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## Development of microbial desalination cells for the treatment of reverse osmosis reject water: A new benchtop approach

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## ABSTRACT

To address the growing global demand for usable water, there is an immediate necessity to enhance wastewater treatment systems. A continuous mode adaption of the conventional three-chamber microbial desalination cell (MDC) configuration was used, with gravity facilitating the flow of residential reject water for desalination. Initially, operating in batch mode with a 100 mL treatment volume, the single microbial desalination machine was expanded to 300 mL in continuous mode, capable of treating 5 L of home refuse water over 36 days. The batch mode MDC had a maximum current and power density of 3.81  $\mu\text{A}/\text{cm}^2$  and 0.337  $\mu\text{W}/\text{cm}^2$ , resulting in 76 % desalination and 83.9 % COD eradication rates. Scaling up increased the MDC's performance, reaching a maximum of 0.45  $\mu\text{W}/\text{cm}^2$  and 5.31  $\mu\text{A}/\text{cm}^2$ , which was 1.3 times greater than batch mode operation. The current work demonstrates the feasibility of microbial desalination cells and their novel approach for treating much higher quantities of reverse osmosis (R.O.) saline water in a comparable period of roughly 36 days. It emphasizes the actual limits when dealing with real-world wastewater samples, presenting a unique path for biotechnology by simultaneously generating bio-electricity and tackling future contaminants. Furthermore, incorporating desalination chambers with microbial fuel cells increases efficiency and opens up new options for

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enhanced wastewater treatment, resource recovery, and bioenergy generation. This pioneering strategy uses innovative membrane technologies and microbial optimization approaches to push the limits of desalination.

## 1. Introduction

Water is necessary for all life forms on Earth. Compared to seawater, the amount of freshwater available to people is highly restricted, making it vital to develop ways to raise the volume of water suitable for drinking, industrial and agricultural operations, and finally meet everyone's needs (He et al., 2021). Desalination is a dependable and realistic means of satisfying the world's growing water needs. Dissolved salts from treated salty water (varying from brackish to saline) are removed to produce fresh water. Desalination is currently utilized in 149 countries, and 4 million people rely on it every day (He et al., 2021; Okampo and Nwulu, 2021).

People usually believe that desalination is a safe method for supplying drinking water. However, it, like many others, can have the same environmental repercussions as any other treatment approach (Okampo and Nwulu, 2021). In addition to freshwater recovery, a discharge stream known as refuse is created, which is dangerous to the environment due to its hypersaline content. Currently, rejects are tossed into the sea, producing severe environmental issues (Gaber et al., 2022). Desalination systems also require a significant amount of power, which is currently produced using fossil fuels. This raises the demand for electricity as well as the pollution emissions caused by the use of fossil fuels (Khan et al., 2024). Energy generation utilizing microorganisms in conjunction with wastewater treatment is an interesting field of research to address the worldwide energy and water crisis (Anand et al., 2021; Sirohi et al., 2023). Furthermore, as the human population increases, the amount of solid and liquid waste also increases. The most frequent wastewater is home reverse osmosis (RO) reject water (Fujioka et al., 2012). R.O. reject is a sophisticated mixture of many chemicals and salts condensed from the intake of water, contributing significantly to residential wastewater (Chandrasekhar et al., 2020). Approximately, 82% of the water sent to houses is returned as sewage. Although injection into deep wells and surface water disposal are conventional procedures for disposing of R.O. rejects, these activities have generated considerable environmental problems (El-Saadony et al., 2023). Wastewater salvage and reuse are critical options for completing the water cycle, reducing scarcity, and minimizing negative environmental consequences. However, the majority of these technologies cannot tolerate the high salinity and dissolved heavy metals found in R.O. reject (Punia et al., 2022). Vigneswaran et al. (2021) confirmed the negative effects of raw reject water from RO plants dumped into the ground. The findings demonstrated the steady deterioration of soil and subsurface water quality caused by the accumulation of ions (Vigneswaran et al., 2021).

As a result, the uncontrolled explicit release of domestic R.O. reject onto the earth could affect soil fertility (Kankarla et al., 2021). Furthermore, because the energy cost changes with water salinity, microbial fuel cell-based desalination is highly suitable for brine wastewater treatment. This will minimize our reliance on nonrenewable fuel sources in the long run. The limited quantity of fossil fuels may last until 2080 (Bridgeland et al., 2022). Nonrenewable energy sources are not expected to meet the world's energy demands. The most difficult task is determining how to provide enough energy while decreasing the global carbon impact (Marazzi, 2017). As a result, establishing a more efficient, effective, and sustainable energy system to replace nonrenewable energy sources is an essential step towards expanding global energy supply.

Microbial fuel cells (MFCs) are renewable energy generation devices that immediately convert chemical energies into electrical ones (Rabaey et al., 2003). A microbial fuel cell can create energy by biological catabolism in the presence of a biologically active catalyst, which favors proton transfer between electrodes, resulting in energy production. The MFC is a potent sustainable energy source that transfers chemical energy from the organic substrate to electrical energy in a biologically active chemical system using active biocatalysts such as microorganisms and enzymes (Gilani et al., 2024; Naik and Jujjavarappu, 2020).

Geobacter and Shewanella species are the two most important bacteria being studied for extracellular electron transfer (EET) mechanisms (Bond and Lovley, 2003; Marsili et al., 2008). Both bacteria have evolved separate EET methods. The first method involves direct electron transfer (DET), and the second is mediated electron transfer (MET). *G. sulfurreducens* and *S. oneidensis* produce a dense biofilm on the electrode's external surface, making them the most intensively researched exo-electrogens in any MFCs. These exo-electrogens use a variety of carbon sources as a substrate for bio-energy generation. Microorganisms including *R. ferrireducens* (Chaudhuri and Lovley, 2003), *E. coli* (Feng et al., 2018), *G. sulfurreducens* (Stöckl et al., 2019), *S. cerevisiae* (Duarte and Kwon, 2020), *P. aeruginosa* (Zhang et al., 2019), *B. velenzensis* (Dongre et al., 2022b, a) and *P. fermentans* (Pal and Sharma, 2019) etc. are also able to produce electricity when used in an MFC setup (Huang et al., 2011).

Microbial desalination cells (MDCs) are a form of MFC designed for saline water desalination. MDCs provide significant energy savings over typical membrane-based processes like distillation and evaporation. Compared to reverse osmosis facilities, MDCs save 4.5 kWh/m<sup>3</sup>, but the savings are significantly higher when linked with thermal technologies such as multistage flash or MSF distillation, reaching 200 kWh/m<sup>3</sup> (Bazargan, 2018). Under anaerobic circumstances, microorganisms (most commonly bacterial strains) are put into the anodic chamber, where they bio-degrade organic compounds present in wastewater via diverse oxidation processes, releasing electrons (Tawalbeh et al., 2020). When the cathode reacts with atmospheric air, reduction activities occur, resulting in the combination of hydrogen and oxygen to generate water and the eventual transport of the liberated electrons to the appropriate electrode (cathode) (Tawalbeh et al., 2020). Between the two electrode chambers (anode-cathode), a third compartment can be added. The middle or third chamber is called a desalination chamber because it contains saline water. The proposed structure is considered a microbial desalination cell (MDC) (Morris and Jin, 2009).

