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Revolutionizing Water Treatment with Microbial Desalination Cells: Current Trends and Future Directions

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Abstract: The Microbial Desalination Cell (MDC) emerges as an innovative solution for power generation and wastewater treatment, operating without external energy input. MDCs utilize Electroactive bacteria (EAB), which oxidize organic matter and transfer electrons to an anode. These electrons subsequently traverse an external circuit to a cathode, where they react with protons and oxygen. The MDC setup incorporated three distinct chambers: anode, desalination, and cathode. Wastewater samples were placed in the anode and cathode compartments, while the desalination chamber contained saline water. The MDC operated for 30 days continuously. A digital multimeter was employed to regularly monitor and log the generated voltages. In the anode, the concentration of No_2 decreases from 28.15 mg/l to 1.56 mg/l, while in the cathode, the concentration of No_2 decreases from 29 mg/l to 2.92 mg/l. In the anode, the concentration of No_3 decreases from 37.95 mg/l to 2.09 mg/l, in the cathode, the concentration of No_3 decreases from 39.1 mg/l to 3.96 mg/l. the concentration of So_4 in anode decrease from 1314 mg/l to 110 mg/l in cathode decrease from 1710 mg/l to 122 mg/l. An MDC presents notable benefits, including the concurrent treatment of wastewater, production of renewable energy, and desalination of water. This comprehensive method makes them a sustainable and economically feasible option for tackling water scarcity and energy challenges. Nonetheless, issues such as scalability, efficiency, and membrane fouling need to be resolved before they can be widely implemented. Current research is dedicated to optimizing MDC designs and boosting their performance for effective use in real-world scenarios.

Keyword: Microbial Desalination Cells (MDCs). Bioelectrochemical systems (BES). Wastewater treatment, Sustainable desalination.

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Introduction

Earth's surface is 70% water (1). Saltwater comprises 97.5%, while freshwater exists as ice caps or soil moisture (2). Only 1% of freshwater is available for human use (3). "Wastewater" refers to water polluted with organic and inorganic compounds after industrial or domestic use (4). Petrochemical, leather, food, and paper industrial processes generate organic-rich wastewater with high sulfate and nitrogen levels (5).

Sulfate/sulfide-rich effluents harm health and ecosystems (6). Anaerobic treatment with sulfate-reducing bacteria (SRB) treats sulfate-laden wastewaters (7). SRB convert SO_4^{-2} to sulfide (S_2^-), serving as an electron source for NO_2^- denitrification (8). Excess nitrogen causes eutrophication and health hazards (9). Eutrophication remains a

critical global water pollution challenge (10). This causes algal growth, reducing oxygen content and aquatic life mortality (11). Nitrogen pollution stems from industrial treatment, sewage, agricultural runoff, livestock, and aquatic organisms' metabolism (12).

The conventional nitrification-denitrification is the major process for nitrogen removal in municipal WWTPs (13). It removes nitrogen efficiently, prevents pollution and eutrophication, and deploys bacteria rather than expensive chemicals. However, this process requires energy and carbon addition, with potential N_2O emissions (14). Biological denitrification is preferred over physicochemical approaches for its effectiveness (15).

Materials and Methods

MDC manufacturing and system setup

MDC was designed and manufactured in the engineering process laboratory of the Institute of Genetic Engineering and Biotechnology for post-graduate studies, using local material and acrylic sheets. The design of the MDC was based on three cubic shapes, which represent three chambers: anode, middle membrane desalination unit, and cathode, as illustrated in Figure 1. The desalination unit was separated by two types of membrane, anion-exchange membranes (AMI-7001S) and cation-exchange membranes (CMI-7000) (10*10 cm). Membranes were purchased from Membranes International Inc., Ringwood, NJ, USA. AEM was placed to separate the anode and middle chambers, while a CEM was placed to separate the middle and cathode chambers. The cell net volume was 550 ml for the anode, 550 ml for the cathode, and 350 ml for the desalination unit. Gaskets were placed between the cubic chambers to seal them and to ensure no water leakage. The three chambers were clamped together with screws which were fastened tightly. Graphite plates (7 mm thickness, 6× 8 cm) were placed in the cathode and anode chambers. It was connected to an electric wire to determine the generated voltage by a digital multimeter.

