increase was noted during the first 6 h of operation, followed by a decline after 20 h. Fig. 1 shows that the voltage difference between the two electrodes (cathode and anode) increased with increasing external resistance. The performance of voltage generation when the bio-cathode was used is shown in Fig. 2. The effect of light on voltage generation in macroalgae is described in section 3.3. In the day, oxygen is used as an electron acceptor. In the dark, CO_2 is used as an electron acceptor. The maximum power obtained when utilising the chemical cathode (Fig. 3) was 3209.66 and 4512 mW/m^3 under the resistance of 50 kΩ. The initial concentrations of total dissolved solids in the desalination chamber were 15 and 25 g/L, respectively. Under 100 kΩ and 15 and 25 g/L initial TDS in the middle compartment, the highest power density was 4169.25 and 4235 mW/m^3, respectively. Under 150 kΩ and 15 and 25 g/L initial TDS in the middle compartment, the highest power density was 3654.171 and 3721.22 mW/m^3, respectively. The maximum power densities when the bio-cathode was used (Fig. 4) were 7 and 10 mW/m^3 under 50 kΩ. When the initial TDS concentrations in the desalination chamber were 15 and 25 g/L respectively, under 100 kΩ and 15 and 25 g/L initial TDS in the middle compartment, the highest power densities were 10.1 and 100.5 mW/m^3 respectively. When using 150 kΩ and 15 and 25 g/L initial TDS in the middle compartment, the highest power densities were 10.9 and 100.9 mW/m^3, respectively. The present study achieved a maximum voltage of 202 mV, which surpassed the 136 mV reported by Wen et al. (2012) for the same MDC equipped with an aerobic biocathode. This difference in voltage could be attributed to the use of macroalgae in our study, whereas Wen et al. (2012) used bacteria as a catalyst. Additionally, the current study's external resistance was higher than that described by Wen (2012).

3.2. COD removal

Throughout all studies, the overall chemical catholyte solution content was fixed at 1350 ppm of simulated sewage in each of the three resistance values (50, 100 and 150 kΩ). The highest percentage of COD concentration removal in anode chamber was 51 %. As anticipated, this condition resulted from a massive energy loss due to a large external load, as indicated by the extremely low CE, which was less than 2 %. Table 1 displays the percent of elimination in studies with varying resistance values. Table 1 shows the level of removal efficiency in the unit, influence of starting TDS values inside the middle compartment and influence of outer electrical resistance on the percentage of COD removal. Table 1 show that the percentage of COD removal was influenced by the initial concentration of salts. Specifically, an increase in salt concentration led to the increase in the amount of energy generated by bacteria in the anode chamber. Some of the electrons may be diverted to other processes instead of being used to eliminate salts (cathode reactions) due to factors including external resistance and the electrolysis process. Therefore, as the initial salt concentration in the desalination compartment grew, chemical oxygen was removed to enhance the desalination process by increasing the number of electrons. The external resistance had an immediate influence on the COD removal procedure. The external resistance with the highest magnitude resulted in the greatest amount of COD removal. This was observed at 150 kΩ, because of the production of the highest amount of energy, as shown in Table 1. The results of COD removal from wastewater from the use of macroalgae in the cathode chamber gave low removal values, which may be due to the lower voltages generated compared with the use of chemical cathodes (Table 1).

The current study dealt focused on deriving an equation for

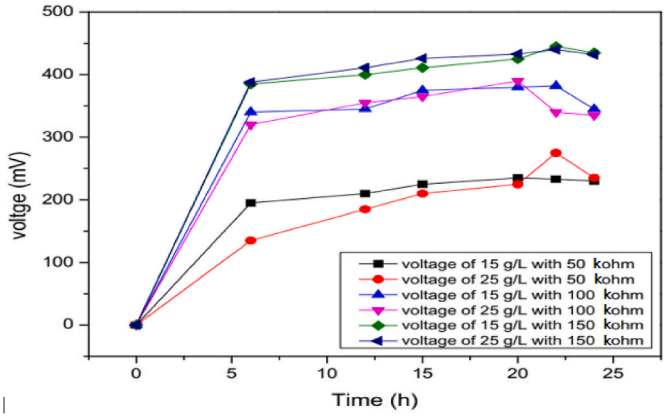

Fig. 1. The voltage generation in the MDC with chemical cathode
*A, Power density vs. time when 50 kΩ and 15, 25 g/L TDS concentration for chemical cathode.
*B, Power density vs. time when 100 kΩ and 15, 25 g/L TDS concentration for chemical cathode.
*C, Power density vs. time when 150 kΩ and 15, 25 g/L TDS concentration for chemical cathode.

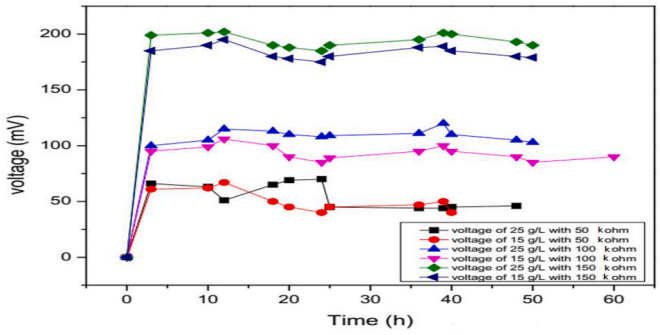

Fig. 2. The voltage generation of MDC with bio-cathode
*A, Power density vs. time when 50 kΩ and 15, 25 g/L TDS concentration for bio-cathode.
*B, Power density vs. time when 100 kΩ and 15, 25 g/L TDS concentration for bio-cathode.
*C, Power density vs. time when 150 kΩ and 15, 25 g/L TDS concentration for bio-cathode.

