SIMULTANEOUS WASTEWATER AND SALINE WATER TREATMENT WITH POWER GENERATION USING PHOTOSYNTHETIC MICROBIAL DESALINATION CELL

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A thesis submitted in partial fulfillment of the requirement for the degree of Master of Science in Environmental Science

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"Simultaneous Wastewater and Saline Water Treatment with Power Generation Using Photosynthetic Microbial Desalination Cell"

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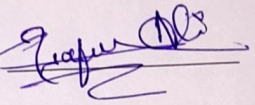
Signature of student: 

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Dedication

This research is dedicated to my parents, siblings and uncle, whose unwavering love, endless encouragement, and boundless support, efforts and sacrifices have made my dream of having this degree a reality. Words cannot adequately express my deep gratitude to them.

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Syeda Safina Ali

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ACRONYMS

ABR	Anaerobic Baffled Reactor
AEM	Anion Exchange Membrane
ASP	Activated Sludge Process
AMNRC	Advanced Microbial Nutrient Recovery Cell
BBM	Bold's Basal Media
BCMDC	Bio-Cathodic Microbial Desalination Cell
BES	Bio-Electrochemical System
BG11	Blue Green 11 Media
BOD	Biological Oxygen Demand
CDI	Captive Deionization
CE	Coulombic Efficiency
CEM	Cation Exchange Membrane
CNP	Carbon Nitrogen Phosphorous
COD	Chemical Oxygen Demand
CPMDC	Continuous Photosynthetic Microbial Desalination Cell
DO	Dissolved Oxygen
EC	Electrical Conductivity
EDI	Electro Deionization
EDTA	Ethylenediaminetetraacetic Acid
EDX	Energy Dispersive X-Ray Spectrometry
EGSB	Expanding Granular Sludge Bed
ER	External Resistance
FO	Forward Osmosis
HCl	Hydrochloric Acid
HRT	Hydraulic Retention Time
IEMs	Ion Exchange Membranes
IR	Internal Resistance
MDC	Microbial Desalination Cell
MEDC	Microbial Electrolysis and Desalination Cell
MEDCC	Multiple-Effect Distillation Coupled With Capacitive Deionization
MFC	Microbial Fuel Cell

mg/L	Milli gram per liter
MODC	Microbial Osmotic Desalination Cell
MSF	Multi-Stage Flash Desalination
mV	Millivolts
NaCl	Sodium Chloride
OCV	Open Circuit Voltage
OLR	Organic Loading Rate
ORP	Oxidation Reduction Potential
ORR	Oxygen Reduction Reaction
PBS	Phosphate Buffer Solution
PCRWR	Pakistan Council of Research for Water Resources
PMDC	Photosynthetic Microbial Desalination Cell
RO	Reverse Osmosis
SBR	Sequence Batch Reactor
SEM	Scanning Electron Microscopy
SMDC	Static Microbial Desalination Cell
SMDDC	Submerged Microbial Desalination-Denitrification Cell
SWRO	Seawater Reverse Osmosis
TDS	Total Dissolved Solids
TEA	Terminal Electron Acceptor
TF	Trickling Filter
UF	Ultra Filteration
UASB	Upflow Anaerobic Sludge Blanket
UNDP	United Nations Development Program
WWAP	World Water Assessment Programme

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ABSTRACT

Traditional microbial desalination cells (MDCs) face challenges related to expense and reduced efficiency due to the necessity of expensive catalysts or aeration for reduction reactions at the cathode. A possible way to overcome these challenges is by employing microalgae as a biocatalyst in MDC's cathode chamber. This research aims at comparing the performance efficiency of photosynthetic MDC (PMDC) inoculated with a biocatalyst, Scenedesmus obliquus, in the cathode chamber against the conventional aerated MDC. The performance of these systems was examined by using various substrate concentrations of 2000, 4000 and 6000 mg/L at the anode and different salt concentrations of 15, 25 and 35 g/L at the desalination chamber. The results demonstrated that PMDC performed better than MDC in all the experiments, and optimum efficiency was attained at 4000 mg/L anode substrate concentration and 25 g/L salt concentration. PMDC achieved an average working voltage of 354 mV, 90.5% anode COD removal, and 27% desalination, which was 38.1%, 7.5%, and 17.4% higher than MDC at 4000 mg/L. Moreover, using 25 g/L salt concentration yielded an average working voltage of 354 mV, 90.5% anode COD removal, and 27% desalination in PMDC, which was 38.1%, 7.5%, and 17.4% higher than MDC. Furthermore, the PMDC system offers the additional benefits of nutrient removal and biomass production at the cathode chamber. These findings proved that, PMDCs outperformed MDCs in treatment efficiency, power generation, and desalination, making them a sustainable treatment option.

Keywords: Wastewater treatment, Photosynthetic Microbial desalination cell, Nutrient removal, Desalination, Biocathode, Substrate concentration

CHAPTER 1

INTRODUCTION

This chapter introduces the central theme of water treatment, highlighting the escalating demand for water driven by a growing global population and the consequential surge in wastewater production. It discusses the challenges faced by traditional water and wastewater treatment methods. This chapter then highlights the potential of Microbial Desalination Cells (MDC) as a noteworthy water and wastewater treatment option.

1.1 Background

The ever-growing population, urbanization, and industrialization globally have resulted in a massive demand for sustainable energy, an eco-friendly environment, and clean water (Jafary et al., 2020). Water is present abundantly on the Earth's surface, i.e., 1.4×109 km^3. However, 97% of this water has a high salinity of 35,000 mg/L (Zahid et al., 2022a). Of the remaining 3%, which is freshwater, only 1% is usable and unevenly distributed globally (Barahoei et al., 2021). Climate change and poor water management are also causing global freshwater ecosystems to decline and become saltier (Almutairi et al., 2021), straining the available freshwater reserves (Gujjala et al., 2022). According to the World Water Assessment Programme (WWAP), a 40% global water deficit by 2030 due to climate change is projected, emphasizing the importance of treating seawater, brackish water, domestic and industrial effluent, and other similar source to meet global water demands (Patel et al., 2021).

To meet global water demands, water procurement typically involves desalinating seawater and treating industrial and domestic wastewater (Akash et al., 2022). Traditional water and wastewater treatment technologies are often costly and energy-consuming (Barroso Soares, 2017). Such as the actual energy needed to desalinate typical saltwater (35 g/L of total dissolved solids) ranges from 1.8 to 2.2 kWh/m^3 (Sayed et al., 2020a), while the energy consumption for treating wastewater ranges from 0.3–2.1 kWh/m^3 (Gandiglio et al., 2017). Such high fossil fuel usage raises the costs and has detrimental effects on the environment. Therefore, there is a pressing need to explore alternative energy-efficient sources that can lower treatment costs and save the environment. One of the unconventional yet untapped

sources with immense potential is wastewater. It is particularly significant because it has an energy content of 1.8 to 2.1 kWh/m^3, which is similar to that required for typical seawater desalination. In contrast, it also contains nearly 5 to 10 times the energy needed for wastewater treatment. Thus, harnessing the energy from domestic wastewater for desalination and wastewater treatment purposes would offer both environmental and energy benefits (Sayed et al., 2020).

To date, several water and wastewater treatment technologies have been developed, encompassing traditional methods suitable for both portable and non-portable purposes. These include thermal and membrane-based desalination, chemical treatment, aerobic treatment, and anaerobic digestion (Gujjala et al., 2022; Guo et al., 2020). However, these methods have drawbacks, such as high energy consumption in desalination processes, particularly in thermal and membrane-based methods, leading to increased operational and water costs (Sayed et al., 2020a). Aerobic wastewater treatment also consumes substantial energy due to oxygen supply requirements and produces more sludge (Zahmatkesh et al., 2022). Some technologies, like anaerobic digestion, have energy-producing potential. Still, currently, only a small fraction of this potential (1.6-2.2%) is utilized for biogas production (World Biogas Association, 2019), with much of the produced biogas being flared instead of used for energy production (Gandiglio et al., 2017). Therefore, looking for cutting-edge technology that can offer many benefits in one place is crucial.

Microbial desalination cells (MDCs) represent an innovative bio-electrochemical system that utilizes the energy present in wastewater for co-energy production, wastewater treatment, and seawater desalination in a single device (Jatoi et al., 2022; Sevda et al., 2015; Yang et al., 2023; Zahid et al., 2022b). The MDC system operates through the collaborative functioning of its anode, cathode, and central desalination chamber. The anode biofilm degrades organic pollutants, aiding electron transport via an external circuit toward the cathode. An electrode potential difference drives desalination and energy recovery processes (Abd-almohi et al., 2022; Danaee et al., 2023; Ghasemi et al., 2022; Prakash et al., 2022; Rahman et al., 2022; Yang et al., 2019). In traditional MDCs, oxygen primarily acts as an oxidizing. However, its sluggish reduction kinetics require expensive platinum catalysts, resulting in reduced cost-effectiveness. Various other electron acceptors like

ferric cyanide, potassium permanganate, phosphate, and bicarbonate buffers are also utilized, but their high cost and toxicity pose limitations. Therefore, identifying alternative catholytes or catalysts is crucial for real-time MDC application (Imoro et al., 2021). Microalgae are recognized as potential bio-cathodes in MDCs, transforming them into PMDCs (Photosynthetic Microbial Desalination Cells). This shift eliminates mechanical aeration through the oxygenic photosynthesis conducted by microalgae (Prakash et al., 2022), using carbon, nitrogen, and phosphorous for biomass production while employing wastewater for cultivation. These features make PMDC a sustainable technology for energy generation and resource recovery.

Despite several advantages, MDCs and PMDCs are influenced by various critical factors, including substrate type and concentration, saline water conductivity, concentration gradient, operational modes, pH variations, membrane type and inter-membrane distance, and internal and external resistors.