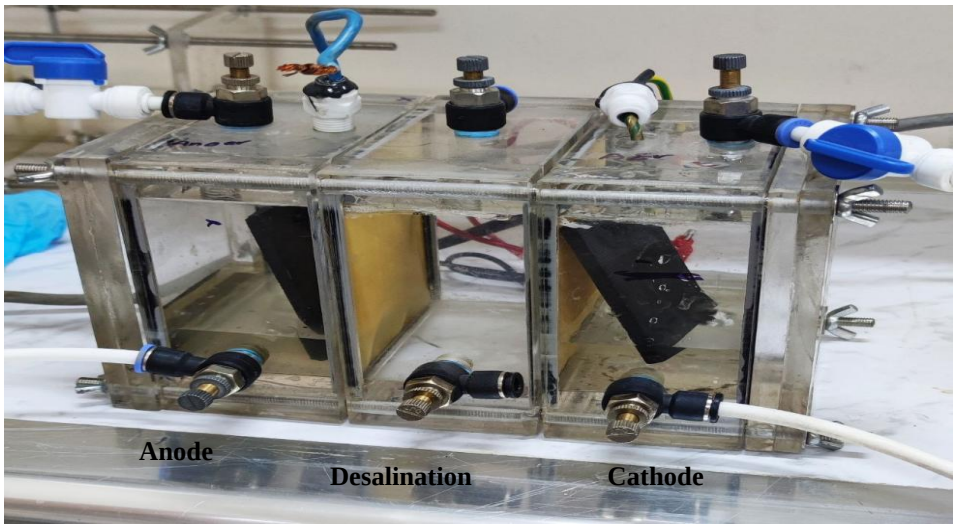


Figure (1): Microb ial desalination cell configuration.

Wastewater was used to feed both the cathode and anode chambers. Wastewater utilized represents a bacterial and nutrient source to

stimulate the MDC. Wastewater samples were collected from the AL-RUSTUMIA wastewater treatment plant, Baghdad/Iraq. In this experiment, two sources of wastewater were used: the anaerobic wastewater sample, which was collected from the anaerobic wastewater tank, and the aerobic wastewater sample, which was collected from the aerobic digester. Furthermore, anaerobic and aerobic sludge were collected from both sites, and it was added to the wastewater used in this experiment to enrich the microbial community. These samples were characterized by general parameters (Table 1). The wastewater and sludge samples were kept in a refrigerator at 4 °C before use. Saline water was prepared at a concentration of 20 g/L NaCl using distilled water.

The microbial desalination cell was run as a continuous reactor. The anaerobic condition was maintained in the anode chamber by using a 1 L feeding tank container, which used sparged nitrogen gas to keep the anaerobic condition. On the other hand, aerobic condition was maintained in the cathode chamber using a 1 L feeding tank, which was aerated constantly to maintain aerobic conditions. The middle membrane desalination unit was fed with saline water. A peristaltic pump was used for influent and effluent for all chambers at a constant flow rate of 200 ml/day. The effluents of the three chambers were collected in separate containers, and samples were taken periodically for further tests. MDC was operated at room temperature, and it was pre-run for 5 days to ensure no leakage occurred and to acclimate the utilized microbial community.

Table (1): wastewater parameters

NO	Characteristics	Anaerobic wastewater	Aerobic wastewater
1	No_3–N (mg/l)	2501	24.05
2	No_2^- (mg/l)	18.62	17.84
3	So_4^{-2} (mg/l)	114	152

The experiment parameters

Voltages across the external resistor were recorded periodically using a digital multimeter (DT-830D). Nitrate of the water inlet and outlet of the cell were measured according to the standard methods of the Cadmium Reduction Method (Powder pillow procedure) by DR3900. Nitrite of water inlet and outlet of the cell was measured according to the standard methods of Ferrous Sulfate Method1 (Powder pillow procedure) by DR3900, and Sulfate of water inlet and outlet of the cell was measured according to the standard methods of USEPA1 SulfaVer 4 Method2 by DR3900.