Furthermore, various ions' charges and molecule sizes might vary greatly, influencing the transfer behavior of distinct ions in saline

water. The MDC performance was reduced when artificial saline water was employed in the desalination chamber instead of pure NaCl (Morris and Jin, 2009). However, no study on membrane fouling or multiple ion transport behaviors in MDCs has been reported. In this work, we analyzed the transport of different ions during MDC operation and characterized the effects of various ions on the performance of MDC reactors in regard to desalination efficiency, power generation, and organic matter removal (Dongre et al., 2022a,b).

MDCs are still a relatively new concept, with numerous arrangements in the developmental stages. Many experiments are being performed to develop this technology and make it suitable for commercialization. MDCs provide great promise for saltwater desalination and can save significant amounts of energy compared to typical techniques such as evaporation, distillation, membrane-based separations, etc. Compared to membrane-based R.O. treatment methods, MDCs conserve about 5 kWh/m<sup>3</sup> of power (Tawalbeh et al., 2020). As a result, MDCs offer immense potential as long-term desalination systems and should be encouraged for use in establishing environmentally sustainable strategies for water recovery and reuse. The current study intends to take an existing MDC batch configuration and grow it up into a continuous mode of operation. All of this is to compare the batch mode to the endless mode to determine the possibilities of improving critical points in energy generation or desalination efficiency as a proof of concept for serially linking desalination chambers in MDCs. A cutting-edge approach that improves desalination while also opening up new opportunities for wastewater treatment, resource recovery (Qi et al., 2021), and even biofuel. Advanced membrane technology and microbial optimization power this discovery, surpassing previous attempts at stacking chamber. Over all this study presents a new benchtop approach for the development of microbial desalination cells tailored specifically for the treatment of RO reject water. The results demonstrate the feasibility and effectiveness of benchtop MDCs in reducing salinity levels and removing contaminants from RO reject water, offering a sustainable solution for water reclamation and resource recovery. Future research directions may include optimization of system design, exploration of novel electrode materials, and scale-up studies to evaluate the feasibility of deploying MDCs at pilot and full-scale applications.

## 2. Materials and methods

### 2.1. Microbial desalination cell (MDC) construction

The suggested unit for desalination was made up of three horizontally stacked, six-inch-diameter polypropylene tube sections with about 100 mL of volume individually (see [supplementary material](#)). The electrodes in the anode and cathode chambers were sheets of graphite (graded FC-GR347B) measuring 4 × 3 × 0.5 cm. Anion as well as cation exchange membranes (Fumasep FAS PET 75 and Fumasep FKB PK 130) for partitioning all three chambers, namely anode, desalination and cathode chambers, respectively serving as separators for the three chambers spanning a surface area of 28.27 in.<sup>2</sup> individually (Dongre et al., 2022). Electrodes and membranes were obtained from Fuel Cell Store (Texas, USA).

## 3. Experimental setup

The microbe-based desalination unit used the *B. velenzensis* strain AD1-ELB culture broth as the anolyte, and a cow dung slurry 5 % (w/v) concentration with nutrient broth (single strength) as the catholyte, making it a microbial desalination cell using biocathode. From a local dairy farm, fresh cow dung, of about 700 g in weight, was collected and air-dried in an open container at room temperature for at least 72 h. After air drying, the surface, rigid coating of cow dung was removed and the inner layers sample was obtained to make the previously mentioned catholyte solution (Thiagarajan et al., 2018).

This desalination unit was kept at 30 °C while in operation for about 31 days with an external load ranging from 820 kΩ to 100 Ω along with 31 cm<sup>2</sup> electrode surface area and 28.27 cm<sup>2</sup> of membrane cross-section. This was done to test the whole setup's ability to work as a battery. This change of resistors determines how effectively the battery maintains its voltage under load, which is more essential than simply monitoring the resting voltage. The load is applied for a 15–30 s or until stable reading is observed and monitor the voltage drop. A substantial dip implies a poor battery, which may not function well in real-world scenarios. Preceding the inoculation procedure, the desalination unit was sterilized by employing standard autoclaving parameters of 15 psi pressure, achieving 121 °C of temperature for at least 20 min using a concealed airtight box and followed by soaking individual components in sterile 90 % (v/v) ethanol-water solution for at least 600 seconds. The autoclaved box containing the sterile desalination units was placed inside a laminar airflow cabinet underneath UV light, subsequently drying the components with air flow along with UV light for at least 30 minutes to guarantee proper ethanol vaporization and sterility for the desalination unit upon assembly. This procedure is comparable to the start-up strategy used by Borjas et al. (2017) and is used consistently in the study (Borjas et al., 2017).

The domestic R.O. reject water used in the desalination units was first sterilized using the previously mentioned parameters in an autoclave. Also, a sterile NaCl solution of 0.5 M concentration was used to pre-wash both ionic membranes separately before being stored in sterile distilled water to remove any surface additives, proceeding with its installation in the desalination units using stabilizing spacers in between the respective chambers in-turn assembling the microbial desalination unit.

The anolyte containing single-strength nutrient broth was inoculated with 2 mL inoculum of a pure broth culture of *B. velenzensis* strain AD1-ELB (OD<sub>600</sub> = 1) for the desalination unit along with cow dung slurry, as previously mentioned, being used as catholyte. After assembling the complete desalination unit, including electrodes and membranes in their respective places along with their respective chamber solutions, the desalination unit was maintained inside a BOD incubator set at 30 °C for three days, allowing microbes to thrive on the exterior of the electrode. The domestic R.O. reject was added into the middle chamber following the incubation. The desalination cycle began with replacing the R.O. reject water in the middle chamber with a fresh sample after the electrodes became stable and showed the slightest fluctuation in terms of current output. Once the conductivity of the middle chamber

dropped to 1000  $\mu\text{S}/\text{cm}$ , the desalination cycles were regarded as complete because this was presumed to be the safest score for water quality initiatives (Council Directive 75/440/EEC, 2019; Li et al., 2019).