While the previous studies have independently investigated PMDCs for the treatment of wastewater from both domestic and industrial sources, there is a notable lack of research on their combined treatment. This gap is significant given the prevalence of sucrose-based wastewater in industries like sugar, beverages, and brewery production. Furthermore, *Scenedesmus obliquus* remains relatively unexplored as a biocatalyst for treating domestic wastewater in the cathode chamber of PMDC. Additionally, there is a lack of comprehensive understanding regarding the impact of substrate concentration on microbial desalination cells, particularly in PMDC with high-strength wastewater. Most of the studies have predominantly examined anolyte wastewater with low anode substrate concentration in PMDC. Also, there is a notable lack of research investigating the influence of highstength wastewater treatment (sucrose/sugar) on the desalination performance of PMDCs, especially concerning various salt concentrations. Therefore, there is a need to examine the impact of substrate and salt concentration on PMDC performance, particularly for treating medium to high-strength wastewater, as substrate availability is crucial for maintaining steady-state operation.

1.2 Objectives of Research

The objectives of the current study include;

Main objectives

To design and construct two three-chambered rectangular MDCs for treating industrial and domestic wastewater and saline water using chemical and biological catholytes and compare their treatment efficiency and power production.

Specific objective

- To study the effect of different substrate concentrations of anode on the performance of PMDC.
- To study the effect of different saline water concentrations on the performance of PMDC.

1.3 Significance of the Research

This research addresses a crucial gap in understanding the impact of substrate and salt concentration in anode and desalination chambers on the performance of Photosynthetic Microbial Desalination Cells (PMDCs). It particularly focuses the pollutant removal and power generation for high-effluent wastewaters, with a focus on sugar industry wastewater as an example for industrial applications. The study delves into different MDC operational modes, emphasizing the potential advantages of utilizing fed-batch mode in both anode and cathode operations to enhance PMDC stability and performance. A novel aspect of the research involves investigating the use of microalgae, specifically *Scenedesmus obliquus*, in the cathode chamber of PMDC for the secondary treatment of domestic wastewater for nutrient recovery.

1.4 Scope of the Study

The scope of this current study includes;

- Construction of lab-scale rectangular three-chamber MDCs.
- Use of anaerobic sludge as an inoculum in anode and microalgae in the cathode.
- Use of sugarcane wastewater as a sole carbon source for anode (Substrate).
- Use of two different catholytes (bio-catholyte and PBS) for the best performance.
- Analysis of the MDC performance for power production, COD removal, desalination, and internal resistance.

CHAPTER 2

LITERATURE REVIEW

The chapter underscores the significance of water and wastewater treatment methods, with a specific focus on Pakistan's water and wastewater management, particularly addressing the effluent produced by the sugar industry. Within this framework, it highlights the potential of Microbial Desalination Cells (MDC) as a noteworthy treatment option. The chapter delves into the applications of MDCs since their inception in 2009, examining various studies conducted on different types of MDCs concerning their configurations, electrodes, membranes, and other factors. Moreover, it discusses research endeavors aimed at modifying MDC systems to enhance desalination rates, exploring studies focused on component adjustments and operational conditions.

2.1 The Water-Energy Nexus

Nearly 8 billion people live on Earth today, and by 2040, that number is expected to rise by 21% (Naseer et al., 2021). The ever-growing population, urbanization, and industrialization have elevated many concerns about water demand; thus, more wastewater has been produced anthropogenically, and freshwater is rapidly being depleted due to human and natural causes in many countries, including Pakistan. Nearly 25% of humanity today struggles with a lack of access to fresh water (Islam et al., 2018). Pakistan is on the brink of transitioning into a water-scarce nation, as indicated by the notable reduction in per capita water availability from 5260 m^3 in 1951 to 1000 m^3 in 2016 (Zhang et al., 2021). Projections by the United Nations Development Program (UNDP) and the Pakistan Council of Research for Water Resources (PCRWR) suggest that by 2025, this figure could further decrease to about 700 m^3, signifying a critical shift in the country's water availability status (Tahir et al., 2022). The situation could exacerbate in regions beyond the Indus basin, where the annual per capita water availability is less than 1000 m^3. Specifically, in areas like the drought-affected zones of Sindh Province, individuals without access to fresh water are compelled to utilize brackish water for domestic use (Azizullah et al., 2011). Nevertheless, according to the(O. P. Sahu & Chaudhari, 2015) World Bank (2019a), Pakistan has its water resources (Caldera et al., 2021) including zones

of fresh, brackish, and saline aquifers (Hasan et al., 2017) that can be used to meet the rising water demand.

Pakistan has a lengthy coastal line exceeding 1,046 km, providing access to diverse oceanic resources (Tahir et al., 2022). The World Bank claims that a water management strategy is investing more money in desalination infrastructure. Pakistan's total desalination capacity was about 188,168 m3 per day in 2015. 25% of the online capacity was provided by seawater reverse osmosis (SWRO), but almost half of the online capacity comprises brackish water desalination plants; brackish desalination is preferred because of the lower investment and energy costs. The issues with brackish desalination, however, still include the scarcity of sources of suitable feedwater and the locations for brine discharge disposal (Caldera et al., 2021). Therefore, it is essential to look for other unconventional water resources to meet the rising water demands; one such unconventional water resource is "wastewater" which remains an "untapped" and "undervalued" resource that is broadly defined as "used" water that has been contaminated as a result of human activities (Jones et al., 2021) and due the surge in population and industrialization has amplified the generation of wastewater, a significant portion of which is discharged into the environment without proper treatment. According to estimates, 359 billion cubic meters of wastewater are generated annually around the globe (Jones et al., 2021). In Pakistan, 4.36 billion m^3 of wastewater is generated annually, of which 3.06 billion m3 is domestic wastewater and 1.30 billion m^3 is industrial wastewater ("Wastewater Treatment Technology to Be Introduced in Major Cities," 2021.). Notably, residential sources contribute 73% of the wastewater, while the agriculture sector contributes 16%, and the industrial and commercial sectors contribute 6% and 5%, respectively. (Rasheed et al., 2020). It is estimated that around 70% of industrial wastes in developing countries are released untreated into water bodies, thereby contaminating existing water supplies (Qureshi et al., 2015)

2.2 Sugar Industry and Wastewater Production

Sugarcane production is experiencing rapid growth in response to the escalating demand for sugar and bioethanol. As a major agricultural sector, the sugar industry plays a crucial role in contributing significantly to the economies of numerous emerging nations (Fito et

al., 2019), including Pakistan. The well-organized sugar industry provides livelihoods for almost 10 million people in Pakistan, making up 16% of the country's agricultural economy. Pakistan is home to an estimated thirty sugar refineries along the Indus River's left bank (Qureshi & Mastoi, 2015). With every tonne of cane processed, the industry requires 1500–2000 dm^3 of water and generates 1000 dm^3 of wastewater. The primary sources of wastewater are floor washing, condensation, leakage, and the improper disposal of sugarcane, syrup, and molasses within various sections (Sahu & Chaudhari, 2015). In addition to having high COD, BOD, and TDS (Kushwaha, 2015), sugar industry wastewaters also contain small traces of metals and nutrients (Sahu, 2019). The significant mineral, sugar, and carbohydrate content in these wastewaters makes them ideal substrates for bacterial cultivation, leading to the generation of valuable products such as organic acids, enzymes, biomass, and biogas (Wang et al., 2020). Effluents from sugar processing typically have a BOD5 of 1,700 to 6,600 mg/L for untreated cane and 4,000 to 7,000 mg/L for beetroot. COD values range from 2,300 to 8,000 mg/L in cane processing and can reach 10,000 mg/L in beetroot processing. They may also contain high ammonium levels and up to 5,000 mg/L of TSS (Sahu & Chaudhari, 2015). Moreover, sugar-processing wastewater typically contains significant amounts of organic and particulate matter that often surpass environmental discharge standards, exacerbating its detrimental impact (Sahu, 2019b). Managing industrial wastewater has become a serious challenge worldwide due to the diverse range of pollutants generated by industrial processes, which are difficult to treat and come with high costs.

2.3 Conventional Wastewater Treatment Technologies

Different approaches and strategies are utilized in the treatment of municipal and industrial wastewater, commonly incorporating primary, secondary, and tertiary treatments (Gedda et al., 2021). The primary treatment primarily aims to eliminate organic and inorganic solids, whereas the secondary treatment focuses on the removal of fine suspended solids, dispersed solids, and dissolved organics. Tertiary treatment, also known as advanced treatment, further enhances the quality of water before it is discharged, employing biological, physical, and chemical methods. Depending on the techniques employed, the purification levels of water can reach up to 95%, and in certain instances, even 98-99%

(Zinicovscaia, 2016)

Physicochemical methods are essential in water treatment, including coagulation, flocculation, precipitation, adsorption, ion exchange, and membrane separation. Coagulation-flocculation plays a vital role in removing solids, color, and organic matter. However, the use of metal coagulants can result in the formation of chemical sludge and reduce disinfection efficiency (Teh et al., 2016). Precipitation effectively eliminates metal ions and phosphorus compounds but generates bulky sludge (Zinicovscaia, 2016). Ion exchange is an effective technique for removing heavy metals but faces challenges with high concentrations (Saleh et al., 2022). Adsorption, particularly with activated carbon, proves to be efficient but comes at a higher cost (Rashid et al., 2021). Membrane technologies like ultra filteration (UF), reverse osmosis (RO), and nano filteration (NF) offer selective removal of pollutants but are limited in widespread use due to cost and maintenance challenges (Iwuozor, 2019; Shoshaa et al., 2023; Zahid et al., 2022c; Zinicovscaia, 2016).

Biological treatment stands as a remarkably encouraging technology for the treatment of wastewater, demonstrating its effectiveness in treating nearly all types of wastewaters containing biodegradable components and a BOD/COD ratio of 0.5 or higher (Sandra et al., 2017). This approach effectively employs both aerobic and anaerobic processes, showcasing its versatility and potential. Aerobic treatment employs oxygen to convert organic waste into biomass and CO2, whereas anaerobic treatment breaks down organic waste into methane, CO2, and water in the absence of oxygen. Aerobic treatment is typically recommended for low-strength wastewaters (COD < 1000 mg/L), while anaerobic treatment is commonly employed for high-strength wastewaters (COD > 4000 mg/L) (Sangamnere et al., 2023). Although aerobic methods are effective in treating organic wastewater, they demand substantial energy and result in the generation of large amounts of sludge. Conversely, anaerobic treatment prioritizes resource recovery, requiring less energy and producing less sludge (Wang et al., 2022).