Nitrate

Cadmium metal reduces nitrate in the sample to nitrite. The nitrite ion reacts in an acidic medium with sulfanilic acid to form an intermediate diazonium salt. The salt couples with gentisic acid to form an amber colored solution. The measurement wavelength is 500 nm for spectrophotometers or 520 nm for colorimeters. Cadmium Reduction Method, Method 8039, 0.3 to 30.0 mg/L NO3 –N (HR) Powder Pillows

Powder pillow procedure For Nitrate Measure

Start program 355 N, Nitrate HR PP. Prepare the sample: Fill a sample cell with 10 mL of the sample. Add one NitraVer 5 Nitrate Reagent Powder Pillow. Put the stopper on the sample cell. Start the instrument timer for 1 minute. Shake the cell hard until the timer stops. Some powder may not dissolve, but this is okay. Start the

instrument timer for 5 minutes. If nitrate is present, the sample will turn amber. Prepare the blank: When the second timer stops, fill another sample cell with 10 mL of the sample. Clean the blank sample cell. Insert the blank into the cell holder. Press ZERO. The display will show 0.0 mg/L NO3 —N. Clean the prepared sample cell. Within 1 minute after the timer stops, insert the prepared sample into the cell holder. Press READ. Results will show in mg/L NO3 —N.

Nitrite

This method uses ferrous sulfate in an acidic medium to reduce nitrite to nitrous oxide. Ferrous ions combine with the nitrous oxide to form a greenish-brown complex in direct proportion to the nitrite present. The measurement wavelength is 585 nm for spectrophotometers or 560 nm for colorimeters.

Powder pillow procedure For Nitrite Measure

Start program 373 N, Nitrite HR PP. Prepare the sample: Fill a sample cell with 10 mL of the sample. Add one NitriVer 2 Nitrite Reagent Powder Pillow. If nitrite is in the sample, a greenish-brown color will appear. Put the stopper on the sample cell and shake it to mix the reagent. Start the instrument timer for a 10-minute reaction. Keep the sample cell still on a flat surface to avoid low results. Prepare the blank: Fill another sample cell with 10 mL of the sample. Clean the blank sample cell. Insert the blank into the cell holder. Press ZERO. The display will show 0 mg/L NO2 –. After 10 minutes, gently turn the prepared sample over twice. Too much mixing can cause low results. Clean the prepared sample cell. Insert the prepared sample into the cell holder. Press READ. Results will show in mg/L NO2.

Sulfate

Powder Pillow Procedure For Sulfate Measure

Start program 680 Sulfate. Prepare the sample: Pour 10 mL of the sample into a sample cell. Add one SulfaVer 4 powder pillow to the cell. Swirl the cell to mix. If there is undissolved powder, it won't affect the results. If sulfate is present, the mixture will turn cloudy white. Start the timer on the instrument. Wait for 5 minutes without moving the cell. Prepare the blank: Pour 10 mL of the sample into another sample cell. When the timer stops, clean the blank sample cell. Put the blank into the cell holder. Press ZERO.

The display will show 0 mg/L SO_4^{-2}. Clean the prepared sample cell. Within 5 minutes after the timer stops, put the prepared sample into the cell holder. Press READ. The results will show in mg/L SO_4^{-2}. Clean the sample cells with soap and a brush.

Results and discussions

Power production by Microbial Desalination Cell (MDC)

The voltage generated by MDC was increased slowly during the first week, as elucidated in Figure 2 . In this figure, the voltage generation was monitored periodically. Furthermore, it rose gradually up to 638 mV when the cell ran for 24 days. Whereas the voltage generated trendline was retreated from this point onward to about 460 mV.

The experiment's recorded voltage trendline demonstrated that the cell is a suitable method for electricity production linked to the desalination process. Moreover, the entire procedure contributes to the creation of environmentally friendly, sustainable energy by utilizing wastewater as a source of nutrients and microorganisms. The observed gradual increase in generated voltage was likely due to the adaptation of the microbial community

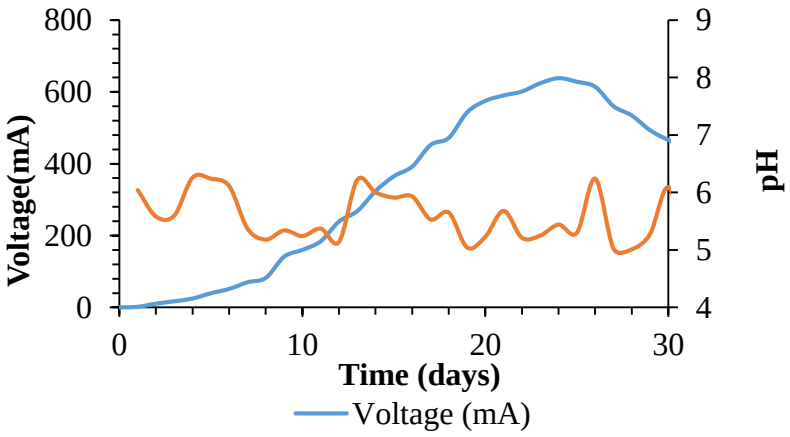
and the development of biofilm on both the anode and cathode surfaces (see Figure 3).