#### 4. Scale up microbial desalination cell

The three identical units of MDC (Biocathode) were serially connected, i.e., the anode and cathode were connected in series, to demonstrate a scale-up system for practical applications in treating domestic R.O. reject water. Furthermore, all three desalination chambers of the respective identical MDC units were serially connected, with the domestic reject water reservoir feeding reject water into the desalination chamber of MDC unit (a), which then fed into the desalination chamber of MDC unit (b), through MDC unit (c), which finally emptied into a treated water reservoir at a constant flow rate (see [supplementary material](#)). This is managed by the retainer clamp controller for the micro-intravenous line-set at 20 mL/hr, converting the previously batch mode (100 mL) MDCs to continuous mode reactor for treating a larger volume (5 L) of domestic R.O. reject water. The retainer clamp controller controls This gravity-driven liquid flow in conjunction with the micro-intravenous line. The power production, as well as changes in COD, conductivity, and the initial and final concentrations of sodium, potassium, and calcium ions, as well as TDS, were measured in batch mode for 30 days using a stop clamp and then in a continuous way using a controller clamp for 6 days (see [supplementary material](#)). For the first 30 days, the entire setup was run in batch mode, with readings recorded simultaneously for all three devices. After 30 days, readings were taken for the initial input water vs output water, alongside the batch mode being converted into a three-unit continuous MDC setup. Also, both the chambers, namely the anode and cathode of the MDC inoculated by a previously enriched *B. velzensis* strain AD1-ELB and cow dung slurry of a previous MDC (50 %, v/v) in nutrient broth to accelerate power generation and bacterial growth (Ebrahimi et al., 2017).

#### 5. Electrochemical calculations

A portable digital multi-meter model Haoyue DT830 made in India was used to record the open circuit voltage every 24 h for the entire study duration.

Using Ohm's law, current (I) was estimated as the proportion of cell voltage (V) to resistance (R) throughout the various resistors (820 k $\Omega$  to 100  $\Omega$  range):

$$I = V/R$$

The power (P) was determined as a product of cell voltage and current:

$$P = V \times I$$

Similarly, the distribution of current (j) and power (p) was determined by multiplying by the surface area of the respective electrode (A<sub>es</sub>) (Sonu et al., 2020).

$$j = \frac{I}{A_{es}}$$

$$p = \frac{P}{A_{es}}$$

The percent change in chemical oxygen demand for the desalination unit's middle chamber is calculated as:

$$\text{COD}\% = \frac{\text{COD}_f - \text{COD}_i}{\text{COD}_i}$$

Where COD<sub>f</sub> represents the end COD value and COD<sub>i</sub> represents the starting COD value.

In microbial desalination cells (MDCs), current (I) follows Ohm's law:  $(I = \frac{V}{R})$ , where V denotes voltage and R is resistance. Voltage (V) adheres to Faraday's law:  $(V = IR)$ , with I as current and R as resistance. Power (P) results from the multiplication of voltage and current:  $(P = VI)$ . Current density (J) represents current per unit area:  $(J = \frac{I}{A})$ , where I is current and A is the electrode surface area. Power density (PD) is calculated as  $(PD = \frac{P}{A})$ , denoting power per unit area. Flow rate is determined experimentally based on the volume of water passing through the system per unit time. SI units- COD (mg/L), P ( $\mu\text{W}$ ), I ( $\mu\text{A}$ ), R (k $\Omega$  and  $\Omega$ ) j ( $\mu\text{A}/\text{cm}^2$ ) and p ( $\mu\text{W}/\text{cm}^2$ )

#### 6. Analytical methods

All conductivity measurements were recorded at 25 °C using a conductivity meter of HACH model HQ11D installed with micro-probes (Ibrahim et al., 2019). For each COD evaluation, a 5 mL sample was stored at 4 °C unless the COD was measured via the dichromate reflux approach, which involves adding a limited amount of an oxidant followed by boiling the mix for 20 minutes to ensure thorough digestion. The oxidant aims to oxidize the entire COD of the targeted sample. The baseline quantity of organic species is obtained after 20 min of digestion/oxidation, by estimating the amount of left over oxidizing agent. Using a spectrophotometer at 670 nm, the concentration of the oxidant was estimated after two hours of the sample being refluxed in a strong acid solution containing 4.903 g of K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> in the presence of a 1 g Ag<sub>2</sub>SO<sub>4</sub> along with HgSO<sub>4</sub>, in which the chromium (VI) species are reduced to

chromium (III) ones during COD experiment (Zendehtel et al., 2022). Samples were sent to the CEG test house at Malviya Nagar in Jaipur for Inductively Coupled Plasma Mass Spectrometry (ICP-MS) examination along with chemical categorization being carried out at Jagdamba Laboratories-(Bagru) Jaipur, estimation of various salts and ions, using methods enforced in IS 10500:2012 and IS:3025 for drinking water standardized by the government of India.

## 7. Result and discussion

The current work aims to treat the domestic R.O. reject water in a batch mode MDC and then transcend the similar MDC setup into a scaled-up continuous mode of operation. This is merely an attempt to improve the performance before as a scale-up microbial desalination cell for treating domestic R.O. reject water and efficient desalination.

## 8. Domestic R.O. reject water desalination

Domestic RO rejects water's chemical analysis, revealing various ions and salts (Table 1). The batch mode MDC achieves the highest power density of  $0.331 \mu\text{W}/\text{cm}^2$  (Fig. 1a), and the current density declined from  $4.63 \mu\text{A}/\text{cm}^2$  (at day 2) to  $1.15 \mu\text{A}/\text{cm}^2$  after 30 days (Fig. 1b). This, in turn, corresponds to about 79 percent reduction in conductance of the desalination compartment for the batch mode MDC processing domestic R.O. reject water (Fig. 2a) (Dongre et al., 2022a).

This implies that the many electron transmission mechanisms occurring at the biocathode's surface, which are aided by microbial activity and diverse chemicals, contribute to changes in chemical oxygen demand (COD) and conductivity (Naaz et al., 2023). As electrons are transported during microbial metabolism, certain metabolic end products are oxidized at the electrode, which influences overall COD reduction. Furthermore, the interaction of these molecules and proteins influences conductivity by changing the ion concentration in the solution, which reflects changes in the electrolyte's electrical conductivity. As a result, the described electron transmission mechanisms play an important role in regulating COD and conductivity levels in the system (Ebrahimi et al., 2018).

This entire process is achievable because the biocathode approach is made up of a community of heterogeneous bacteria that metabolize a range of organic substrates uniformly (Randhawa and Kullar, 2011). It's important to keep in mind that the salt exclusion for batch mode MDC was over 75 %, which is encouraging when compared to earlier partial desalination experiments that employed oxygen reduction at the cathode and only managed to reduce salt by about 50 % measured as drop-in COD of the domestic R.O. reject water (Fig. 2b) (Moruno et al., 2018; Zhang and He, 2015). This result may be explained by the high electro-migration potential, which is made possible by using ion-selective membranes in both MDC designs. The capacity of domestic R.O. rejects water to buffer and opposes ion back-diffusion, minimizing a significant limitation (Ebrahimi et al., 2018). Thus, a net salinity balance of zero in the desalination chamber (Ping et al., 2016; Xie et al., 2021; Yang et al., 2021). The provision of a desalination compartment midst the cathode and anode chambers, in addition to the domestic R.O.'s buffering capacity, given the variety of ions and salts in water, minimizes the suppressive effect of  $\text{O}_2$  diffusion (Ebrahimi et al., 2018). The reduction fraction of COD increases proportionally with the sodium chloride salt, which supports the idea that chloride ions have a greater capacity to neutralize any passive oxidizing elements that tend to accumulate on or near the anode at higher chloride ion concentrations, limiting anode dissolution and enhancing microbial desalination cell performance (Kadier et al., 2016).