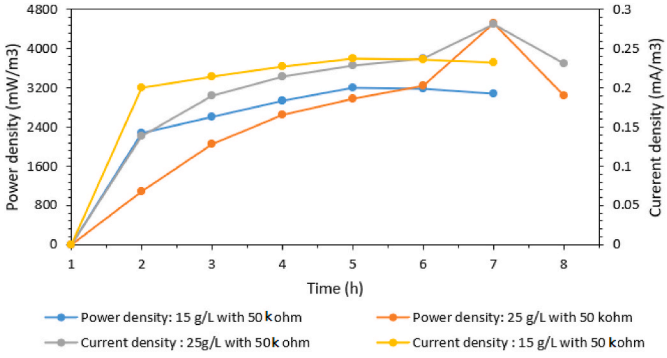

Fig. 3. The power density generation in (MDC) with different external resistance
*A, Power density vs. time when 50 kΩ and 15, 25 g/L TDS concentration for chemical cathode.
*B, Power density vs. time when 100 kΩ and 15, 25 g/L TDS concentration for chemical cathode.
*C, Power density vs. time when 150 kΩ and 15, 25 g/L TDS concentration for chemical cathode.

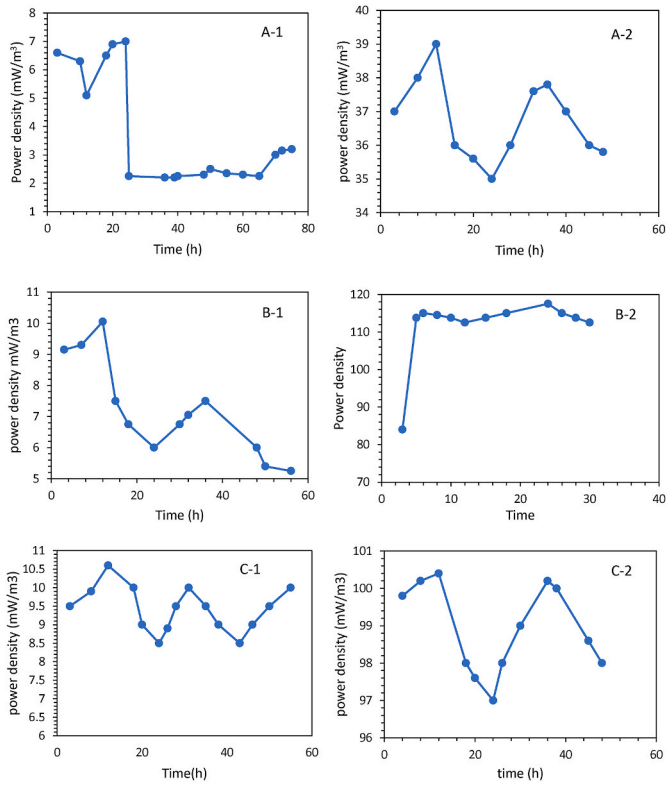

Fig. 4. Effect of day and dark on the power density in the PMDC
*A, Power density vs. time when 50 kΩ and 15 (A-1), 25 (A-2) g/L TDS concentration for bio-cathode.
*B, Power density vs. time when 100 kΩ and 15 (B-1), 25 (B-2) g/L TDS concentration for bio-cathode. *C, Power density vs. time when 150 kΩ and 15 (C-1), 25 (C-2) g/L TDS concentration for bio-cathode.

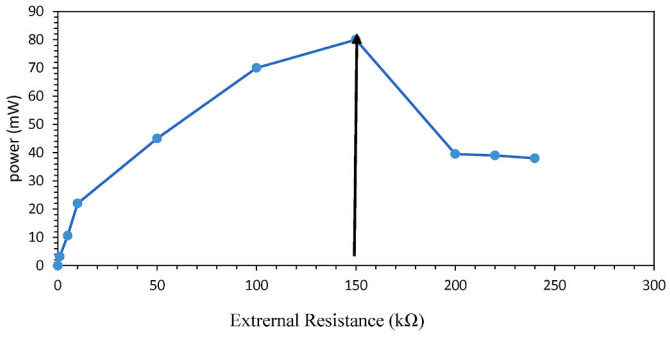

Fig. 5. The maximum power achieved with mixing effect at 150 kΩ.

removing COD from wastewater by using microorganisms in anode chambers. BBD was utilised to determine the influence of duration, initial COD concentration and initial TDS concentration levels on the percent of COD removal inside the anode compartment of MDC. Table 2 displays the information on the BBD. This study's data were validated using ANOVA type III (Table 4) with incomplete sums of squares. At $p < 0.05$, the process variables were shown to be statistically significant. Table 2 displays the distributions of studies for three independent variables together with the findings. All parts were present, and all relevant factor interactions were highlighted. Table 4 shows the ANOVA results for the quadratic model for desalination response. ANOVA revealed that P-values less than 0.05 indicated that model terms were significant. In this case, A, C and A^2 were significant model terms. P-values greater than 0.05 that indicated the model terms were not significant. The model was significant so that the quadratic model fit the desalination response. The fit statistics indicated that R^2 was 0.9894. The predicted R^2 of 0.9704 was in reasonable agreement with the adjusted R^2 of 0.8307; the difference was less than 0.2. Fig. 6 shows the relationship between the actual and predicted experiments of the COD removal process (see Table 3).

Final Equation in Terms of Actual Factors:

$$\textbf{\textit{COD removal}} = 6.5 - 0.04C - 0.03T + 0.8t + 0.0004CT + 0.0002CT - 0.008Tt + 3.9\,e-05C^2 - 0.001T^2 - 0.005t^2 \quad (6)$$

C: COD concentration (mg/L); T: TDS concentration (mg/L); t: Time (h).