During the initial weeks of the experiment, the voltage production in MDC remained below 100 mV. This can be attributed to the gradual adaptation of microbes. Additionally, the time required for microorganisms to develop the biofilm, which establishes the bacterial community, is illustrated in Figure 3 . This observation aligns with the findings of Kokabian and colleagues, who noted that in their study, the lag phase of microorganisms influenced extracellular electron transfer mechanisms, potentially enhancing biofilm formation on the electrodes (16).

Throughout the experiment, the maximum voltage observed reached 638 mV. Despite this, numerous wastewater influent samples were taken during the study, and the decline seen in the last week was likely attributed to shifts in the nutrient levels of the wastewater throughout the experiment.

Studies suggest that the microbial desalination cell (MDC) power output is a key indicator for the overall performance of a microbial desalination system (MDS), which increases the device's desalination efficiency (17). From observation, it is noticed that with time, the capacity of electricity generation increases, and this may be due to the biofilm growth on the anode. As described by Jaroo and coworkers, the ion migration between chambers drives the enhancement of power generation (18). Furthermore, bacteria are important in the power-generating activities. The Geobacter, one of the dominant bacterial species in the MDCs, is suggested to be the most impactful on the power output. Dongre's study established a relationship between the voltage output of MDCs and the time by which the microbial population had developed in them.

638 mV was recorded as the maximum voltage in this study. This result is consistent with findings from other studies in the field. For instance, research conducted by (19) reported a maximum voltage of 639 mV. In a separate investigation, (20) achieved a highest voltage of 600 mV during MDC operation, using an initial salt concentration of 20 g/L. Interestingly, a different study by (21) focused on power generation, recording a value of 3.55, 9 mA.



Figure(2): shows the generated voltage by the microbial desalination cell.