This might be because the potential gradient decreases due to anode substrate consumption during each batch, and the low potential/current at the batch end is insufficient to counteract the osmosis pressure created across the multiple chambers. This effect was seen when a reverse electro dialysis device was run in an open circuit (Post et al., 2009). As will be demonstrated later, constant flow for the desalination chamber will aid in resolving such challenges. ChatGPT

The likely cause of the significant disparity in current density and cycle duration between the cations-MDC and anions-MDC was membrane scaling. Further investigation is necessary to characterize the differences in transport behavior between divalent cations and anions. The materials used in MDC reactors, like as membranes and electrodes, might be another cause. Before the cation/anion

**Table 1**  
Chemical attributes of domestic R.O. reject water treated in MDC setup 2 in different configurations.

S. No.	Characteristics	Unit	Domestic R.O. water (standard)	Treated water (batch MDC)	Treated water (Scale up MDC)	Drinking water range (WHO)
1	Turbidity	NTU	Nil	Nil	Nil	Nil
2	pH	-	8.23	7.3	7.7	6.5–8.5
3	Total Dissolved Solids	mg/L	12,660	3443	9115	250 – 200
4	Electrical Conductivity	$\mu\text{S}/\text{cm}$	6390	1379	3837	200 – 800
5	Calcium	mg/L	824.18	220	675	135
6	Magnesium	mg/L	214.70	68	199	11
7	Chloride	mg/L	2824.65	564	2202	150 – 600
8	Sulfate	mg/L	1130.80	372	960	250
9	Sodium	mg/L	1890.40	352	1474	270
10	Potassium	mg/L	850.70	200	714	8
11	Phosphate	mg/L	4.66	3.59	4	0.1
12	Chemical Oxygen Demand (COD)	mg/L	43.82	9.71	33	4.5



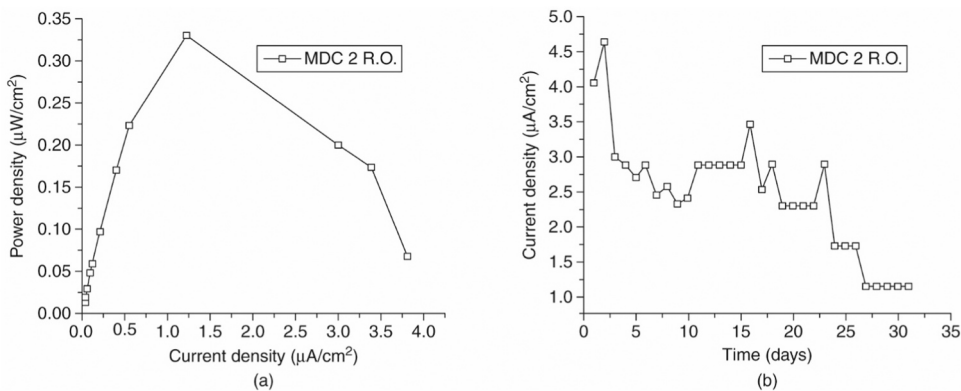


Fig. 1. (a) Power density ( $\mu\text{W}/\text{cm}^2$ ) vs current density ( $\mu\text{A}/\text{cm}^2$ ) and (b) current density ( $\mu\text{A}/\text{cm}^2$ ) vs time in days observed in batch MDC using domestic R.O. reject water.

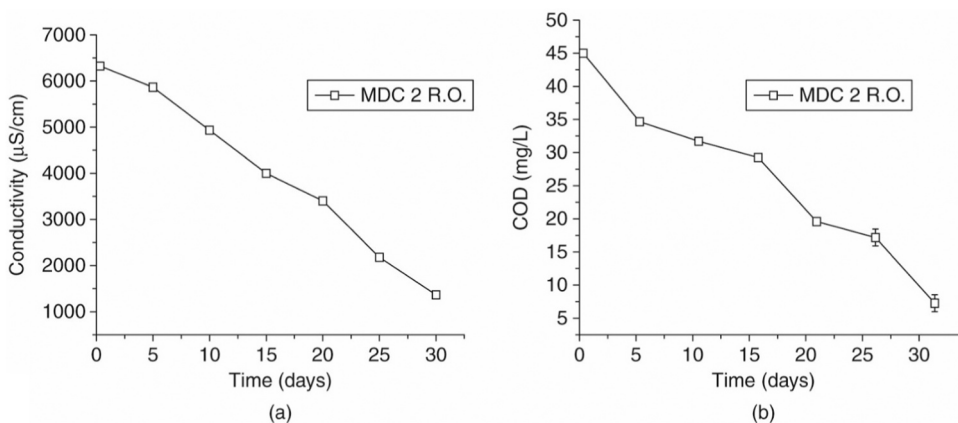


Fig. 2. Shifts in conductivity (a) and COD (b) of domestic R.O. reject water in desalination chamber of batch MDC during 30 days of initiation.

examination, each MDC system underwent control trials using pure NaCl solution in the middle chamber to potentially correlate such effects. The initial feeding solution conductivity was set to the same level as the working MDCs to provide equal solution resistance (Jacobson et al., 2011).

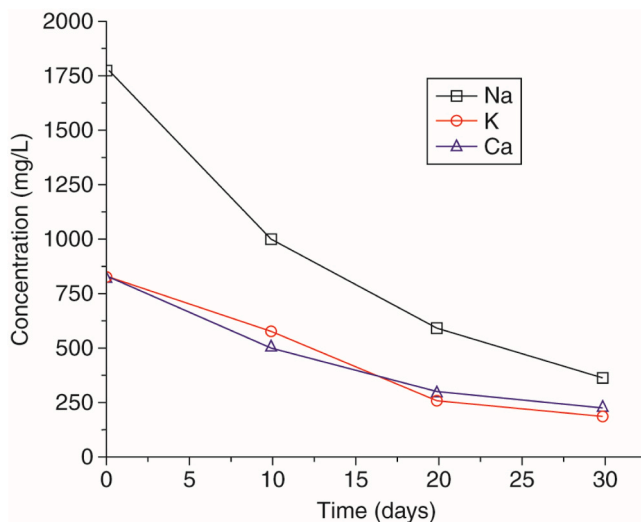


Fig. 3. Shifts in the proportions of Na, K, and Ca in batch MDC treating domestic R.O. reject water over 30 days, as determined by ICP-MS.

These findings support Jacobson et al.'s discovery that the salt removal efficiency of pure NaCl solution was better than that of artificial seawater, which is made from sea salt and contains numerous ions. The study indicates that the accumulation of divalent cations in the interim chamber hampers both MDC power production and salt removal efficiency. Additional research is warranted to elucidate their transport mechanisms, essential for the advancement and practical implementation of MDC technology (Jacobson et al., 2011).