Fig. 7 shows that the effect of COD removal increased when the initial concentration of COD in the anode compartment increased, and the amount of COD removal increased when the initial concentration of NaCl salts increased in the desalination compartment of the MDC. These results proved that high initial concentrations resulted in an increase in the overall resistance of the system and a greater potential difference. This led to an increase in the effectiveness of bacteria to oxidise organic materials in the anode chamber and enhanced removal of COD. Fig. 8 shows that the effect of COD removal increased when the initial concentration of COD in the anode chamber increased, and the amount of COD removal increased when the working time of the system was extended. When the initial concentrations of COD increased with the running time of the system, the removal of the COD concentration increased. Fig. 9 shows that the effect of COD removal increased when the working time of the system increased, and the amount of COD removal increased when the initial concentration of NaCl salts in the desalination compartment of the MDC increased. Thus, increasing the initial concentrations, the overall resistance of the system as heightened, resulting in a larger voltage difference. This led to an increase in the effectiveness of microorganisms in oxidising organic materials in the anode chamber and an increase in COD removal. In addition, the increase in the amount of operational time for the system gave excellent results and higher efficiency compared with the results at short times or hours. In a study by Khazraee et al., the starting concentration of COD in the anode chamber was 1000 mg/L, and the rate of TDS removal was utilised as a bio-cathode in the cathode chamber. The highest amount of salt removed per day was 0.341 g/L (14.2 mg/L·h). Although the current study's TDS removal rate was high (226 mg/L·h), this result might be explained by the type of algae, the MDC setup, or the high external resistance employed [41].

3.3. *Effect of illumination*

The impact of illumination on the power density generation in the PMDC for the initial COD concentration of 1350 mg/L over 3 days is illustrated in Fig. 4. During the light and dark cycle studies with an external resistance of 50 kΩ, the maximum power density obtained was 7 mW/m^3 after 24 h of operation. Three stages were detected, namely, slow rise, stable stage and sharp decline. The first stage was due to the existence of new dissolved oxygen caused by algae in the cathode chamber. During the stationary period, the electrical productivity lasted for about 20 h, suggesting the formation of biofilms on the anode membrane. When using macroalgae, a biofilm layer is not expected to form on the cathode membranes, which may indicate an increase in the transfer of Na^+ electrons from the desalination chamber to the cathode chamber. The decrease in power density was due to the depletion of oxygen in the algae due to the reduction in the inorganic carbon source in the cathode chamber and the decrease in the organic substrate by bacteria in the anode chamber, leading to an increase in pH in both chambers. The sinusoidal function represented the voltage generation of PMDC with 12/12 h day/night cycles. Fig. 4 illustrates that the power density decreased slightly during the dark period because of the insufficient light for photosynthesis, leading to a decrease in oxygen

Table 1
The COD removal in the anode chamber for the chemical cathode.

MDC Type	The experiments sign	TDS concentration (g/L)	Period of the cycle (h)	The external resistance	COD in the anode chamber		COD removal (%)
					In.	Out.	
Chemical	A	15	24	50 kΩ	1350 ± 100	928	31
		25	24	50 kΩ	1350 ± 100	780	42
	B	15	24	100 kΩ	1350 ± 100	875	35
		25	24	100 kΩ	1350 ± 100	715	47
	C	15	24	150 kΩ	1350 ± 100	828	38
		25	24	150 kΩ	1350 ± 100	650	51
Algae	A	15	48	50 kΩ	1350 ± 100	1279	5.2
		25	48	50 kΩ	1350 ± 100	1100	18
	B	15	48	100 kΩ	1350 ± 100	1105	18.1
		25	48	100 kΩ	1350 ± 100	1065	21
	C	15	48	150 kΩ	1350 ± 100	1061	21.4
		25	48	150 kΩ	1350 ± 100	1010	25

Table 2
The information of BBD for COD removal.

Build Information:			
File Version	12.0.3.0		
Study Type	Response Surface	Subtype	Randomized
Design Type	Box-Behnken	Runs	15
Design Model	Quadratic	Blocks	No Blocks
Build time (mS)	1.0000		
Factors:			

Factor	Name	Units	Type	Minimum	Maximum
A	COD concentration	mg/L	Numeric	300.00	1200.00
B	TDS concentration	g/L	Numeric	15.00	35.00
C	Time	h	Numeric	4.00	24.00

Table 3
The distribution of experiments using three parameters with outcomes of the tests.

		Factor 1	Factor 2	Factor 3	Response 1
Std	Run	A:COD concentration	B:TDS concentration	C:Time	COD removal
		mg/L	g/L	h	%
6	1	1200	25	4	25
13	2	750	25	14	10.9
10	3	750	35	4	4
1	4	300	15	14	5.5
12	5	750	35	24	15
15	6	750	25	14	10.8
7	7	300	25	24	10
8	8	1200	25	24	36
3	9	300	35	14	6
2	10	1200	15	14	28
5	11	300	25	4	2
9	12	750	15	4	3.8
14	13	750	25	14	10.88
11	14	750	15	24	18
4	15	1200	35	14	35

production by algae. By contrast, the power density increased when light was restored during daytime due to the restoration of the activity of algae and photosynthesis. The initial COD concentration was 1350 mg/L and external resistance was 100 kΩ for duration of 48 h. Despite the decrease in power density during night, it did not reach zero because algae produce carbon dioxide instead of oxygen at night as a result of non-photosynthetic respiration. Carbon dioxide is considered a photoreceptor, but with much less efficiency than oxygen. Fig. 4 clearly explains the impact of illumination on power density generation in PMDC with 150 kΩ. In the cycle, the highest power density obtained was 100.9 mW/m^3, which was 2.3 times higher than the maximum power density achieved in the previous experiment. The maximum salt removal achieved in this test with an initial concentration of 25 g/L NaCl in the desalination chamber reached 45 % after 48 h, with a rate of 225 mg/L h^{-1}. The intensity of light may significantly affect algae, influencing their growth, reproduction and function of their chloroplasts. High light intensity can hinder algal cell division and disrupt the secretion of enzymes involved in carbon dioxide fixation.