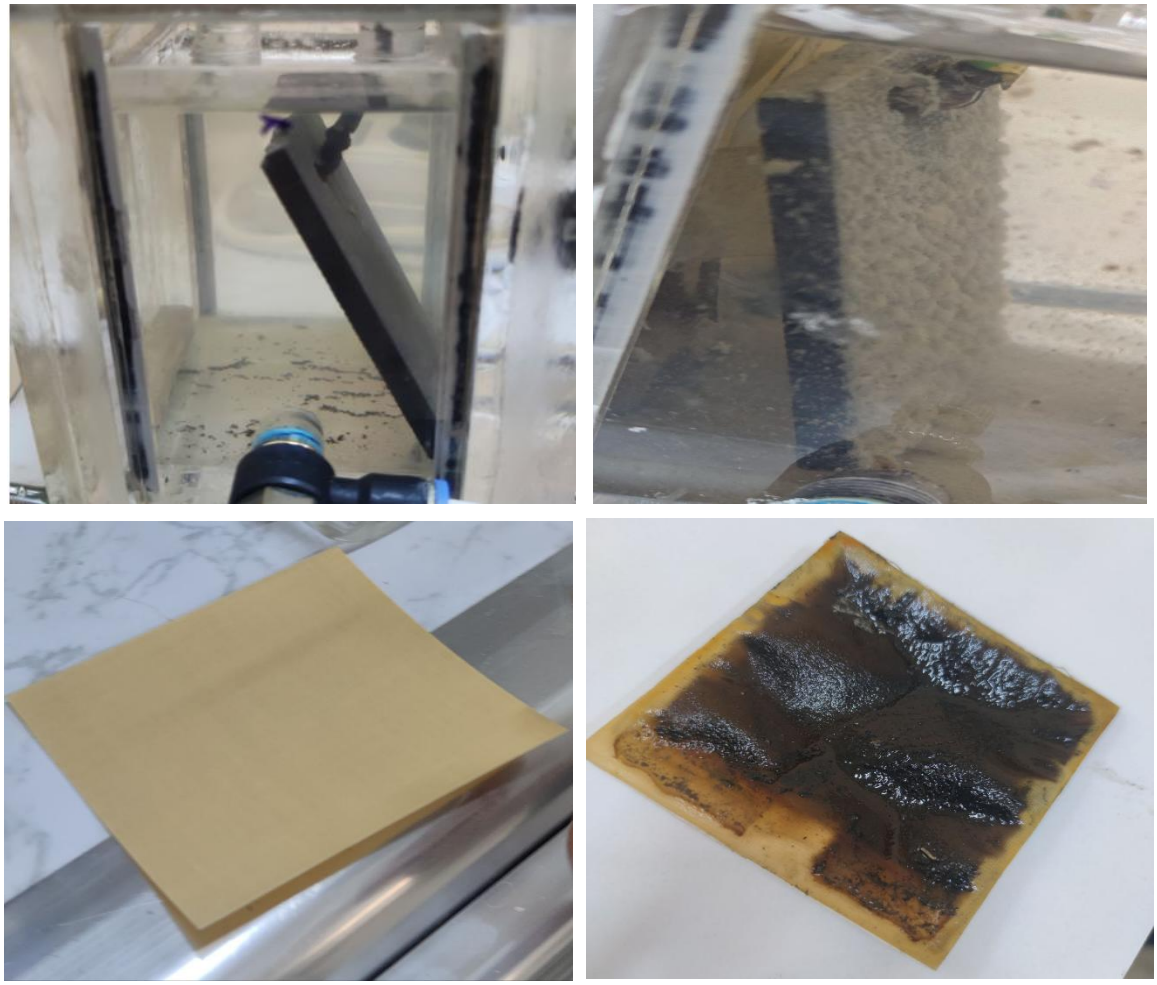


Figure (3): A and B highlight the biofilm on the graphite electrode before and after treatment, while C and D are the membrane before and after treatment.

The concentration of nitrate and nitrite

The nitrite concentration in the anode decreased with time, as highlighted in Figure 4. In the beginning, there was a leveling off in the anode during the first week of the experiment. However, during this time, a noticeable decline was highlighted in the cathode, then this decrease continued from day 16mg/l to about 18mg/l. After two weeks, there was a dramatic decline in nitrite concentration in the anode to about 8.88mg/l. By the end of the experiment, nitrite concentration dropped to 1.56mg/l and 2.92mg/l in the anode and the cathode, respectively.

The observed decrease in nitrite levels within both the cathode and anode chambers could be a result of the combined actions of nitrification and denitrification bacterial populations. The finding was confirmed by Madde and his research group when he attributed the decline in nitrogen by nitrite-oxidizing bacteria (22). Additionally, the decline in nitrite concentration was also observed by Kokabian, who observed a decrease in nitrite levels during the desalination process .

The primary mechanism for nitrogen elimination is the transformation of ammonia into nitrogen gas. A desalination apparatus, submerged in groundwater, facilitates nitrate migration. Initially, nitrate moves toward the anode, followed by its

transfer to the cathode. At the cathode, nitrate undergoes a reduction reaction, yielding nitrogen (23).

Denitrification-based nitrogen removal is a conventional biological process requiring two linear aerobic stages. The first step involves the transformation of ammonium (NH_4^+) to nitrite (NO_2^-) via bacteria such as Nitrosospira and Nitrosomonas (ammonium-oxidizing bacteria) and involves oxygen as the terminal electron acceptor.

In the second stage, nitrite (NO_2^-) is oxidized into nitrate (NO_3^-) by bacteria that oxidize nitrites (NO_3^-), such as Nitrobacter. This conversion is linked to the nitrification of reduced nitrogen compounds in the presence of O_2 (20). Denitrification, in which (NO_2^-) or ($N0_3^-$) are converted to gaseous nitrogen, can take place in both anoxic and non-anoxic systems. This conversion is carried out by many microorganisms, as it was reported in previous studies.

Nitrates (NO_3^--N) and nitrites (NO_2^--N) are two of the major organic pollutants in the water system, which pose a risk to water organisms, land animals, and human beings (24).

The nitrification-denitrification process is the most commonly used technique between different methods for nitrogen removal .

The traditional nitrogen removal processes are generally composed of two successive steps: aerobic nitrification, followed by anaerobic denitrification. There is another from of nitrogen removal known as anaerobic ammonia (NH_4^+) (Anammox). This process oxidizes (NH_4^+) to nitrogen gas (N_2) by using nitrite (NO^-) as an electron acceptor .