Over 30 days, the concentration of calcium ions drops from 817 mg/L to 220 mg/L, sodium ions from 1760 mg/L to 352 mg/L in the domestic R.O. reject desalination process, and potassium ions from 825 mg/L to 187 mg/L, for a respective reduction of 73, 79, and 77 percent as measured by ICP-MS (Fig. 3) (Dongre et al., 2022a). Enthusiastically, a COD reduction (84 %) (Fig. 2b) and median desalination percent of Na, K, and Ca anions (76 %) (Fig. 3), identifies an encouraging direction for salt-containing wastewater remediation, along with the availability of a buffering zone between both electrode compartments, housing the domestic R.O. reject water (Ebrahimi et al., 2018; Kadier et al., 2016). Because of the broad spectrum of ions constituting domestic R.O. reject water (Table 1) and the variety of microorganisms within cow dung that may eventually use it, the biocathode-based MDC may accomplish the optimum desalination for domestic R.O. reject water (Dongre et al., 2022a).

Compared to prior studies, where two MDC setups 1 (Ferricyanide redox) and MDC setup 2 (Bio-catholyte) were examined at the laboratory size, this conclusion is consistent and productive. Experiments were conducted using simulated seawater (NaCl 5 g/L) with an initial electric conductivity of 9010 S/cm. Furthermore, the MDC setup 2 proved more effective in salt removal while treating home R.O. reject water with an initial electric conductivity of 6390  $\mu\text{S}/\text{cm}$  (Dongre et al., 2022a). MDC setups 1 and 2 reported maximum power densities of 0.212 and 0.221  $\mu\text{W}/\text{cm}^2$  and maximum current densities of 3.117 and 3.291  $\mu\text{A}/\text{cm}^2$ , respectively (Dongre et al., 2022a). This was recorded after 31 days of using the MDC. When compared to MDC setup 1 (ferricyanide redox), MDC setup 2 (Bio-catholyte) showed an increase of 5.6 % and 4.2 % in current and power density, respectively (Dongre et al., 2022a). This may be due to the mixed culture of microorganisms in the cow dung slurry, which can aid in the cathode reactions, providing more capacity to run the desalination process (Dongre et al., 2022a).

The chemical evaluation of residential R.O. reject water revealed a diversity of ions and salts (Table 1). This residential R.O. reject water was treated in MDC setup 2 and revealed more salt removal than simulated seawater treatment (MDC setups 1 and 2) containing only NaCl salt (Dongre et al., 2022a). In 31 days, MDC setup 2 treated domestic R.O. reject water to a maximum power density of 0.331  $\mu\text{W}/\text{cm}^2$  and a current density of 4.63  $\mu\text{A}/\text{cm}^2$  (Dongre et al., 2022a). We evaluated the desalination performance of both systems. We identified the substantial technological limitations while treating simulated seawater and home R.O. reject water using two equivalent MDC experimental setups with different cathode techniques (ferricyanide redox and biocathode) (Dongre et al., 2022a).

Despite having one order of magnitude higher salt removal rates than a ferricyanide redox method, the biocathode approach surpasses ferricyanide redox in simulated sea water. When using household R.O. reject water, the biocathode MDC approach also produced good results, adding to the reproducibility of the experimentations.

## 9. Scale up domestic R.O. reject water treatment

Furthermore, three identical batch mode MDC, which address domestic R.O. reject, are arranged in a diagonal stack, and the desalination chambers and the electrodes of all three are linked in series, denoted MDC (a), (b), and (c) (see supplementary material). A 5 L reject water reservoir supplies into the desalination chamber of MDC (a), which flows into the desalination chamber of MDC (b), which goes into the desalination chamber of MDC (c), and so on until it reaches a treated water reservoir. When the desalination chamber is connected initially in series with a predetermined flow rate (20 mL/hr), and the flow is retracted by a stop clamp for 30 days, all three MDCs produce a total treating volume of 300 mL. The stop clamp is only disengaged on day 30, so when electrolyte conductivity in each of the three separate desalination chambers reaches 1000  $\mu\text{S}/\text{cm}$ , linking the desalination chambers of MDCs (a), (b), and (c) in serial flow rate of 20 mL/hr. This switches all three MDCs from batch to continuous flow mode, with a hydraulic

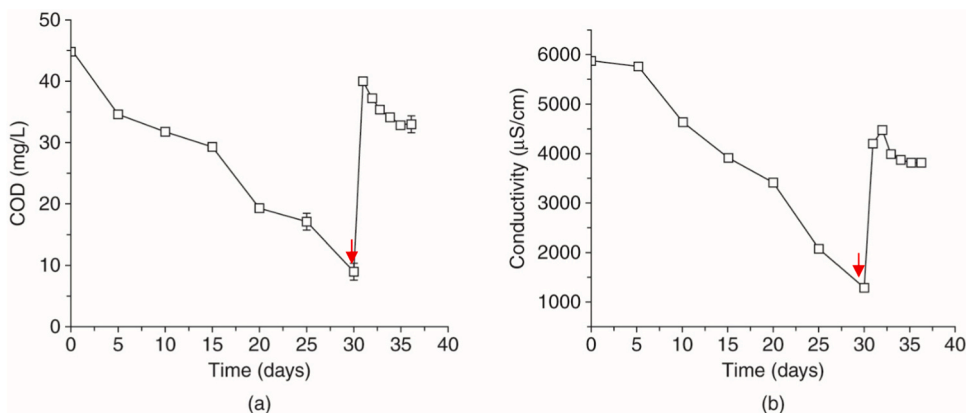


Fig. 4. Change in (a) COD (mg/L) and (b) conductivity ( $\mu\text{S}/\text{cm}$ ) vs time (days) for scale-up MDCs (arrow marks the point at which the MDCs were shifted to continuous mode).

retention time of 500 mL per day. This is done to maximize microbial activity in the anode and cathode chambers of the MDC while recharging the ionic strength in the desalination chamber, which is a limiting factor for the MDC's power output. The retainer clamp controller, in conjunction with the micro-intravenous line, controls this gravity-driven flow of liquid. The flow rate is regulated to 20 drops per minute (5 drops/15 seconds) by physically counting the drops in the drip chamber connected to the R.O. reject reservoir before the MDC (a). The micro-intravenous tube has a standard flow rate of 60 drops/mL (Pierce et al., 2013).