Over the years, a multitude of technologies have emerged for the treatment of wastewater. These technologies comprise aerobic oxidation ponds, trickling filters, activated sludge processes, and sequence batch reactors. Conversely, anaerobic wastewater treatment

systems encompass up-flow anaerobic sludge blanket reactors, expanded granular sludge bed reactors, anaerobic baffled reactors, anaerobic fixed bed reactors, integrated bio-methane reactors, and membrane bioreactors (Gujjala et al., 2022).

Aerobic oxidation ponds, trickling filters (TF), activated sludge, and sequence batch reactors (SBR) are all efficient in decreasing wastewater BOD levels. Nevertheless, aerobic ponds may face limitations due to land availability and specific climate requirements, whereas trickling filters might encounter blockage problems. Activated sludge processes (ASP) may not effectively manage resources and sludge, and newer versions of sequence batch reactors, such as continuous flow SBRs or membrane SBRs, necessitate skilled supervision (Sikosana et al., 2019). On the contrary, anaerobic treatment technologies such as upflow anaerobic sludge blanket (UASB) and anaerobic baffled reactors (ABR) offer alternative solutions by creating compact sludge beds to facilitate efficient biological processes and accommodate a wide range of effluent strengths. Nevertheless, these technologies may encounter challenges associated with fluctuations in biomass and the presence of odors. Expanding granular sludge bed reactors (EGSB) reactors improve the interaction between substrate and biomass, but they exhibit slow nutrient removal and the potential for odorous emissions. ABR provide versatility, yet they may struggle with biomass fluctuations and occasional odors. Anaerobic Fixed Bed Reactors (AFBR) are effective in handling varying organic loads, although careful management is necessary to prevent clogging. Integrated Bio-Methane Reactors, which combine anaerobic digestion and methane recovery, may face difficulties in gas production and maintaining stability. While anaerobic membrane bioreactors excel in pollutant removal, they are susceptible to fouling and require regular maintenance (Gedda et al., 2021; Sandra et al., 2017; Sangamnere et al., 2023).

2.4 Microbial Desalination Cell – An Emerging Technology

The Microbial desalination cell is an emerging technology introduced by (Cao et al., 2009). It functions as a bio-electrochemical system and serve as an extension to modified microbial fuel cell (MFC) technology, which typically consists of two chambers: an anode chamber and a cathode chamber (Imoro et al., 2021). Unlike the two-chambered MFCs, the MDC incorporates a third chamber called the desalination chamber. This chamber is

situated between the anode and cathode chambers with anion and cation exchange membranes positioned at a specified distance(s). The diffusion of ions across a membrane, guided by concentration gradients, serves as the driving force behind desalination process(Ghasemi et al., 2022). To further enhance the efficiency of desalination processes, microbial desalination cells (MDCs) have been devised based on the principles of microbial fuel cells (MFCs). These MDCs not only facilitate the treatment of wastewater and desalination of salt water but also contribute to energy generation, thereby reducing the reliance on electricity for desalination purposes.MDCs have gained considerable importance in the present era due to their ability to address two crucial challenges: wastewater treatment and water desalination. By harnessing the energy content present in wastewater, MDCs offer a unique advantage of combining both processes in a single device (Sevda et al., 2015; Zahid et al., 2022b). MDCs offer substantial energy savings compared to conventional methods like evaporation, distillation, and membrane processes. For instance, MDCs can save up to 4 kWh/m^3 of energy in comparison to RO, and the savings are even more substantial, reaching 196 kWh/m^3 when compared to thermal technologies like multi stage flash distillation (MSF) (Patel et al., 2021). The MDC technology offers a vivid representation of the water-energy nexus concept Through the utilization of energy produced by microbes in wastewater treatment, the MDC has the potential to operate autonomously without the need for external energy sources. Additionally, surplus energy generated by MDCs can be redirected to support other desalination technologies like RO, capative deionization (CDI), and electrodionization (EDI) systems (Imoro et al., 2021).

2.5 Working Mechanism of MDC

The system is structured with three chambers: the anode, desalination, and cathode In these chambers, electrical energy is generated by the movement of electrons outside the cell, when organic materials in the anode chamber undergo oxidation due to the presence of anaerobic bacteria on the anode electrode. This oxidation process produces carbon dioxide, protons, and free electrons. The free electrons then travel from the anode to the cathode through an external circuit. In the cathode chamber, the electrons are consumed in reactions involving a terminal electron acceptor. On the one hand, the electron transfer results in the production of bioelectricity, while the potential difference within the internal circuit

prompts the migration of ions across the exchange membranes in the desalination chamber, ultimately facilitating water desalination in the central chamber. The anode-cathode potential difference facilitates electron flow through the external electrical circuit, allowing for self-powered operation (Abd-almohi et al., 2022; Danaee et al., 2023; Ghasemi et al., 2022; Prakash et al., 2022; Yang et al., 2019), as shown in Fig. 2.1.

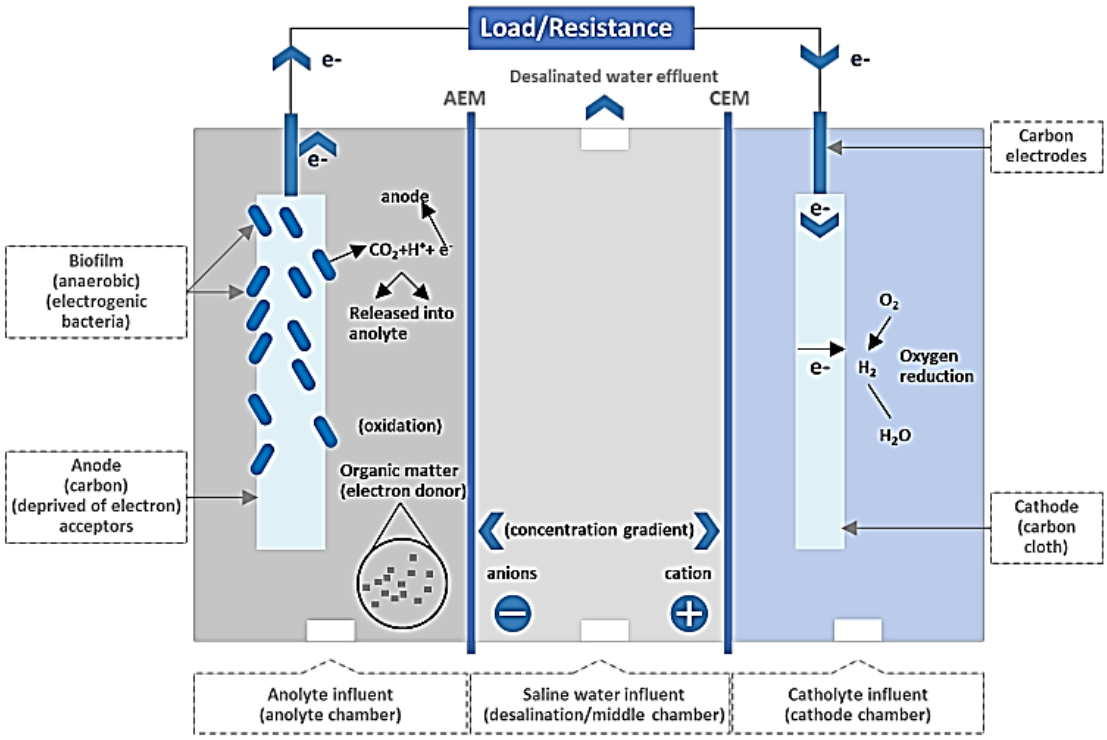


Figure 2.1 Traditional MDC configuration, source:(Jingyu et al., 2017).

2.5.1 Bio-electrochemical process in MDC

In the presence of bacteria, organic matter undergoes oxidation at the anode in wastewater, resulting in the release of carbon dioxide and protons into the anolyte. The electrons generated during this oxidation process (Eq 1) flow through the external electrical circuit, creating electricity. Oxygen in the cathodic chamber then accepts these electrons, causing reduction and the production of water (Eq 2). Hence, a potential gradient is established between the anode and cathode, causing anions like Cl^{-1} and SO_4^{2-} to move from seawater/saline water from middle chamber towards the anode through the anion exchange membrane for maintaining electroneutrality. This results in the production of a strong acid in the anode chamber, making it acidic (Eq 3). Meanwhile, the positively charged ions,

such as Na^+ and Ca^{2+}, migrate towards the cathode by passing through the cation exchange membrane. This migration results in the formation of a highly alkaline environment in the cathode chamber (Eq 4). As a result of the varying charges in the two chambers, anions and cations pass through their respective membranes, elevating the salt concentration in the anodic and cathodic compartment while reducing it in the central saline compartment (Eq 5). (Ashwaniy & Perumalsamy, 2017; Gujjala et al., 2022; Zahid et al., 2022b). However, the composition of seawater is not solely limited to Na^+ and Cl^- ions; it can also include HPO_4^-, PO_4^{-2}, Cl^-, K^+, and NH_4^+. Furthermore, when utilizing real wastewater, it may consist of several complex compounds such as $CaSO_4$, $CaCO_3$, $MgCO_3$, $Ca(NO_3)_2$, $Mg(NO_3)_2$, and $MgSO_4$ (Abd-almohi et al., 2022).

The following Chemical reactions take place at the respective chambers of MDC as described above

$$(CH_2O)\ n + nH_2O \rightarrow nCO_2 + 4ne^- + 4nH^+ \quad (1)$$

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

$$H^+ + Cl^- \rightarrow HCl \quad (3)$$

$$Na^+ + OH^- + e^- \rightarrow NaOH \quad (4)$$

$$NaCl \rightarrow Na^+ + Cl^- \quad (5)$$

2.5.2 Electron acceptors in MDC

In traditional MDCs, oxygen serves as the primary electron acceptor, but its reduction kinetics are sluggish, necessitating the use of expensive platinum to enhance redox kinetics. This approach is not cost-effective for real-time MDC applications. Other electron acceptors, such as ferric cyanide, potassium permanganate, phosphate buffer, bicarbonate buffer, etc., are also utilized in MDCs. However, the high cost and toxicity of these chemical catholytes restrict the widespread application of MDC. Therefore, identifying alternative catholytes or catalysts is crucial for practical real-time MDC implementation (Bejjanki et al., 2021). Hence, microalgae are recognized as potential bio-cathodes in the cathode compartment of MDCs, transforming them into PMDCs (Photosynthetic Microbial Desalination Cells). This shift brings several advantages, including eliminating mechanical aeration through the oxygenic photosynthesis conducted by microalgae. Notably, the in-situ oxygen production is an effective electron acceptor for bioelectricity generation

(Prakash et al., 2022).