3.4. Effect of TDS concentration

The TDS concentration in the desalination chamber influenced voltage generation and salt removal by algae in the cathode chamber in the PMDC. The two concentrations in the study were 25 g/L, which referred to the concentration found in seawater, and 15 g/L, which referred to the concentration found in brackish water. These concentrations were tested with three different external resistances (50, 100 and 150 kΩ) for about 48 h of operation, and the initial COD concentration was fixed at 1350 ppm in the anode chamber. Fig. 2 shows the voltage generation by algae with 25 g/L in the desalination chamber with external resistance of 50 kΩ. The maximum voltage achieved was 71 mV, and the salt removal was 32 %, with TDS removal rate of 0.164 g/L h^{-1}. Fig. 2 show clearly the voltage generation with initial 15 g/L TDS with 50 kΩ external resistance the highest voltage achieved was 67 V and the percentage TDS removal was 15 % afterward 48 h operating time with 0.051 g/L h^{-1} rate of removal; Fig. 2 the beginning TDS concentration was 25 g/L with 100 kΩ external resistance the highest voltage generation was 120 mV after 24 h of operating and the salts removal was 33 % with 0.167 g/L h^{-1} rate of removal; Fig. 2 the initial TDS concentration was 15 g/L with 100 kΩ external resistance the maximum voltage reached was 106 mV after 12 h operating time and the percentage of removal was 16 % with 0.054 g/L h^{-1} rate of TDS removal. When the initial TDS concentrations were 25 and 15 g/L with external resistance of 150 kΩ, the maximum voltages achieved were 202 and 195 mV, respectively, and the percentages of TDS removal were 43 % and 23 %, respectively, with TDS removal rates of 0.226 and 0.0708 g/L h^{-1}, respectively. In previous experiments, a rise in the voltage in the system was observed during operation, which was attributed to an increase in the internal resistance of the system, thereby affecting the overall electrical circuit of the system and leading to an increase in the electrical voltage in the system. The increase in the NaCl concentration in the middle chamber led to an increase in TDS removal from the chamber due to Fick's law (the movement of molecules from a high concentration area to a low concentration area, driven by the concentration gradient that promotes diffusion). During the experiments, increasing the amount of external resistance in the system had a positive effect. It led to an increase in the desalination rate in the system and an increase in the voltage generated in the PMDC (Fig. 2). The voltage generation was compared using different external resistances (50, 100 and 150 kΩ) with a concentration of 25 g/L NaCl in the desalination chamber. The high voltage resulted in a significant increase in

Table 4
ANOVA with quadratic design reaction on Chemical Oxygen Demand Removal effectiveness.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1762.13	9	195.79	51.93	0.0002	significant
A-COD concentration	1262.53	1	1262.53	334.83	< 0.0001	
B-TDS concentration	2.76	1	2.76	0.7323	0.4312	
C-Time	244.20	1	244.20	64.77	0.0005	
AB	10.56	1	10.56	2.80	0.1550	
AC	2.25	1	2.25	0.5967	0.4747	
BC	2.56	1	2.56	0.6789	0.4475	
A^2	230.87	1	230.87	61.23	0.0005	
B^2	0.0750	1	0.0750	0.0199	0.8934	
C^2	0.9888	1	0.9888	0.2622	0.6304	
Residual	18.85	5	3.77			
Pure Error	0.0056	2	0.0028			
Cor Total	1780.98	14				

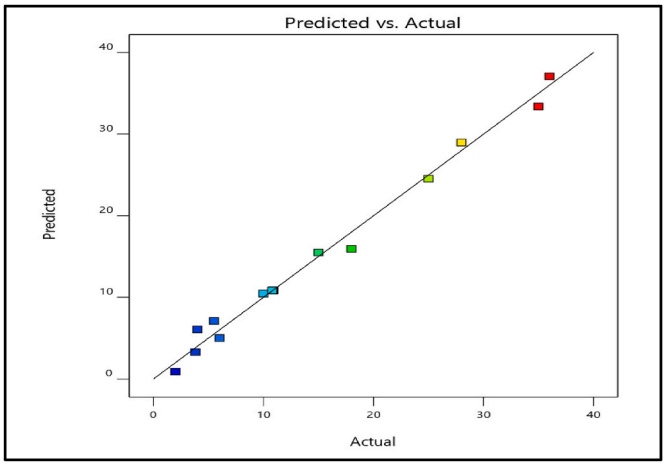

Fig. 6. The predicted vs. actual experimental of COD efficiency.

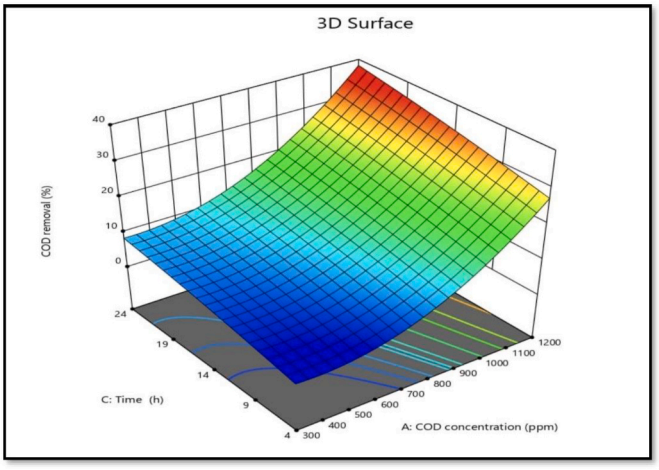

Fig. 8. Effect of initial concentration of COD and Time on COD removal efficiency.