This assembly is then run for 6 days to measure fluctuations in COD and conductance in all of the MDC's desalination chambers and, after day 6, in the treated water reservoir. The starting COD and conductivity were 44.5 mg/L and 5899 (S/cm) in the R.O. reject reservoir. The COD and conductivity of the desalination MDC were around 9.12 mg/L and 1312  $\mu\text{S/cm}$  on day 30 of the three units MDC starting, right before the three MDC arrangement was switched to a continuous mode with a 20 mL/hr flow rate. The COD and conductivity in the desalination chambers of the scaled-up MDC increased to 40.7 mg/L and 4213  $\mu\text{S/cm}$ , accordingly, on day 31 (Fig. 4a and b). The inflow of R.O. caused this reject water in MDC units and the desalinated electrolyte passed through and drained into the treated water reservoir. The MDCs maintained a steady range of COD and conductivity from day 2 onwards but in a declining manner. The overall flow rate was 20 mL/hr, which resulted in 500 mL of R.O. reject water being treated every day starting on day 31, which brought the total volume of treated wastewater up to 3 L in 6 days with only a 25 % and 34 % drop in COD and conductivity, correspondingly (Fig. 6a and b). Compared to the 100 mL batch reactor MDC, this technique reduces the time necessary to treat wastewater while increasing the volume of treated wastewater at the expense of a substantial reduction in desalination rate.

During the primary 30-day batch mode operation, the three matchings serially connected MDCs with their separate desalination chambers, also associated in series with a steady flow rate of 20 mL/hr exhibited a maximal power density of 0.45  $\mu\text{W/cm}^2$  and a current density of 5.31  $\mu\text{A/cm}^2$ . During the continuous mode of operation, the highest current and power density were around 0.33  $\mu\text{W/cm}^2$  and 3.24  $\mu\text{A/cm}^2$ , respectively (Fig. 5a). The power and current densities exhibited a consistent pattern for the following four days, with slight fluctuations indicating a continuing desalination process treating R.O. reject water.

In contrast, the OCV pattern indicates that microbial culture growth in MDCs has decreased till day 30 (Fig. 5b). The strain *B. velzensis*, which was inoculated in the anode chamber as well as a subset of the cow dung slurry (was isolated from the exact slurry), has the prospects for salt tolerance and elevated auto-aggregation on hydrophobic interfaces, suggesting the potential to non-specifically bind to hydrophobic interfaces in aqueous solutions, such as the graphite electrode utilized in the MDC setups (Emam and Dunlap, 2020). *B. velzensis* has this ability, which aids in the creation of stable biofilm and the inclusion of multicellular biofilm, as in the instance of cow dung slurry. The serially connected MDC setup was converted to continuous mode after day 30 along with the addition of enriched *B. velzensis* strain AD1-ELB and cow dung sludge (50 % v/v) and removing the stop clamp, resulting in a flow rate of 20 mL/hr across all three desalination chambers. The *B. velzensis* strain AD1-ELB strain used in the entire study in the anode chamber has been isolated and identified from the same cow dung sample by culturing in a single chamber microbial fuel cell setup with aluminum electrodes. The visualization of bacterial biofilm formation on the electrode of the batch MDC via FESEM imaging reveals increased microbial biofilm growth on day 30 compared to day zero, providing evidence of microbial viability throughout the experiment (see supplementary material). During the first screening of isolates from cow dung, 8 isolates were discovered and kept as glycerol stocks before being treated to the ferrocyanide reduction test separately, with only AD1-ELB efficiently reducing ferrocyanide to ferro-thiocyanide (Dongre et al., 2022a,b). The isolate AD1-ELB tested positive for the Voges-Proskauer test as well as hydrolyzed starch (Dongre et al., 2022). It also shows the ability to metabolize citrate, xylose, dextrose, glucose, fructose, and mannitol (Dongre et al., 2022b). The isolate AD1-ELB's morphological characterization and physiological and biochemical studies showed a link to methylophilic bacillus (Dongre et al., 2022a,b). According to the findings, isolate AD1-ELB is connected to the *B. velzensis* strain. Based on nucleotide homology and phylogenetic analysis, the 16 s RNA study showed 99 % of the same. The strain AD1-ELB's 16 s rRNA gene sequence (1467 bp) was submitted to NCBI GenBank under the accession number MN006624 (Dongre et al., 2022b).

This reduced OCV from 512 mV to 456 mV as the enriched culture was introduced and adapted in both electrode chambers throughout the following period. The OCV then climbed to 502 mV and remained steady until day 36, when it decreased somewhat (Fig. 5b). On day 31, the current and power density rises, followed by a similar fall pattern. During the 36 days, the maximal power and

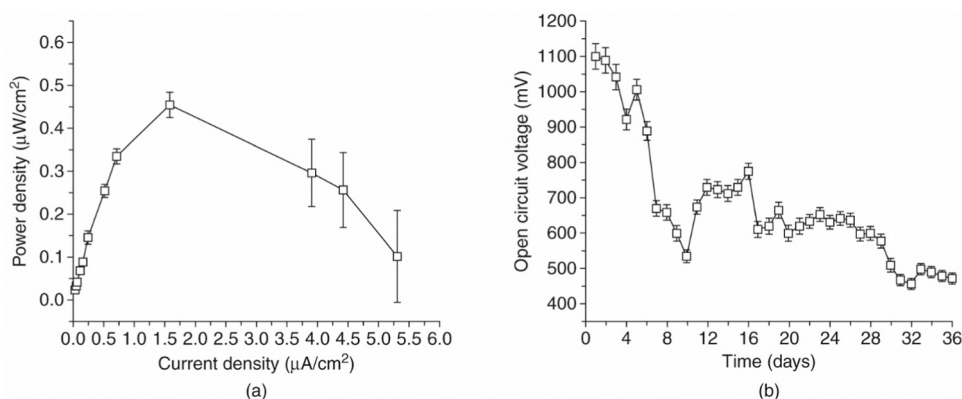


Fig. 5. Change in current density vs power density (a) and open circuit voltage (b) for scale-up MDC (arrow marks the point at which the MDCs were shifted to continuous mode).



current density were  $0.45 \mu\text{W}/\text{cm}^2$  and  $5.31 \mu\text{A}/\text{cm}^2$ , respectively (Fig. 5a). For the domestic R.O. reject water after 6 days of treatment, there was a 25% and 34% reduction in COD and conductivity (Figs. 4a and b), accordingly, as well as a drop in TDS from 12,660 mg/L to 9115 mg/L (Fig. 6a). This also comprises the 22, 18, and 16 percent decreases in sodium, calcium, and potassium ion concentrations in domestic R.O. reject water (Fig. 6b).

The analysis involved comparing the transport rates of various ions in the MDC system by examining the variations in the ratio of residual concentration over the beginning concentration in the middle chamber as a function of time. The ions transfer out of the middle chamber, causing the normalized concentrations to drop.  $\text{Ca}^{2+}$  concentration fell somewhat quicker than  $\text{Na}^+$  concentration. The size and charge effects may account for the transport competition and selectivity of monovalent and multivalent ions in the cations-MDCs because bigger ions have been shown to be sterically inhibited while traveling through the membranes (Van der Bruggen et al., 2004). As per a similar study, the radius of the hydrated  $\text{Mg}^{2+}$  ion is 0.429 nm, the biggest of the three cations, while the radius of the hydrated  $\text{Na}^+$  ion (0.365 nm) is slightly more significant than the radius of the  $\text{Ca}^{2+}$  ion (0.349 nm) (Firdaous et al., 2004). It should be noted that precipitation of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  from the solution might affect the transport behavior of divalent ions predicted using the hydrated radius. Because the anions-MDC had similar radii ( $\text{SO}_4^{2-} = 0.300 \text{ nm}$ ,  $\text{Br}^- = 0.330 \text{ nm}$ ,  $\text{Cl}^- = 0.332 \text{ nm}$ ), the association between ion size and transfer rate was not visible, most likely because the pore size of the typical grade membranes is more significant than the examined ions (Firdaous et al., 2004). This scale-up method resulted in a reduction in overall time, a rise in the amount of water treated and an increase in MDC process life at the expense of a decreased desalination rate. This can be further enhanced by installing more units to the present arrangement with optimal flow rate and recirculation techniques.