LOH (25) describe that the nitrification process of ammonia to nitrate is carried out by two groups of bacteria: ammonia- oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB).

Denitrification, on the other hand, is the process through which hete rotrophic denitrifying bacteria convert nitrate to dinitrogen gas under oxygen-limited conditions.

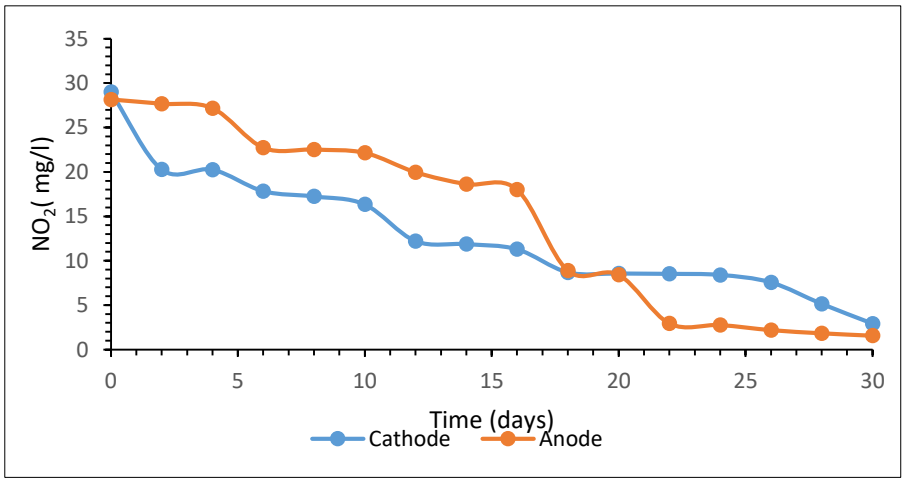


Figure (4): The concentration of Nitrite in both the anode and cathode.

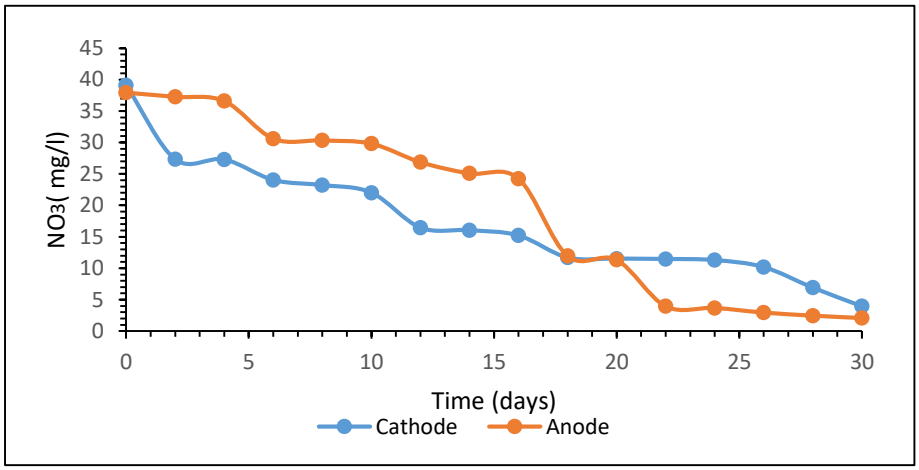


Figure (5): The concentration of Nitrate in both the anode and the cathode.