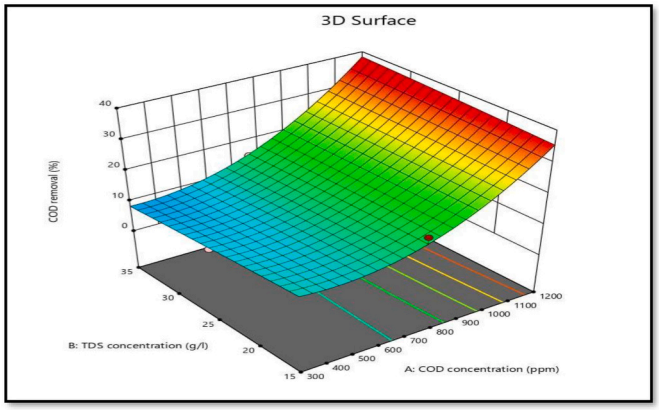

Fig. 7. The effect of initial concentration of TDS and initial COD concentration on the COD removal efficiency.

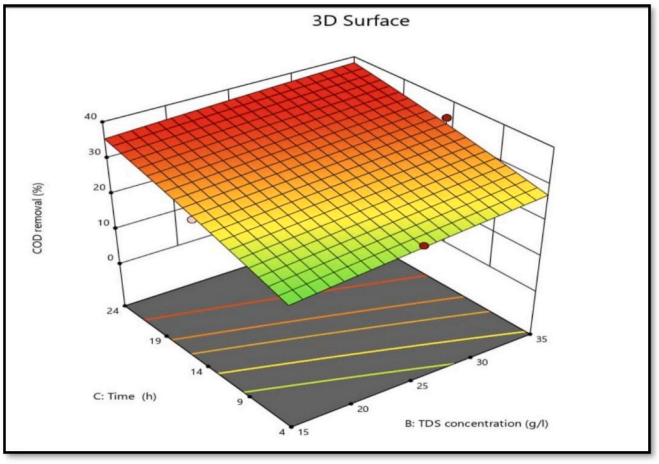

Fig. 9. Effect of Time and initial concentration of TDS on the COD removal efficiency.

desalination, which could be attributed to the stimulation of ion movement from the desalination chamber to the anode and cathode chambers. The disparity in high salt concentrations between the chambers of the system enhanced the movement of ions between the positive electrode in the anode compartment and the negative electrode in the cathode compartment, leading to a rise in water desalination efficiency [18–20]. Table 5 summarises the effect of resistance and TDS concentration on the desalination percentage in the PMDC.

In addition to the chemical catholyte used in MDC, the two-concentration study referred to a concentration of 25 g/L found in seawater and a concentration of 15 g/L found in brackish water. These concentrations were tested with three different external resistances, namely, 50, 100 and 150 kΩ, for about 24 h. The initial COD concentration was fixed at 1350 ppm in the anode chamber. In the desalination chamber, when the concentration of salt was 25 g/L and an external resistance of 50 kΩ was applied, the removal of salt was 22.8 % with a

Table 5
Effect of resistance and NaCl concentration on desalination percentage.

Types of MDC	No.	Anode chamber COD (ppm) pH	Desalination chamber NaCl (g/l)	Time of operation (h)	External resistance (kΩ)	NaCl removal (%)
PMDC	1	1350 8.16	25	48	50	32
	2	1350 8.16	25	48	100	33
	3	1350 8.16	25	48	150	43
	4	1350 8.16	15	48	50	15
	5	1350 8.16	15	48	100	16
	6	1350 8.16	15	48	150	23
Chemical of MDC	1	1350 8.16	25	24	50	6
	2	1350 8.16	25	24	100	22.8
	3	1350 8.16	25	24	150	27
	4	1350 8.16	15	24	50	28
	5	1350 8.16	15	24	100	30
	6	1350 8.16	15	24	150	32

TDS removal rate of 0.24 g/L h^{-1}. When the initial concentration of TDS was 15 g/L and the same external resistance was used, the maximum percentage of TDS removal was 6 % after 24 h of operation, with a rate of removal of 0.045 g/L h^{-1}. When the initial TDS concentration was 25 g/L and an external resistance of 100 kΩ was applied, the removal of salts was 28 % after 24 h of operation, with a rate of 0.28 g/L h^{-1}. When the initial concentration of TDS was 15 g/L with an external resistance of 100 kΩ, the percentage of TDS removal was 27 % after 12 h of operation, with a rate of 0.19 g/L h^{-1}. The initial TDS concentrations of 25 and 15 g/L with external resistance of 150 kΩ led to percentages of TDS removal of 33 % and 31 %, respectively, and the rates of TDS removal were 0.34 and 0.29 g/L h^{-1}, respectively. All results of TDS removal are summarised in Fig. 10. Table 6 shows the comparison of the results of this study with the results of previous studies.

By comparing the percentage of COD concentration elimination in the current study with the study conducted by Bahrareh (2013), it was found that using bio-cathode MDC led to the greatest removal of COD (65.6 %). Other MDC setups or varying external resistance levels may contribute to more COD removal beyond what was observed in the current experiment [31].