Moreover, due to design constraints, the variation in the desalination chamber was analysed in batch mode in initial experimentation. The study's main goal was to record the change in pH, COD, etc. of the R.O. reject water to determine whether it is effectively desalinated by the microbial desalination cell.

The voltage reversal is a serious challenge in serially connected microbial desalination cells, reducing power output and damaging individual cells. Fortunately, for this study, the MDCs units were connected with identical components, resulting in all units having equivalent internal resistances and fuel capacity, reducing potential imbalances. In addition, the flow rate of the R.O. reject water was closely optimized to provide even distribution to all cells, preventing localized ion depletion, while proton exchange membranes with excellent selectivity were used to minimize internal resistance and reversal potential. Future research will include all data pertaining to change in all three chambers at the same time including microbial parameters as well and the biofouling process of the membranes in use.

Yogamoorthi et al. (2018) proved the viability of creating renewable energy attributed to mainly two aspects — First, the composition of the cow manure employed as a growing medium, and second, the catholyte's potency (2 %  $\text{KMnO}_4$  solution) they utilized (Thiagarajan et al., 2018; Yogamoorthi et al., 2018). Fresh cow manure was treated before being used in the MFC (5% w/v slurry) to standardize the composition of the cow dung used in this experiment. Cow excrement was gathered and kept at room temperature for three days in an uncovered dish. The cow dung's hardened surface helped create an anaerobic environment beneath the top layer. Fresh cow dung's anaerobic microbial population increased after this pre-treatment (Thiagarajan et al., 2018). Given the acidic climate, only a specific type of microbial growth could occur, and those microbes flourished by digesting the organic stuff in the cow waste. Abubakari et al. (2019), reported that their MDC reduced COD by up to 49 %, which could be attributed to methane production and fermentation. Other electron acceptors such as oxygen ( $\text{O}_2$ ) and nitrates ( $\text{NO}_3^-$ ) have also contributed towards lower coulombic efficiency being reported. In a standard MDC batch experiment in their research, the MDC eliminated about 1 % of the nitrate because of heterotrophic denitrifying bacteria. Also, approximately 10 % phosphorus elimination was witnessed, which was linked to the presence of polyphosphate-accumulating bacteria (Yogamoorthi et al., 2018). Because of this, it's possible that phosphorus reduction took place at the start of the trial before even the MDC's anaerobic conditions were achieved (Dongre et al., 2022a).

In an MDC arrangement, cow dung-based bacteria may use various ions. Furthermore, cow dung is not expensive; if biocathode MDC demonstrates an improved efficiency towards salt reduction along with power generation in a microbial desalination setup, it would have the added benefit of being replenished inexpensively at the moment of its exhaustion (Sethia et al., 2015).

Cow dung, the waste of bovine animals such as domestic cattle, yak, and water buffalo, consists mainly of undigested plant matter

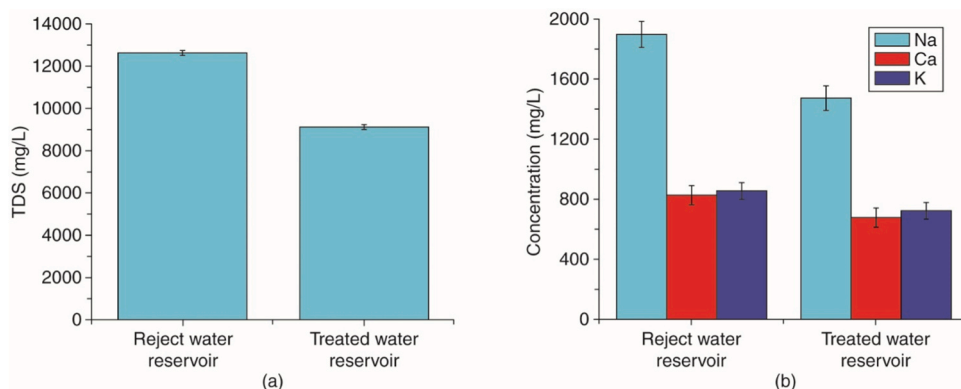


Fig. 6. Change in (a) TDS (mg/L) and (b) (Na, Ca and K) ions (mg/L) for scale-up MDC from day 31 to day 36.

(80 %), residues (14.4 %), and microbes (5.6 %) post-digestion. Its pH typically ranges between 7.1 and 7.4 (Radha and Rao, 2014; Garg and Mudgal, 2007). The part of fecal matter obtained from bovine rumen enriches cow dung components with bile pigments (biliverdin), intestinal microorganisms, and mucus. Biologists have been interested in the microbiological diversity of cow dung, known as coprophilous organisms (Kim and Wells, 2016). Cow dung contains beneficial microorganisms, primarily bacteria (bacilli, lactobacilli, and cocci), actinomycetes, fungi, and yeast (Sharma and Singh, 2015). It harbors approximately 60 species of bacteria (*Lactobacillus* spp., and *Corynebacterium* spp.), fungi such as *Aspergillus*, about 100 species of protozoa, and yeasts like *saccharomyces* (Gupta et al., 2016).

Although bacteria and fungi play a role in the composting of cow dung, bacteria are more predominant (Holman et al., 2016; Zhou et al., 2023). *Bacillus*, *Bifidobacterium*, and *Lactobacillus* are among the bacteria typically found in the cow stomach (Wang et al., 2023). Based on 16 s rRNA gene sequence analysis, Velázquez et al. (2004) isolated *Paenibacillus Flavivirus*, a unique bacterium, from fresh and aged cow dung (Velázquez et al., 2004). Adegunloye et al. (2007) explored compost microbiological analyses utilizing cow dung as a booster. Various microbes, including *Bacillus species*, *Proteus mirabilis*, *Enterobacter aerogenes*, and others, were found in compost and cow dung (Adegunloye et al., 2007). *Bacillus safensis*, *Bacillus cereus*, *Lysinibacillus xylanilyticus*, and *Bacillus licheniformis* were identified from cow dung, potentially forming an electrogenic microflora consortium (Radha and Rao, 2014).