4.5 The concentration of sulphate

Figure 6 describes the sulphate concentration during the MDC experiment. However, the sulphate concentration was monitored only in the cathode and anode chambers. The collected data highlighted that in the anode chamber, the sulphate concentration was dramatically decreased during the first week, nonetheless, the concentration of sulphate was continuously decreased until day 26. Then a sudden drop in concentration on day 28, and a gradual decrease on day 30. In the cathode chamber, a dramatic decrease in the So4 concentration for the first 2 days, then a gradual decrease until day 6. followed by a slight decrease until day 22, and the concentration was around 940mg/l. After that, a significant decline in sulphate concentration was detected up to 110mg/l. Similar behavior was noticed in the anode, as the first concentration for sulphate was 1340mg/l, and the decline in the concentration was continued for 26 days. In the latest week, there was a sharp drop in the sulphate concentration, reaching 110mg/l. The decline in sulfate concentration can be attributed primarily to bacterial activity, which constitutes the main biological factor. Wastewater typically contains high levels of ammonium and sulfate (26). In aquatic environments, sulfate represents the most significant form of sulfur (S). The harmful effects of sulfate are linked to its impact on metal solubility and its tendency to alter pH through acid formation. This phenomenon may explain the observed acidic pH range in both the anode and cathode compartments. Recent research highlights the development of sustainable methods for sulfate removal, with a growing focus on bioremediation techniques for SO_4, particularly in wastewater treatment. These approaches, which utilize microorganisms, are gaining attention as viable and eco-friendly solutions (27). The current study has accomplished three primary objectives: water desalination, wastewater treatment, and most significantly, green energy production. Sulfate-reducing bacteria (SRB) are the main actors in this
reaction(28). The microbes utilize the source of energy and carbon from organic molecules and sulfate as the final electron acceptor. This results in sulfide (H_2S, HS^-, S_2^-) and bicarbonate formation, according to the following equation:

$$2\ CH_2O + SO_4^{2-} \rightarrow H_2S + 2\ HCO_3$$

In addition to organic compounds and sulfate, sulfide is also widespread (29). Cheng addressed that sulfate reduction began after a lag phase of approximately two weeks. Thereafter, the sulfate removal rate increased steadily (30). This could be the reason for the gradual decline with time compared with the significant drop in sulfate concentration, which was spotted in Figure 6. A newly published paper indicates that sulfate-reducing bacteria (SRB) are the key players in the process of sulfate reduction. This process can potentially lead to the formation of hydrogen sulfide gas (H_2S), a dangerous secondary product, in environments lacking oxygen (31).

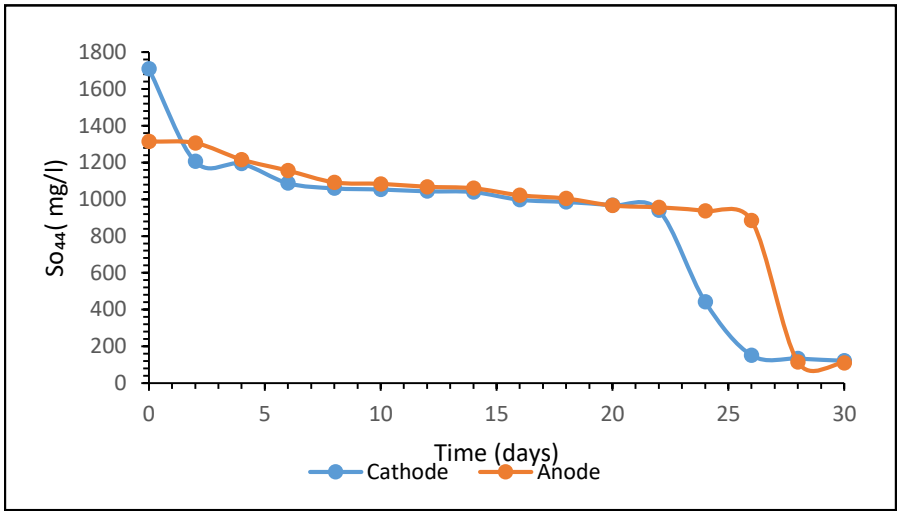


Figure (6): The concentration of sulfate in both the cathode and anode.

Conclusion

The research identified MDC as an environmentally sustainable approach for generating power from organic waste, while simultaneously addressing water scarcity through desalination and wastewater treatment. Exoelectrogens play a crucial role in MDC systems, significantly influencing their effectiveness. The conditions that impact these microorganisms' growth and viability directly or indirectly affect the MDC system's overall efficiency. This investigation demonstrated an additional power generation, coupled with appropriate salt removal capabilities. Additionally, the study found that the sulfate (SO_4^{-2}), nitrate (NO_3^--N), and nitrite (NO_2^--N) compounds had conversion rates higher than 90%. Compared to traditional desalination methods, MDCs have several benefits over traditional desalination methods, such as the possibility of resource recovery and reduced energy consumption. The main goals of recent developments are to optimize microbial communities, increase system scalability, and improve ion-exchange membrane performance. Despite ongoing issues like biofouling and high startup costs, MDCs offer a viable way to manage water resources sustainably in areas with limited resources.

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