3.5. Effect of mixing

The mixing study was performed to improve the desalination rate in the middle chamber. The effect of mixing in the two experiments with a fixed external resistance of 150 kΩ was examined. The external resistance was fixed at 150 kΩ, because of the high power achieved in this value (Figs. 2 and 4). The external resistance was applied to two TDS concentrations (15 and 25 g/L), and the initial COD concentration was fixed at 1350 ppm in the anode chamber in PMDC. The mixing speed and temperature in the desalination chamber were fixed at 250 rpm and 25 °C, respectively. After 48 h of operation with 150 kΩ and 25 g/L, the maximum TDS concentration removal from the middle chamber was 45 %. The rate of removal reached 0.23 g/L h^{-1}. Before mixing, the rate of removal was 0.226 g/L h^{-1} with 43 % of TDS removal. Fig. 11 clearly illustrates the difference in TDS concentration removal between the experiments with and without mixing. In the second experiment with 150 kΩ and 15 g/L, the highest percentage of TDS concentration removal from the middle compartment was 42 %. The TDS concentration removal rate increased and reached 0.13 g/L h^{-1} in the experiment after mixing in the desalination chamber, resulting in 23 % of TDS concentration removal.

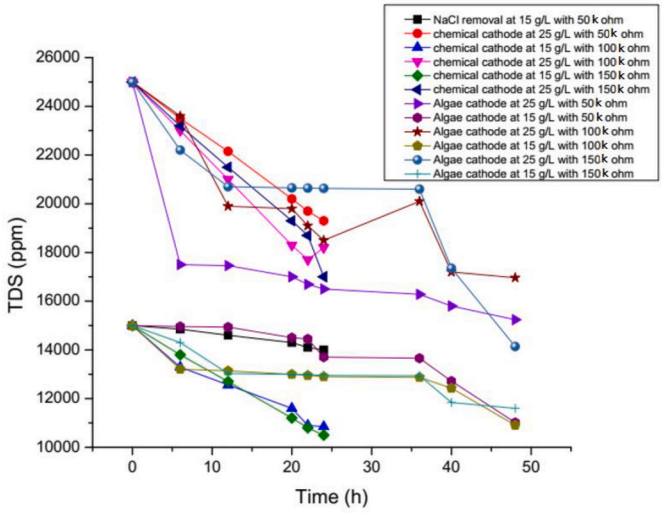

Fig. 10. The TDS concentration removal between the experiments with and without mixing for 150 kΩ as external resistance.

The mixing study was conducted to improve the desalination rate in the middle chamber for the chemical cathode in MDC. The effect of mixing in the two experiment was studied with 150 kΩ (the external resistance was fixed at 150 kΩ because high power was achieved at this resistance, as shown in Figs. 1 and 3) and two TDS concentrations (15 and 25 g/L). The initial COD concentration was fixed at 1350 ppm in the anode chamber in MDC. The mixing speed and temperature were fixed at 250 rpm and 25 °C, respectively, in the desalination chamber. After 48 h of operation with 150 kΩ and 15 and 25 g/L, the results were observed. The maximum TDS concentration removal from the middle chamber was 42 %, with the removal rate reaching 0.23 g/L h^{-1}. Prior to mixing, the removal rate was 0.226 g/L h^{-1} with 32 % of TDS removal. Fig. 12 clearly illustrates the difference in TDS concentration removal between the experiments with and without mixing. In the second experiment with 150 kΩ and 15 g/L, the highest percentage of TDS concentration removal from the middle compartment was 41 %. The TDS concentration removal rate reached 0.13 g/L h^{-1}. By contrast, the experiment without mixing in the desalination chamber only achieved a 30 % TDS concentration removal (see Fig. 13).

Comparing the previous results provided insight into how the desalination rate could be enhanced. When comparing the results of the experiments with and without mixing in the desalination chamber, it was observed that mixing led to an increase in the rate and percentage of salt removal in the system. This increase was attributed to a greater transfer of NaCl from the desalination room to the cathode and anode rooms, as well as an increase in mass transfer. The mixing process facilitated the movement of salts in the room and improved mass transfer according to Fick's law. The act of mixing resulted in an elevation in voltage and a greater degree of COD elimination, as compared with experiments conducted without mixing, during the course of the operation.

Table 6
Comparison between the previous studies and this study.

Configuration	Anolyte/Catholyte With volumes	Anode/cathode Material and AEM/CEM Material	Desalination chamber fed NaCl concentrations	External Resistance, (Ω)	Total Desalination Rate (TDR)	COD Removal, (%)	Power Density/ Desalination Efficiency, (%)	Ref.
PMDC	Synthetic Wastewater with 180 ml/ Mineral solution with microalgae as biocatalyst with 180 ml	Graphite paper/Graphite paper And AMI 7001/CMI 7000	10 g/L	10000	6.7 mg/l	65.6	84 mW/m^3/ 40 %	[42]
Biocathode MDC	Dewatered Sludge/Soil solution in deionized water. 1400 ml/500 ml	Graphite fiber brush/ Graphite brush and Ultrex AMI-7001/ CEM, Ultrex CMI-7000	5,10,35 g/L	1000	–	25.71 ± 0.15	–	[43]
Three Chamber MDC	Sodium Acetate (1.6 g/L) /Ferricyanide 11ml/11 ml	Carbon Felt/Carbon Felt CMI7000, Membrane International	5,20,35 g/L	200	–	–	-/90	[11]
PMDC	Sodium acetate with oil sludge/ mineral solution with macroalgae as biocatalyst 350 ml/350 ml	Graphite plate/Graphite plate and AMI 7001/CMI 7000	25,15 g/L	50 kΩ		12 %, 9 %	/32 % and 15 %	This study
PMDC	Sodium acetate with oil sludge/ mineral solution with macroalgae as biocatalyst 350 ml/350 ml	Graphite plate/Graphite plate and AMI 7001/CMI 7000	25,15 g/L	100 kΩ		15 %,9 %	/33 %, 16 %	This study
PMDC	Sodium acetate with oil sludge/ mineral solution with macroalgae as biocatalyst 350 ml/350 ml	Graphite plate/Graphite plate and AMI 7001/CMI 7000	25,15 g/L	150 kΩ		24 %, 18 %	/43 %, 23 %	This study