A microorganism's ability to generate electricity or utilize electrons remains an intriguing and fast-expanding topic. One impediment to discovering novel electrogenic microbes is a scarcity of methods for reliably determining the electrogenic ability of a microbial species (Slate et al., 2019). Electroactive microorganisms are phylogenetically diverse, making it challenging to establish ribosomal RNA-base molecular biology techniques to help in their identification. Furthermore, unlike *ppk1* or *amoA* gene markers are specific for phosphorous-accumulating or ammonia-oxidizing microbes (Logan et al., 2019).

Technical applications based on electron-transporting microbes are still in their infancy, but breakthroughs in novel materials emerged. As the science of electron transfer strategies advances, established MFC and MEC innovations are expected to improve, leading to the development of novel applications targeting the various extraordinary abilities of these remarkable electroactive microbes (Dhanda et al., 2023). This progress will be achieved while harnessing bioenergy (electricity) from the treatment of saline reverse osmosis reject water and focusing on biofilm engineering and electrode optimization in future research (Dhanda et al., 2023). As previously indicated, most MDCs incur significant costs for reagents and materials. As a result, stacking desalination chambers in MDCs can be a game-changer. In turn improving performance and bioenergy generation through a variety of methods. Firstly, it increases the surface area accessible for ion exchange, which improves desalination efficiency (Lin et al., 2023). Secondly, the stacked arrangement allows for sequential treatment of the supply water, which improves the removal of salts and other contaminants (Feng et al., 2023). It also encourages greater utilization of available microbial populations, hence optimizing electron transport and bioenergy production (Manikandan et al., 2023). Furthermore, the stacked chambers allow for effective mixing of reactants and products, which improves mass transfer rates and overall system efficiency (Lv et al., 2023). The 6-day timeframe provides for a quick assessment of early desalination efficiency, microbial activity, and any potential concerns or challenges that arise. It gives a brief idea of the MDC's potential to lower chemical oxygen demand (COD), conductivity, and total dissolved solids (TDS) in a shorter timescale, allowing for faster decision-making on its scalability and real-world applicability. Furthermore, short-duration trials are more resource-efficient and time-saving, allowing researchers to iterate and modify MDC design and operating settings more quickly. This technique is consistent with the agile model commonly used in scientific research, emphasizing iterative testing and rapid feedback loops to accelerate innovation and improvement in microbial desalination technology (Aziz et al., 2024).

Furthermore, the characterization of biofouling in MFCs encompasses parameters such as membrane surface roughness, porosity, and hydrophilicity, which collectively influence microbial biofilm adhesion and development, and these parameters remained unaltered compared to fresh membranes after a 6-day trial period (Ahmed et al., 2023). Thus, future research will examine into this when stacked MDCs are performed for 15 days or longer. Surface modifications to lessen adhesion, regular membrane cleaning or replacement, and the application of antifouling chemicals will all be used to combat biofouling (Elkhatat and Qiblawey, 2024; Zikalala et al., 2023). As a result, operational conditions are improved, allowing MFCs to function longer. Recent advancements in membrane technology and microbial optimization fuel this innovation, pushing beyond previous limitations of stacked chambers. The future research should focus on low-cost and effective methods for the revival and reuse of essential MDC components, most ordinarily as using innovative sources of electrogenic bacteria, inexpensive electron transport intermediaries, etc.

## 10. Conclusion

The present study aimed to enhance the treatment efficiency of domestic R.O. reject water through the implementation of a batch mode Microbial Desalination Cell (MDC), followed by the transition to a scaled-up continuous mode of operation. This approach represents an endeavor to refine the performance of a microbial desalination cell for the treatment of domestic R.O. reject water and to achieve more efficient desalination processes. In the batch mode MDC, a notable reduction in chemical oxygen demand (COD) and conductivity was observed, indicating effective desalination of the reject water (Dongre et al., 2022a). The power density achieved in the batch mode MDC was  $0.331 \mu\text{W}/\text{cm}^2$ , with a corresponding decline in current density over 30 days of operation (Dongre et al., 2022a). This decline in current density was attributed to the electron transmission mechanisms facilitated by microbial activity and diverse chemicals present at the biocathode surface, influencing COD reduction and conductivity (Ebrahimi et al., 2018; Naaz et al., 2023).

Comparatively, previous studies have explored different MDC setups for desalination purposes. For instance, Dongre et al. (2022) compared two MDC setups, with MDC setup 2 employing a biocathode approach, which proved more effective in salt removal compared to MDC setup 1. The use of a biocathode, particularly with a mixed culture of microorganisms from cow dung slurry,

enhanced desalination efficiency, leading to a 5.6 % increase in current density and a 4.2 % increase in power density compared to the ferricyanide redox method (Dongre et al., 2022a). Moreover, the scaled-up continuous mode operation of the MDC showed promising results in maintaining desalination efficiency while increasing the treated water volume. The transition to continuous mode after 30 days of batch mode operation resulted in a steady power and current density pattern, indicating sustained desalination activity (Dongre et al., 2022a). Additionally, the utilization of cow dung-based bacteria, such as *B. velenzensis*, contributed to stable biofilm formation and enhanced desalination performance, as evidenced by the reduction in COD, conductivity, and total dissolved solids (TDS) (Dongre et al., 2022b).

Furthermore, the composition and microbial diversity of cow dung offer unique advantages for microbial desalination applications. Cow dung contains a variety of beneficial microorganisms, including bacteria, fungi, and yeast, which can contribute to electricity generation and electron transport in MDCs (Wang et al., 2023). The diverse microbial population present in cow dung, such as *Bacillus*, *Bifidobacterium*, and *Lactobacillus*, offers potential for bioenergy production and desalination efficiency (Adegunloye et al., 2007; Velázquez et al., 2004). Additionally, the implementation of stacked MDCs provides opportunities for improved desalination performance through increased surface area for ion exchange, sequential treatment of supply water, and enhanced utilization of microbial populations (Lin et al., 2023; Manikandan et al., 2023). Overall, the utilization of cow dung-based bacteria and innovative MDC configurations holds promise for advancing microbial desalination technology and addressing challenges in domestic R.O. reject water treatment (Dongre et al., 2022a; Sharma and Singh, 2015). This study establishes the framework for future research into optimizing MDC technology for more widespread use in water treatment, resource recovery, and bioenergy generation. The respective E-supplementary data for this work can be found in the e-version of this paper online.

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## CRedit authorship contribution statement

**S.L. Kothari:** Conceptualization. **Ashwag Shami:** Formal analysis. **Aman Dongre:** Writing – original draft. **Ahmad Faizal Abdull Razis:** Funding acquisition. **Shahanavaj Khan:** Investigation, Funding acquisition. **Mohammad Alsaad:** Formal analysis. **Salah-Ud-Din Khan:** Investigation. **NITESH KUMAR PODDAR:** Writing – review & editing, Supervision.

## 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.

## Data availability

The authors do not have permission to share data.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version.

## Appendix B. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.eti.2024.103664](https://doi.org/10.1016/j.eti.2024.103664).

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