2.6 Photosynthetic Microbial Desalination Cell

The Photosynthetic Microbial Desalination Cell (PMDC) is a sustainable system that modifies the Bio-Cathodic Microbial Desalination Cell (BCMDC) using algae as a biocathode, as shown in Fig 2.2. This innovative approach harnesses the power of algae to accept electrons, produce oxygen, generate valuable biomass for biofuels, and help reduce carbon dioxide in wastewater treatment. PMDCs efficiently tackle salt removal, wastewater treatment, and nutrient recycling, making them a promising solution for multiple environmental challenges.

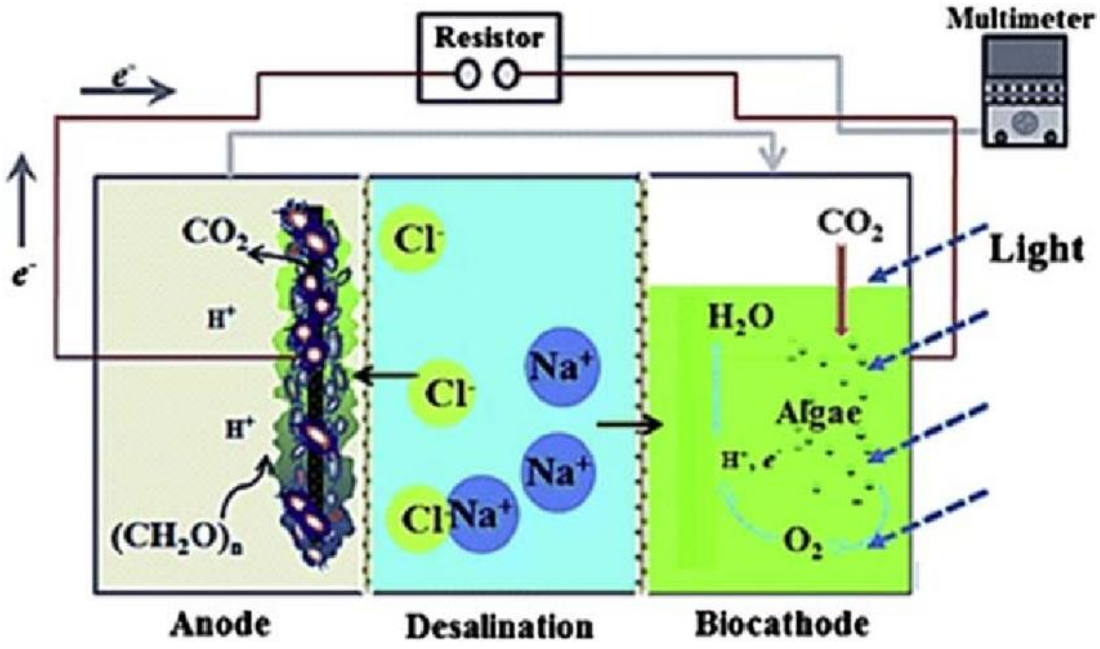


Figure 2.2 Photosynthetic Microbial Desalination Cell (PMDC), source: (Kokabian & Gude, 2019).

2.6.1 Microalgae used in PMDC

The foundational research by Kokabian and Gude (2013) marked a crucial moment in exploring sustainable water desalination and energy generation technologies, explicitly emphasizing the integration of algae as a viable and eco-friendly biocathode in PMDCs. Subsequent studies have since focused on employing algal biocathodes in MDCs, with *Chlorella vulgaris* emerging as the most prevalent algal specie in these investigations

(Arana & Gude, 2018; Barahoei et al., 2021; Ewusi-Mensah et al., 2021; Hui et al., 2020; Jaroo et al., 2019; Kokabian et al., 2018a; Kokabian et al., 2018b; Kokabian & Gude, 2013, 2015; Tyas et al., 2016; Zamanpour et al., 2017). Additionally, various other algal species have been employed to serve as oxygen sources in the cathode of PMDCs, such as *Chlamydomonas* sp. and *Scenedesmus* sp. (Nadzri et al., 2023), *Oscillatoria* sp. (Bejjanki et al., 2021), *C. pyrenoidosa*, (Neetu et al., 2019), *Scenedesmus abundans* (Ashwaniy & Perumalsamy, 2017), *Nannochloropsis salina* (Girme, 2014; Mahdi & Safi, 2016; Saba et al., 2017a), *Scenedesmus obliquus* (Danaee et al., 2023) and mixed culture of *Coelastrella* sp. and *Mariniradius saccharolyticus* (Sadeq & Ismail, 2023). Due to the dual benefits of desalination and bioenergy generation, microalgal-assisted MDCs are acknowledged as having the potential to generate environmental sustainability and economic benefits (Alseroury, 2018), as illustrated in Table 2.1

2.6.2 Different types of catholyte used at PMDC

Although wastewater has the possibility of dual application to be used in the anode and cathode concurrently, only a few studies have examined this feature of PMDC, such as Jaroo et al. (2019) successfully employed oil refinery wastewater as a nutrient medium for algae. However, the primary focus of PMDC studies continuous to be on utilizing wastewater as anolyte and algal cultivation medium as catholyte, such as BG11 medium (Ashwaniy & Perumalsamy, 2017; Neethu et al., 2019, 2019; Barahoei et al., 2021; Bejjanki et al., 2021; Hui et al., 2020; Sadeq & Ismail, 2023), Bold's basal medium (Danaee et al., 2023; Ewusi-Mensah et al., 2021; Nadzri et al., 2023), mineral solution (Arana & Gude, 2018; Kokabian et al., 2018a; Kokabian et al., 2018b; Kokabian & Gude, 2013, 2015; Zamanpour et al., 2017), Growth media with PBS solution (Jaroo et al., 2021) and F2 media (Girme, 2014; Mahdi & Safi, 2016). Since PMDCs use the photosynthetic and electrogenic capabilities of microalgae and bacteria within their anode and cathode compartments to treat two separate types of wastewater simultaneously, in addition to desalination, they stand out from traditional MDCs. Not only that, but PMDCs can take advantage of microalgae's intrinsic ability to extract nutrients (NP) from effluent in the cathode chamber, thereby facilitating its growth.

Table 2.1 Recent studies on Photosynthetic Microbial Desalination Cell.

Anolyte (A) **Catholyte (C)** **Saline Solution (S)**	**Anode Electrode (A) and Cathode Electrode (C)**	**Microalgae**	**Pollutant & Salt Removal** **COD (C)** **Desalination (D)** **NH_4^+-N (N)** **NO^{3-}N (N_A)** **PO_4^{3-}P (P)**	**Maximum Power Density (mW/m^2 or mW/m^3)** **Voltage (mV)**	**References**
A: Real sewage **C:** BG11 medium **S:** Real seawater	**A & C:** Plain graphite material	*Coelastrella* sp. and *Mariniradius saccharolyticus*	**C:** 91% **D:** 72%	430.7 mW/m^3	(Sadeq & Ismail, 2023)
A: Palm oil mill effluent **C:** BBM media **S:** 35 g/L NaCl solution	**A & C:** Graphite felt implanted with a titanium foil	*Chlamydomonas* sp. (UKM6) and *Scenedesmus* sp. (UKM9)	**C:** 53.1% (UKM9) and 49.3% (UKM6) **D:** 44.8% (UKM9) and 32.4% (UKM6)	1942 mW/m^3 (UKM9) and 1714 mW/m^3 (UKM6)	(Nadzri et al., 2023)
A: Synthetic Human fecal sludge **C:** BBM media **S:** 25 g/L NaCl Solution	**A & C:** carbon paper	*Scenedesmus obliquus*	**C:** 48% **D:** 60% **N:** 94% **P:** 8% (NP removal at Anode)	175.2 mW/m^2	(Danaee et al., 2023)

A: Hydroxide solution **C:** BG11 medium **S:** 12 g/L NaCl solution	**A**: Aluminum metal sheet **C:** Carbon felt disk	*Chlorella vulgaris*	**D:** 69%	32.4 W/m^3 (Anodic vol)	(Barahoei et al., 2021)
A: Diluted Dairy wastewater **C:** BG11 medium **S:** 10, 20 and 30 g/L NaCl	**A & C:** Plain graphite plate	Oscillatoria sp.	**C:** 78.2% (20 g/L) **D:** 65.8% (20 g/L)	44.1 mW/m^2 (20 g/L)	(Bejjanki et al., 2021)
A: Physically pre-treated real landfill leachate **C:** BG11 medium **S:** 15 g/L NaCl solution	**A & C:** Carbon fiber brushes	Chlorella vulgaris	C: 95% D: 3.93 mg/L/h	121.57 mW/m^2	(Hui et al., 2020)
A: Synthetic wastewater **C:** BG11 medium **S:** 2.5 and 5 g/L NaCl	**A & C:** Graphite plate	*C. pyrenoidosa*	**C:** 78% (5 g/L) and 72% (2.5 g/L) **D:** 71 % (5 g/L)	45.52 mW/m^2 (5g/L)	(Neetu. et al., 2019)
A: Synthetic wastewater **C:** Mineral medium **S:** 15, 35 and 55 g/L NaCl Solutions	**A & C:** Carbon cloth	*Chlorella vulgaris*	**D:** 40 %-55% (15-55 g/L)	25,080 mV (15 g/L), 16,575 Mv (35 g/L) and 18,062 Mv (55g/L)	(Arana & Gude, 2018)