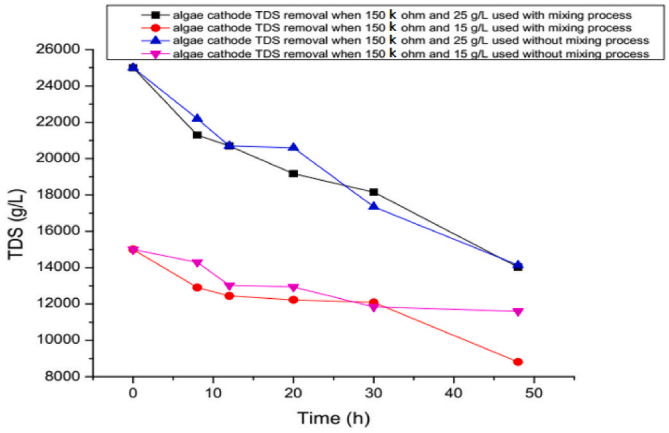

Fig. 11. The comparing the TDS concentration removal before and after using mixing process with algae cathode.

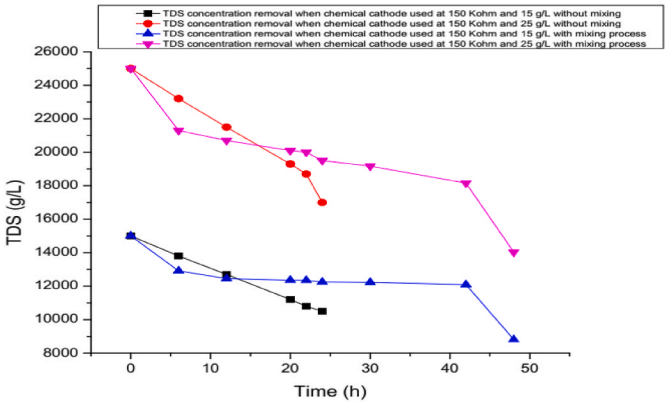

Fig. 12. The comparing the TDS concentration removal before and after using mixing process with chemical cathode.

3.6. Biofouling and biofilm examines

The pore size for the anion exchange membrane (AEM) before and after use is shown in Fig. 15 (see Fig. 14). Fig. 15A and B shows the SEM images for unused and used AEM for the anode chamber, respectively. These two figures clearly demonstrate the distinctive differences between the membrane surface, with a noticeable cracked observed for the new anion exchange membrane. Fig. 15B shows the fouling layer for the used anion exchange membrane. The anion exchange membrane exhibited biofouling caused by bacteria aggregating in the form of rods and inorganic crystalline crusts, which were made by the deposits of inorganic chemicals in simulated wastewater. Fig. 15C and D shows clearly the difference in pore size for the AEM before and after use. In Fig. 15C, the pore size ranged from 26.61 μm to 54.19 μm. After use, the pore size ranged from 28.56 μm to 12.17 μm. This difference in pore size was the main case for the decrease in the rate of desalination during operation. The maximum desalination rate was achieved in the early hours.

3.7. Diagnosis of sludge

The bacteria dominant in the sludge of the anode chamber were detected and obtained from the first gas power plant's wastewater in southern Baghdad, Iraq. The exoelectrogenic bacterium was identified in the biotechnology labs of the University of Baghdad's College of Science. Details on the identification of the microbe from the microbiology chart report for Vitek 2® Compact are shown in Table 7. The predominant exoelectrogenic bacterium detected in the anode chamber sludge was *Pseudomonas fluorescens.*

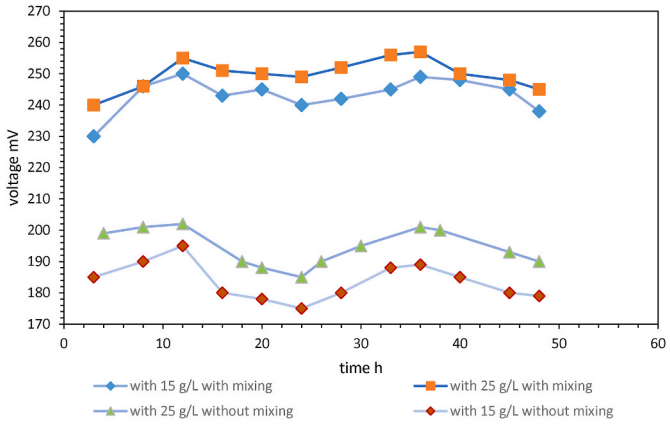

Fig. 13. The effect mixing on the voltage generating in the PMDC.

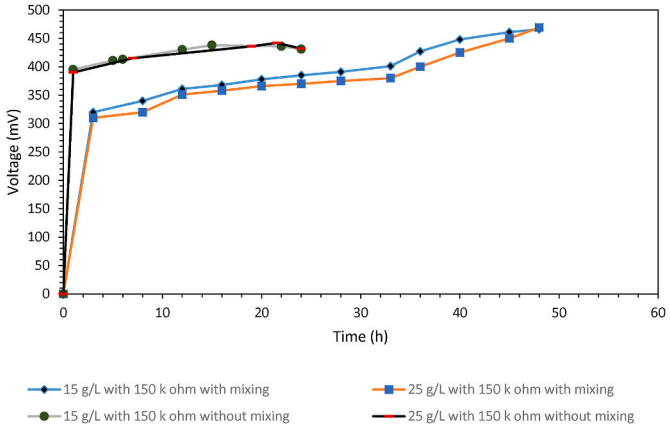

Fig. 14. The effect mixing on the voltage generating in the chemical cathode MDC.