A: Synthetic wastewater **C:** Mineral medium **S:** 5, 10, 20 and 35 g/L NaCl solution	**A & C:** Carbon cloth covered with stainless steel mesh	*Chlorella vulgaris*	**C:** 58% (5 g/L), 63% (20 g/L) and 64% (35 g/L) **D:** 21.4% (5 g/L), 29% (25 g/L) and 32.2% (35 g/L)	285 mW/m^3 (5 g/L), 550 mW/m^3 (20 g/L) and 675mW/m^3 (35 g/L)	(Kokabian et al., 2018a)
A: Synthetic wastewater **C:** Mineral medium **S:** 35 g/L NaCl solution	**A & C:** Carbon cloth covered with stainless steel mesh	*Chlorella Vulgaris*	**C:** 31% (batch) 45.6% (fed-batch) **D:** 22.7% (fed-batch) **N_A:** 42% **P:** 16% (NP removal at the cathode)	753.75 mW m^3 (batch & fed-batch)	(Kokabian et al., 2018b)
A: Real petroleum wastewater **C:** BG11 medium **S:** 20 and 35 g/L NaCl solution	**A & C:** Graphite rods	*Scenedesmus abundans*	**C:** 61.3% (35 g/L) and 68% (20 g/L) **D:** 55.3% (35 g/L) and 42.6 % (20)	654 Mv (35 g/L) and 506 mV (20 g/L)	(Ashwaniy & Perumalsamy, 2017)
A: Synthetic wastewater **C:** Mineral medium+ PBS (PMDC) and (MDC) **S:** 15 and 35 g/L NaCl	**A & C:** Graphite sheet	Chlorella vulgaris	**D:** 0.341 g/L/d (35 g/L) and 0.241 g/L/d (15 g/L)	20.25 mW/m^2 (35 g/L) and 9.12 mW/m^2 (15 g/L)	(Zamanpour et al., 2017)
A: Synthetic wastewater **C:** Water	**A & C:** EC-12 grade EDM blank graphite	*Nannochloropsis salina*	**D:** 45% (35 g/L), 79% (17.5 g/L) and 46% (8.25 g/L)	384 mW/m^3	(Saba et al., 2017b)

S: 8.25, 17.5 and 35 g/L NaCl solution	plates				
A: Synthetic wastewater **C:** F2 media **S:** 7.5 g/L NaCl solution	**A & C:** Mixed of packed and planed electrodes (Granular Activated Carbon (GAC) & graphite plates)	*Nannochloropsis salina*	**C:** 79.4%(PMDC) and 76.4% (Stacked PMDC) **D:** 94.0% (PMDC) and 93.5% (SPMDC)	1.18 W/m^3 a (PMDC) and 0.874 W/m^3. (SPMDC)	(Mahdi & Safi, 2016)
A: Synthetic wastewater (500 and 1000 mg/L COD) **C:** Mineral medium **S:** 7.5 g/L NaCl solution	**A & C:** Circular Carbon papers	*Chlorella vulgaris*	**C:** 76.1% (500 mg/L) and 82.2% (1000 mg/L) **D:** 64.2% (500 mg/L) and 63.5% (1000 mg/L)	0.77 W/m^3 (500 mg/L) and 0.69 W/m^3 (1000 mg/L)	(Kokabian & Gude, 2015)
A: Synthetic wastewater **C:** Mineral medium **S:** 10 g/L NaCl solution	**A & C:** Graphite papers	*Chlorella vulgaris*	**C:** 65.6% **D:** 40.1%	84 mW/m^3 (anode volume)	(Kokabian & Gude, 2013)

2.6.3 Different types of wastewater and saline water treated at PMDC

The efficiency of PMDCs and traditional MDCs in treating various types of wastewater, producing electricity, and desalinating water have been compared in various studies. For instance, the effectiveness of an algae-assisted MDC using Chlorella vulgaris in the cathode, a synthetic salt solution at desalination, powered by dairy effluent at the anode was examined by Zamanpour et al. (2017). Algae-assisted MDC removed 0.341 g/L/d of salt, exceeding the air cathode MDC's removal by 1.5 times. Jaroo et al. (2019) examined PMDC operation using oil refinery effluent at both anode and cathode, employing *Chlorella vulgaris* as the biocathode and 35 g/L of artificial saline water for desalination. They reported organic content removal of 96. 3% at the anode and 77.2% at the cathode, with a total dissolved solids (TDS) removal rate of 159.7 ppm/h over three days. In a study, *Chlamydomonas* sp. (UKM6) and Scenedesmus sp. (UKM9) were tested as biocathodes in PMDC with palm mill oil effluent at the anode and 35 g/L artificial seawater in the middle chamber by Nadzri et al. (2023) The findings indicated that PhMDC-UKM9 and UKM6 acquired 1942 mW/m^3 and 1714 mW/m^3 of power densities, with salt removal of 44% and 32%, and 49% and 53% of COD in the anode chamber, respectively. At the same time, a sewage sludge-fueled anode chamber and mixed microalgae culture dominated by *Coelastrella* sp. and *Mariniradius saccharolyticus* was used in a cathode chamber by (Sadeq & Ismail, 2023). The desalination chamber contained real seawater having 19,000 to 45,000 mg/L TDS. The system achieved a maximum desalination efficiency of 80 ± 1.2% in 180 days of continuous operation with maximum organic content removal of 99.3 ± 0.5 % and a power output of 430.7 ± 0.7 mW/m^3.

2.7 Factors Affecting the Performance of MDC & PMDC

Due to the complex mechanism of MDCs, several factors impact their performance, such as the membrane type and inter-membrane space, mode of operation (Batch, fed-batch, or continuous) and hydraulic retention time (HRT), anodic substrate type and concentration, anode electrode and biocatalyst, cathode electrode and electron acceptors, pH imbalance, Internal and external resistance, salt concentration, and gradient all play crucial roles in determining MDC efficiency (Sayed et al., 2020a; Sophia & Gohil, 2018; Yang et al., 2019; Zahid et al., 2022b).

2.7.1 Membrane type and intermembrane space and IR

The ion exchange membranes (IEMs) are crucial in microbial-based desalination systems as they establish distinct chambers and facilitate ion transport, significantly influencing system performance. However, the intermembrane distance is a critical consideration during MDC construction, as it directly affects the system's internal resistance (IR). A higher intermembrane distance widens the desalination chamber, increasing input salt solution and electrolyte conductivity while decreasing IR, potentially leading to lower cell performance. For example, when the intermembrane distance was set at 0.30 cm, 1 cm, and 1.50 cm, the corresponding IR values were 342 Ω, 222 Ω, and 214 Ω, respectively. IR is inversely proportional to conductivity in saline solutions, meaning that IR and MDC performance decline as conductivity decreases.

2.7.2 Mode of operation and HRT

Additionally, increasing hydraulic retention time (HRT) and employing continuous mode operation enhance IR, affecting MDC efficiency positively. Continuous mode operation, especially in submerged MDCs, outperforms batch and cyclic batch modes due to homogenous substrate distribution, which boosts current generation and desalination efficacy. However, cyclic fed-batch modes surpass batch mode, reducing lag phase time and stabilizing pH. While increasing the HRT (Hydraulic Retention Time) in the middle chamber of a desalination system can enhance the process of desalinating saline water. However, it is important to note that larger middle chambers may lead to an increase in ohmic resistance, which can have a negative impact on the overall performance of the system.

2.7.3 Electrodes

Electrodes are crucial in MDCs, impacting power output through their potential difference. Ideal electrode materials possess high conductivity, a large surface area with accessible pores, enhanced mass transfer, chemical stability, mechanical strength, biocompatibility, low cost, and scalability. Anode surfaces contribute significantly to activation losses and can be optimized for higher current generation by using materials with larger surface areas for biofilm adhesion. Cathodic reactions, like oxygen reduction, can use abiotic (air cathode) or biotic (biocathode) sources. Air cathodes are efficient and cost-effective, while

biocathodes offer sustainability and economic viability, attracting interest for practical MDC applications due to potential cost savings, waste removal, and operational sustainability.

2.7.4 Substrate (anolyte and catholyte)

Electrolytes play a crucial role in enhancing performance by serving as a source of organic matter and nutrients for bacterial metabolism, a reservoir for ion species in processes such as desalination and electrodialysis, and a medium for pH variation. Various researchers have explored different substrates in different concentrations and operational modes, such as acetate, xylose with phosphate buffer solution (PBS), enriched cellulose-degrading rumen microbial consortium, synthetic wastewater containing acetate and glucose, real industrial wastewater, and untreated domestic wastewater, as anolytes to stimulate the activities and by-products of exoelectrogenic bacteria in the anode chamber. On the other hand, catholytes like ferricyanide, PBS with NaCl, and others have also been utilized. However, concentration losses are common due to a nutrient gradient in the substrate decreasing gradually over time as bacteria consume it.

2.7.5 Ions migration and pH fluctuaions

Migration of ions like Cl^- from the desalination chamber to the anode chamber decreases the anolyte pH, inhibiting microorganism activity and limiting desalination cycles. Conversely, Na^+ migration from the desalination chamber to the cathode chamber raises the catholyte pH, resulting in potential voltage losses. MDCs exhibit a distinct anodic bacterial community structure due to the high salinity anode environment in desalination chambers.

2.7.6 Electrolyte and saline water volume

The proportion of electrolytes to saline water volume has been shown to significantly affect overall performance, particularly in terms of desalination efficiency.Effective removal of high salinity levels requires a large volume of electrolyte, especially anolyte, to improve performance; otherwise, only partial removal may be achieved. For example, a microbial desalination cell (MDC) was able to achieve a salt removal rate of 40–60% because the electrolyte volume was only two to three times that of the desalinated water.

2.7.7 Conductivity

Conductivity is the expression of the ion concentration in an electrolyte that enables the transmission of electric charge. It also denotes the capacity of a material's electrodes to conduct electric charge from one point to another. Generally, a higher concentration of saline water corresponds to higher conductivity, creating a greater concentration gradient between the desalination chamber and the electrode chambers, thus enhancing desalination performance When the concentration of salt water is higher than that of the electrolytes, dialysis can enhance desalination efficiency.

Considering all relevant factors, optimizing MDC design and operational parameters is crucial for maximizing performance while minimizing environmental impact and operational challenges.

2.8 Applications of MDC & PMDC

The array of potential applications extending beyond desalination, electricity generation, and organic carbon removal encompasses the following:

2.8.1 Pretreatment and post-treatment of desalination processes

The microbial desalination cells (MDCs) employed in seawater reverse osmosis (SWRO) systems have the potential for a 20% reduction in the energy requirements of the SWRO process when utilizing up-flow MDCs. Additionally, there could be a 30% reduction in energy demands specifically related to the total dissolved solids (TDS) in the feed seawater. Moreover, this integration of MDCs with SWRO systems is anticipated not only to save energy but also to facilitate the generation of electricity during the desalination process. Moreover, multiple-effect distillation coupled with capacitive deionization (MEDCC) exhibits the lowest energy requirements (ranging from 2.85 to 6.14 kWh/m^3) for treating RO concentrate compared to alternative methods such as thermal evaporation, electrodialysis (ED), and electrochemical oxidation (Yang et al., 2019).

2.8.2 Acid & alkali production

When a MEDCC facility is provided with a composite solution comprising of NaCl as saline, crucial secondary substances like alkali, acid, and magnesium can be extracted alongside desalinated saline water. The study suggests that alkali production from

MEDCCs holds significant economic potential, with a projected production cost approximately seven times lower than current market prices. Moreover, electrically driven selective ion separation in MEDCCs can produce hydrochloric acid, caustic soda, malic acid, and formic acid (Odunlami et al., 2023).

2.8.3 Synthesis of bio-hydrogen

MDCs have the potential to efficiently generate H_2 and eliminate NaCl from saltwater through engineering techniques. By altering the cathodic reduction reaction and utilizing an external power source, MDCs can effectively generate either hydrogen gas or hydrogen peroxide at their cathodes. In practice, the microbial electrolysis and desalination cell (MEDC) resembles a typical three-chamber MDC, with a modification in the cathode chamber to facilitate hydrogen gas collection. This setup resulted in the MEDC producing 48.7 mL of H_2 gas and achieving a 98.8% salt removal efficiency over a 96-hour operational period (Imoro et al., 2021).

2.8.4 Water softening and metal ions removal

A specialized Microbial Desalination Cell (MDC) with enzymatic characteristics has been developed to address the need for water softening. This process aims to remove metal cations such as Ca^{2+}, Mg^{2+}, and other ions commonly present in hard water (Sophia & Gohil, 2018). Moreover, MDCs provide a practical approach to treating water that contains copper by utilizing cathodic reduction to convert copper (II) to copper (I) oxide. Another strategy involves using the alkali effluent from the cathode chamber in stacked MDCs to facilitate the precipitation of hydroxide, which aids in the removal of copper. Additionally, a conventional three-chambered MDC has been successfully employed to address issues related to hardness and heavy metals, such as arsenic, copper, nickel, and mercury, through the migration of ions driven by an electric field. Both enzyme-catalyzed and conventional three-chambered MDCs have demonstrated their effectiveness in softening water (Yang et al., 2019).

2.8.5 Nutrient removal and recovery

The efficacy of MDCs in nutrient removal from wastewater has also been showcased. In a 12-hour trial, a submerged microbial desalination-denitrification cell (SMDDC)

successfully. In a 12-hour experiment, a submerged microbial desalination-denitrification cell (SMDDC) effectively eliminated 90.5% of NO_3^- from simulated groundwater and extracted 88% of total ammonia. The elimination of ammonia occurred through two mechanisms: the migration of NH_4^+ driven by the current from the middle chamber to the cathode chamber and the diffusion of NH_3 due to a concentration gradient (Imoro et al., 2021). Through the utilization of an electrical field, the SMDDC system effectively denitrified groundwater in situ by trapping nitrates in the anode chamber and then electrochemically reducing them in the cathode chamber. This denitrification process did not require additional construction (Sophia & Gohil, 2018). Furthermore, an advanced microbial nutrient recovery cell (AMNRC) was developed through the modification of a stacked MDC in order to efficiently eliminate and reclaim nitrogen and phosphorus from wastewater (Yang et al., 2019). Kokabian et al., 2018a introduced the continuous photosynthetic microbial desalination cell (CPMDC), employing algae as an electron acceptor and simultaneously benefiting nutrient removal at the cathode. During a 50-hour observation period, microalgae effectively removed approximately 90% of nitrate, while phosphate removal was relatively lower, at around 20%.

2.9 Challenges and Future Perspectives

2.9.1 Membrane fouling

Membrane fouling occurs when contaminants gather, adhere, or accumulate on the membrane surface, or when membrane pores become blocked and ultimately impacting the membrane's performance over time (Zahid et al., 2022b). Due to its close proximity to the anode, which is a hub of active microbial activity, the anion exchange membrane (AEM) is bound to develop a biofilm over time (Pandit et al., 2018). For instance, during the eight-month operation of the MDC with domestic wastewater, the presence of biofouling on the AEM resulted in a decrease in current density and salt removal. This decline can be attributed to the increased internal resistance(Salehmin et al., 2021; Sayed et al., 2020). The presence of actual wastewater and seawater exacerbates fouling in comparison to simulated wastewater and saline solution, primarily due to their complex compositions. This leads to modifications in solute removal efficiency, permeate flow, and pressure drop across the membrane, consequently impacting the overall performance of MDCs (Zahid et

al., 2022b).

2.9.2 Water safety

When dealing with microbes and wastewater, there is a potential for the microbes to penetrate the AEM and enter the middle chamber. This possibility is dependent on the characteristics of the AEM material. The AEM's pores must be of a small enough size to only permit the passage of ions and water molecules. However, in practical situations, it is often observed that solutes such as acetate and glucose easily pass through the AEM's pores and enter the desalination chamber. Additionally, the osmotic movement of water carries certain organic molecules from the wastewater into the desalinated water. The issue becomes more severe with microbial osmotic desalination cells (MODCs), as the pore size of forward osmosis (FO) membranes is significantly larger, potentially enabling certain microbes to infiltrate the middle chamber. This creates a breeding ground for bacteria in the desalination chamber, fueled by the presence of organic matter. These phenomena contribute to the contamination of desalinated water, necessitating the adoption of appropriate measures to address it. This involves the use of AEMs with minute pore sizes and the implementation of effective downstream treatment processes to purify the desalinated water (Pandit et al., 2018).

2.9.3 High salinity of the treated wastewater

Through bio-electrochemical system (BES) technology, desalination involves the separation of salts from seawater by the movement of ions towards the anode and cathode chambers. This process leads to an increase in salinity levels within both the anolyte and catholyte solutions (Sayed et al., 2020b).

2.9.4 High cost

The primary expenses in MDCs are allocated towards procuring membranes and cathode materials. Additionally, the adoption of MDCs for commercial purposes is occasionally hindered by the utilization of costly or hazardous catalysts and chemicals in the cathode chamber, aimed at enhancing the oxygen reduction reaction (ORR) (Rabiee et al., 2022)

2.9.5 The low desalination rate

When compared to conventional desalination techniques, the desalination rate in an MDC is notably lower, primarily due to the inherent constraints of the current generation caused by a bioanode. This reduced desalination rate indicates that MDC technology may be more suitable for pre-desalination purposes prior to the standard desalination process or for treating low-salinity water (Sophia & Gohil, 2018).

CHAPTER 3

MATERIALS AND METHODS

This research involved the construction of two lab-scale Microbial Desalination Cells (MDCs), operated in two distinct phases. In the first phase, various concentrations of substrates in the anode were tested, while in the second phase, different levels of saline water concentrations were examined for desalination. Inoculum and synthetic wastewater were prepared for all three substrates to facilitate these experiments. Performance parameters were compared for each case. The subsequent sections provide a detailed account of each step in this study, including the calculations and analysis conducted throughout the operational period.

3.1 Construction of Microbial Desalination Cells

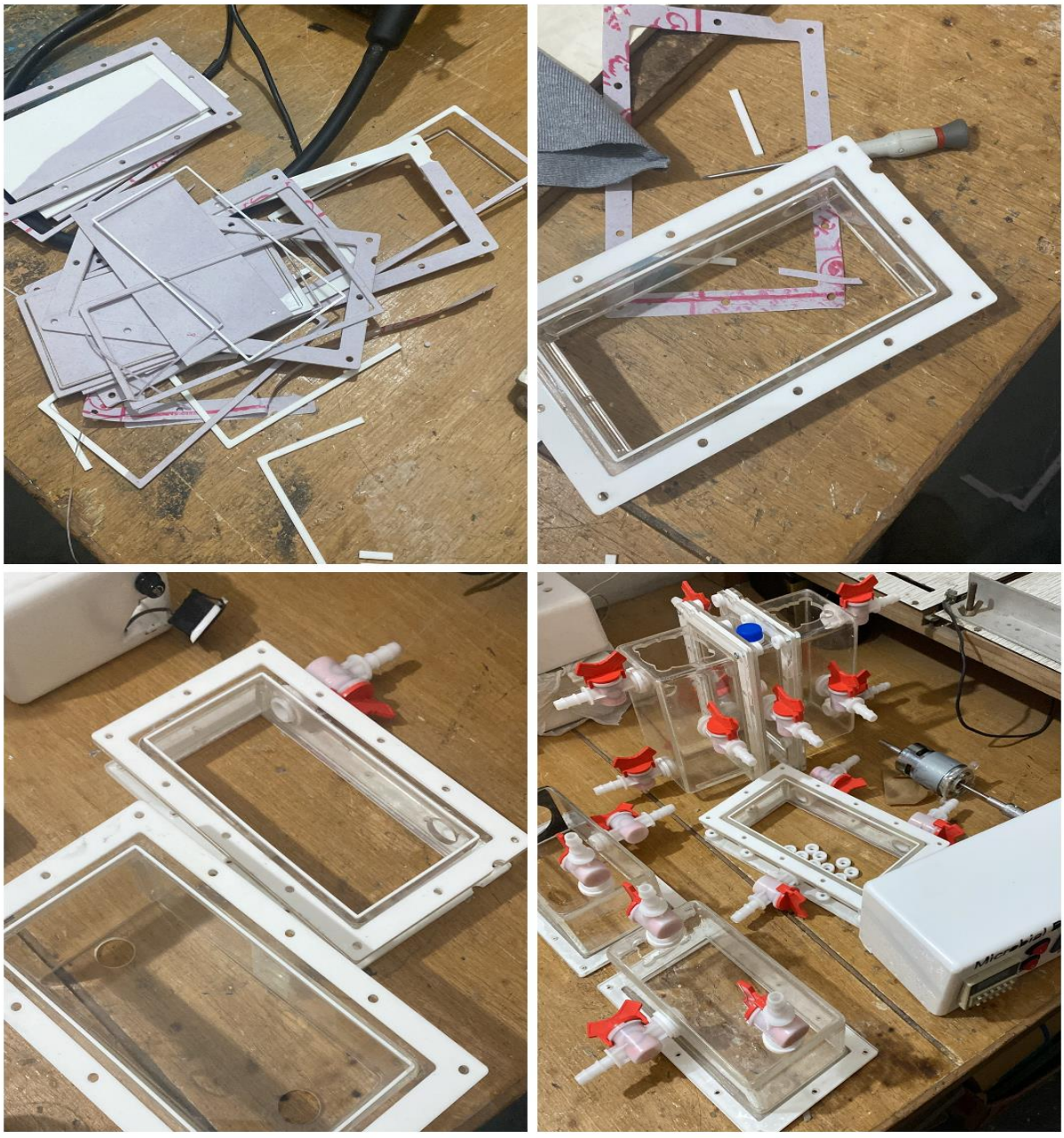
Two lab-scaled three-chambered rectangular MDCs were designed and constructed using acrylic sheets having 5mm thickness with a total volume of 1:0.5:1 for the anode, desalination, and cathode chambers as shown in Fig 3.1 (a, b, c, and d).