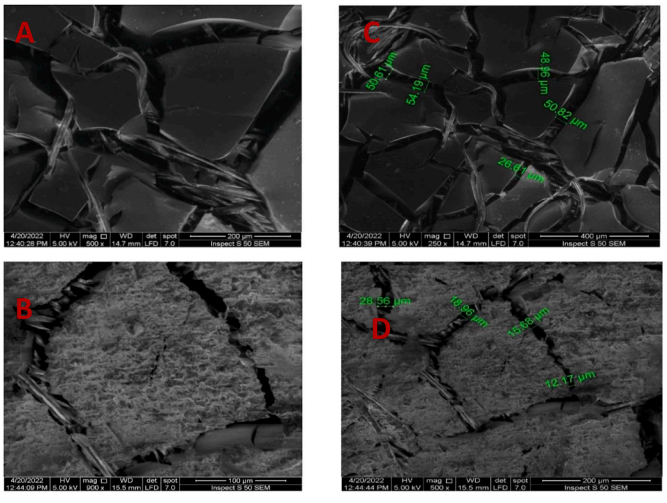

Fig. 15. The SEM analysis and the pore size for the AEM before used and after used.

4. Conclusion

This study observed the efficiency of PMDC in utilising macroalgae in the cathode chamber as a source of oxygen to accept electrons. The study proved that the external resistance increased the activity of microorganisms, which was crucial for the production of electrons through the desalination of desalinate and oxidation process in the MDC. Besides understanding the impact of initial NaCl concentrations in the desalination chamber on desalination, which aids in the separation of ions in saline water to boost efficiency, a mixing procedure was implemented within the desalination compartment of a cell. The migration of salt ions from saline water, as well as the mixing process, facilitated the removal of the TDS deposits that had formed on the ion exchange membranes. This study also compared the performance of the chemical MDC and PMDC in terms of power generation, COD removal and TDS removal efficiency. The results showed that the chemical cathode had a higher performance as an electron acceptor than PMDC. However, the results also indicated that PMDC could be used as electron acceptor without generating any hazardous waste, unlike the chemical catholyte in MDC. Thus, PMDC could be considered as a promising technology for MDC in the future. The ANOVA results indicated an interaction between parameters in MDC, which affected COD removal. Additionally, the mixing process in the desalination chamber affected TDS removal and voltage generation through the movement of particles.

5. Present challenges

1. Increasing voltage generation: One major obstacle is still producing high voltage output reliably at different concentrations of NaCl and external resistances. Although mixing and chemical catalysts have demonstrated potential, both methods still require further development in terms of integration and optimisation.
2. Improving desalination efficiency: The rate of NaCl removal must be increased in a way that is economical and energy-efficient. Although the existing approaches have promise, further advancements are needed before MDCs can compete with traditional desalination techniques.
3. Handling wastewater effectively: The continuous removal of a significant amount of chemical oxygen demand (COD) may be difficult to achieve in a reasonable amount of time. Further research is needed into the integration of sophisticated optimisation approaches, such as Design Experimental® software, to optimise COD elimination.
4. Environmental impact and operating costs: Bio-cathodes can lower environmental impact and operating costs by substituting hazardous chemical catalysts; however, further research is needed to determine how well-suited and scalable bio-cathodes are.

Future studies and advancements pertaining to MDCs have to concentrate on innovative methods for increasing voltage generation and desalination efficiency, as well as the exploration of novel materials for electrodes and membranes, to greatly boost MDC performance. Optimisation and Scale-Up: To ensure the MDC system is commercially viable, sophisticated optimisation techniques must be used and scaled to pilot and industrial levels. COD elimination and overall system efficiency can be improved with further study on the Design Experimental® software. Bio-Cathode Development: Reducing operating expenses and environmental impact may be achieved by advancing the usage of biocathodes, which includes investigating diverse microorganisms and optimising their performance under varied environmental circumstances. The establishment of legislative and economic frameworks that are conducive to the adoption of MDC technology and the stimulation of investment in research and development is imperative.

CRediT authorship contribution statement

Hussein H. Abd-almohi: Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Ziad T. Alismaeel:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Conceptualization. **Mohanad J. M-Ridha:** Software, Resources, Funding acquisition, Formal analysis, Data curation.

Table 7
The microbiology chart report for Vitek 2® compact.

Identification information			Card: GN		Lot Number: 2411807103		Expires: Nov 7, 2022 12:00 AST
			Status: Final		Analysis Time: 6.57 h.		Completed: Jun 23, 2022 02:26 AST
Organism Origin			VITEK 2				
Selected Organism			99 % probability **Pseudomonas Fluorescens**				
			Bionumber: 4001011100100210 Confidence: Excellent identification				

Biochemical Details																
2	APPA	–	3	ADO	–	4	PyrA	+	5	lARL	–	7	dCEL	–	9	BGAL	+
10	H2S	–	11	BNAG	–	12	AGLTp	–	13	dGLU	+	14	GGT	–	15	OFF	–
17	BGLU	–	18	dMAL	–	19	dMAN	–	20	dMNE	+	21	BXYL	–	22	BAlap	+
23	ProA	+	26	LIP	–	27	PLE	–	29	TyrA	+	31	URE	–	32	dSOR	–
33	SAC	–	34	dTAG	–	35	dTRE	–	36	CIT	–	37	MNT	–	39	5 KG	+
40	ILATk	+	41	AGLU	–	42	SUCT	–	43	NAGA	–	44	AGAL	–	45	PHOS	+
46	GlyA	–	47	ODC	–	48	LDC	–	53	IHISa	–	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	–	61	IMLTa	–	62	ELLM	–	64	ILATa	–			

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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