- Three-chambered chemical catholyte MDC known as microbial desalination cell, which (MDC) was called “control.”
- Three-chambered bio-catholyte MDC known as photosynthetic microbial desalination cell (PMDC), referred to as “Experimental.”

The Ion exchange membranes, Anion Exchange Membrane (AEM, AMI-7001, Membrane International, USA), and Cation Exchange Membrane (CEM, CMI-7000, Membrane International, USA) were used to separate the chambers. The graphite rods were utilized as electrodes in the cathode chambers, while the carbon fiber brushes (The Mill-Rose Company, USA) constructed of a core of titanium wire with graphite fibers were utilized as electrodes in the anode chambers. The cathode chamber of PMDC had an electric motor to provide energy for intermittent mixing, while the cathode chamber of MDC had an air sparger. Illumination at the cathode chamber of PMDC was provided using an LED light of 18 watts using a digital timer. Check valves on the anode were made to allow the off-gasses to escape the anode easily. This prevented pressure build-up in the anode chamber and allowed the carbon dioxide produced by the bacteria to escape from the chamber. A

copper wire was linked to each electrode and extended outside the MDC setups to develop an electrical circuit for electron transport at a fixed resistance of 1000Ω. These three chambers were securely joined together using a silicon binder and several layers of rubber gaskets between the AEM and the CEM to prevent gas intrusion to the anode chambers and the remaining leaks from the cells. The inlet and outlet ports were made on all three chambers to facilitate sampling and inoculation.

(a)



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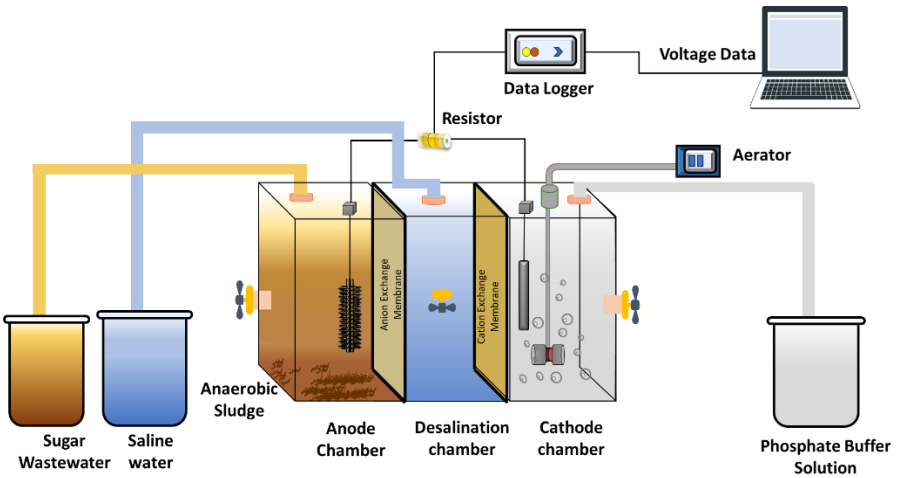
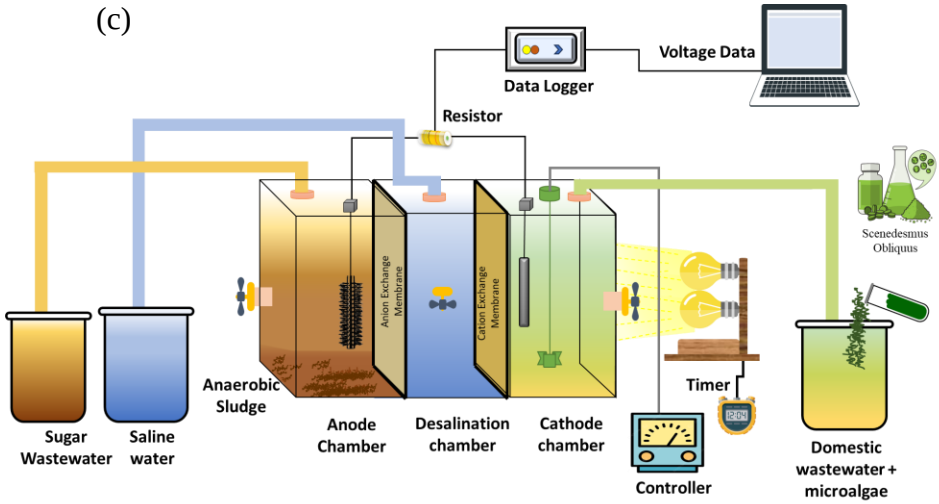


(c)



(d)

Figure 3.1 Construction of MDC& PMDC (a), Schematic diagram of MDC (b) and PMDC (c) Experimental setup of MDC and PMDC (d).

Table 3.1 Reactor Dimensions and Setup.

Reactor design	**Measurements**
Reactors material	Acrylic sheet (5mm)
Dimensions (cm) of Anode	7.6 × 8 ×16.5
Dimensions (cm) of Cathode	7.6 × 8 ×16.5
Dimensions (cm) of Desalination	3.8 × 8 ×16.5
Dimension of Graphite Rods	10 × 0.5
Dimension of Carbon fiber Brushes	5.9 × 6.93 (14.85cm overall length with stem)
Dimension of Ion Exchange Membranes (AEM & CEM)	16.5 × 8
Surface Area of Graphite Rods	32.28 cm^2
Surface Area of Carbon Fiber Brushes	183.13 cm^2
Surface Area of Ion Exchange Membranes (AEM & CEM)	128 cm^2
Aerators (Air supply source)	1 (200–300 mL/min)
Motor and mixing	20 Watts, 145 rpm
LED lights	1 (18 Watts)
Light Intensity	150 μmol/m/S (12/12 hrs.)
Reactor's working volume (Anode: Desalination: Cathode)	900:450:900

3.1.2 Electrodes and membrane pre-treatment

Both membranes were preconditioned by immersing in 5% NaCl solution at 40 C for 24 h and rinsed with DI water prior to use to allow for membrane hydration and expansion as recommended by the supplier.

The graphite rod electrodes were soaked in distilled water for 12 hrs, followed by drying before installation to improve the bacterial adhesion. The carbon fiber brush electrodes were soaked in acetone for 24 hrs., and then the brush was soaked in deionized water and heated at 100^oC for 30 mins, rinsed with distilled water thrice before use for disinfection purposes.

(a) (b) (c)

Figure 3.2 Graphite rods (a), carbon fiber brush (b) electrodes, and membranes (c) used in this study.

3.1.3 Membrane cleaning

To retain membrane efficiency, tap wash water is first introduced through the system for 15 min, followed by cleaning with 1% v/v HCl solution for 30 min, and subsequently, a second wash with tap water was provided (Vadthya et al., 2015). The cleaning procedure was adopted to remove mineral depositions. An extra washing step for 15 min was carried out with 0.5% tetrasodium EDTA aqueous solution to remove stubborn foulants deposited on the membrane surface (Ravikumar et al., 2013).

Table 2.2 AMI-7001 and CMI–7000 membrane technical specifications.

Technical Specification	**AMI- 7001**	**CMI-7000**
Functionality	Strong Base Anion Exchange Membrane	Strong Acid Cation Exchange Membrane
Polymer Structure	Gel polystyrene cross linked with divinylbenzene	Gel polystyrene cross linked with divinylbenzene
Color	Light Yellow	Brown
Electrical Resistance	<40 Ohm.cm^2	<30 Ohm.cm^2
Standard Thickness	0.45 mm	0.45 mm
Thermal Stability	90 ^{o}C	90 ^{o}C

3.2 MDC Inoculation, Wastewater and Saline Solutions

3.2.1 Anode chamber

The anodic chamber utilized a microbial consortium sourced from aerobic sludge at the Islamabad wastewater treatment plant. Before application, the activated sludge underwent 18-20 days of anaerobic conditioning in a nitrogen-purged, air-sealed container. This sludge was comprehensively characterized for total and volatile solids, along with Oxygen Reduction Potential (Table 3.3). It was then employed in both the Microbial Desalination Cell (MDC) and Plant Microbial Desalination Cell (PMDC) anodic chambers in a 2:1 substrate-to-sludge ratio, playing a pivotal role in the experimental setup.

Table 3.3 Characteristics of anaerobic sludge used in anode chambers.

Characteristics	Values
Total Solids	39.7 g/L
Volatile Solids	26.2 g/L
pH	7.1
Oxygen Reduction Potential	-321 mV
Electrical Conductivity	4.03 mS/cm

Figure 3.3 Anaerobic Sludge inoculation in anode chamber.

Synthetic sugar industry wastewater was used as substrate in anode which was synthesized using sucrose ($C_{12}H_{22}O_{11}$) as readily soluble COD source, ammonium sulfate $(NH_4)_2SO_4$, potassium dihydrogen phosphate (KH_2PO_4) according to the modified recipes of (Tanksali,

2013; Zhou et al., 2015) with 350:5:1 CNP ratio, Sodium bicarbonate was used for pH adjustment. The experiment involved three distinct variations of COD concentrations - 2000 mg/l, 4000 mg/l, and 6000 mg/l - sourced from sugar industry wastewater, as outlined in the corresponding table. High-influent COD wastewater was selected to increase the length of the operation cycle and enhance MDC operation stability, allowing the study to concentrate on the MDC's desalination and power generation aspects.

Table 3.4 Compositions of Synthetic Sugarcane Wastewater.

Chemical	Formula	Quantity (mg/L)		
Sucrose	$C_{12}H_{22}O_{11}$	2000	4000	6000
Ammonium Sulfate	$(NH_4)_2SO_4$	135	269.5	404.2
Potassium Di-Hydrogen Phosphate	KH_2PO_4	25.06	50.2	75.3

3.2.2 Cathode chamber

The algal strain (*Scenedesmus obliquus,* also known as *Tetradesmus obliquus*) procured from the Norwegian Algal Culture Center, Norway, was used in the cathode compartment of PMDC as an oxygen source. The stock culture was initially nurtured in Z8 medium at 30±2 ^{o}C using 9 W cool LED lights for illumination at a light intensity of 50 μmol/m^2/s in a 14/10-hour light/dark cycle as per a previous study (Sohail et al., 2023). The selection of Scenedesmus obliquus for this study was driven by its exceptional adaptability to dynamic environments, displaying robust vitality and a high reproductive capacity (Wei et al., 2020).

(a) (b)

Figure 3.4 S.obliquus cultivation in Z8 media (a) and centrifuged known algal biomass for inoculation in PMDC cathode (b).

Before being added to the cathode, the algal suspension was centrifuged at 4500 rpm for 6 minutes. The concentration of *Scenedesmus obliquus* was estimated by optical density at 680 nm. The collected algal biomass was then allowed to grow and reproduce in the cathodic chamber for a period of 4 days in batch mode and 2 days in the fed-batch cycle with the treated synthetic domestic wastewater as a growth medium with 100 mL (0.1M phosphate buffer solution adjusted to pH 7). The Synthetic Domestic wastewater contained (per L) 188 mg $C_6H_{12}O_6$, 134 mg NH_4Cl, 60 mg KH_2PO_4, 200 mg $NaHCO_3$, 111 mg $NaSO_4$, 250 mg NaCl and 1.25 mL trace element solution (Wang et al., 2019). Meanwhile, for MDC, catholyte consists of 0.1 M phosphate buffer solution (pH 7).

3.3.3 Desalination chamber

The desalination chamber was inoculated with synthetic sea and brackish water. Three different salt concentrations of sodium chloride (NaCl) were chosen; 15 and 25 g/L NaCl mimic brackish water, while 35 g/L represented the salinity of typical seawater. These concentrations were achieved by adding the specified amount of NaCl to distilled water.

3.4 Operation of MDC and PMDC

The reactors were operated in two phases;

- Startup Phase: This initial phase was operated in batch mode and served as an acclimatization period.
- Semi-Continuous Phase: This phase, conducted in a fed-batch manner, aimed to assess the impact of varying anolyte substrate concentrations and different salt concentrations on MDC and PMDC performance.

During testing, aluminum foil was used to cover both anode chambers to prevent algae growth and shield the anodic bacteria from exposure to light.

3.4.1 Batch mode (startup)

The reactor initially operated in batch mode, with 600 mL of synthetic sugar industry wastewater and 300 mL of mixed sludge inoculated in the anode. Wastewater replacement occurred every 5 days for the first 4 batches and every 2 days for the subsequent 3 batches. At the onset of each batch, the anodic chamber underwent a 5-minute nitrogen gas sparging to maintain anaerobic conditions. Meanwhile, the cathode was inoculated with 1 g/L of

algae containing synthetic domestic wastewater for 4 days for the first 4 batches and 2 days for the subsequent 3 batches, serving as the growth medium during batch operation. The desalination chamber was filled with a salt concentration of 35 g/L NaCl, simulating seawater. The salt solution in the desalination chamber was refreshed after 4 days. After 22 days of continuous operation, a stable voltage was observed, signifying the successful generation of a biofilm. Subsequently, the mode of operation transitioned from batch to fed-batch. The results obtained from the batch-mode are included in the *appendix.*

Under the ideal operating conditions, a negative control experiment was executed with an initial salt concentration of 35 g/L, but without any electrode potential. The objective was to analyze the difference in salt removal. The results indicated that without the electrode potential, only a minor fraction (1.5%) of salt was eliminated from the desalination chamber. This outcome is probably linked to solute potential, resulting in salt accumulation on the AEM.

The setup was initially operated in an open circuit to attain voltage stability. Polarization curve studies were conducted once the reactors achieved a stable state in open circuit mode. These curves were derived by altering the external resistance across the cells, ranging from 4Ω to 100,000Ω, utilizing a resistance box. After achieving stable voltage in OCV, the circuit was closed by introducing a 1000 Ω resistor connecting the anode and cathode for closed circuit tests. Before the commencement of each batch, the anode compartment underwent a 5-minute sparging with N_2 gas to minimize electron loss to O_2. All experiments were conducted at a consistent room temperature of 30 ± 2 ^oC.

3.4.2 Fed-batch/semi-continuous mode

3.4.2.1 Substrate concentration (objective 1)

After the establishment of anode biofilm, three different substrate concentrations were investigated by varying COD concentrations of anodic wastewater, i.e., 2000, 4000, and 6000 mg/L COD with OLRs of 0.66, 1.3, and 2.0 g COD/L.d. In the fed-batch, 300 mL of wastewater was continuously fed and removed from the anodic chamber. The pH of wastewater was maintained at 7.0 to 7.1 using bicarbonate. The OLR with the highest salt removal and electricity production was chosen for further study.

The rest of the conditions were kept the same as that of batch mode for desalination and

cathode compartments of MDC, but for PMDC, the cathodic chamber was also operated in fed-batch mode with 1 g/L algae concentration was maintained at the start of each feed where necessary new the biomass was added to maintain 1 g/L algae concentration in the reactor's chamber if the concentration of algae was less than 1 g/L while removing daily effluent.

Table 3.5 Operational Conditions for anode and cathode in fed-batch mode.

Chamber	**COD (mg/L)**	**HRT (d)**	**WW Volume (mL)**	**Working vol of tank (mL)**	**Q (mL/d)**	**OLR (g COD/L/d))**	**Operation Time (d)**
Anode	2000	2	600	900	300	0.67	15
	4000	2	600	900	300	1.33	15
	6000	2	600	900	300	2.0	15
Cathode	200	2	900	900	450	0.1	45
Total operational time of anode and cathode							45

3.4.2.2 Salt concentrations (objective 2)

The effect of different salt concentrations in a desalination chamber on MDC and PMDC performance was studied. The desalination chamber was sequentially operated with three different concentrations of NaCl: 15, 25 g/L, and 35 g/L, representing typical brackish and seawater salinity. The optimal condition of 4000 mg/L COD (1.33g COD/L.d OLR) was kept in anode. Meanwhile, cathode chambers were operated in the same manner as described in section 3.4.2.

Table 3.6 Operational Conditions for desalination chamber in batch mode.

Chamber	**NaCl (g/L)**	**HRT (d)**	**WW Volume (mL)**	**Working vol of tank (mL)**	**Operation Time (d)**
Desalination	15	4	450	500	10
	25	4	450	500	10
	35	4	450	500	10
Total operational time of desalination					30

3.5 Morphological Analysis

The surfaces of the anodes, AEMs, and CEMs were subjected for their morphological assesment before and after the experiment. This investigation was carried out using Scanning Electron Microscopy (SEM) to precisely determine the quantitative composition of both the membranes and electrodes. Upon completion of the operation, the reactors were disassembled, and samples measuring 2 cm^2 were precisely collected from the AEMs, CEMs, and anodes for subsequent SEM analysis. The samples were immersed in a 2.5% glutaraldehyde solution overnight, using a 100 mM phosphate buffer solution. Subsequently, the samples underwent sequential dehydration in alcohol for 5 minutes in each solution, starting from 30% and gradually increasing to 50%, 80%, 90%, and finally 99% (Ragab et al., 2019b). The AEM (adjacent to the anode chamber and the desalination chamber) and CEM (adjacent to the cathode chamber and the desalination chamber) samples, along with the anode electrodes, were coated with a 60μm thick layer of gold on both sides before undergoing SEM analysis.

3.6 Electrochemical and Biochemical Measurements

3.6.1 Biochemical analysis

Anodic and cathodic wastewaters and saline water were characterized for their pH and electrical conductivity (EC) using a multimeter (inoLab pH/Cond 720, Germany). Chemical Oxygen Demand, Ammonium nitrogen (NH_4^+-N), and inorganic phosphorous as phosphate phosphorous (PO34- P) were also measured in wastewater in accordance with the standard method outlined in (APHA, 2017). Dissolved oxygen (DO) and pH and Oxidation-reduction potential (ORP) were recorded using an oxygen meter (WTW Multi 9310) and a pH meter (inoLab pH/Cond 720, Germany), respectively. Light intensity was observed through a quantum meter (apogee instrument, Model MQ-500).

3.6.2 Microalgae growth

The microalgal cell concentration was determined every 24 h by spectrophotometer at an optical density of 680 nm. Before measurement, a standard curve was established using different dilutions of known algal concentrations, where dry weight was equivalent to 0.183 times wet weight. The concentration was then determined using a standard curve (y=

0.0004x + 0.0407 and R^2 = 0.9993). Microalgae growth rate was calculated by equaltion 1

$$Microalgae\ Productivity = \frac{Xfi\ - Xin}{Tc} \qquad (1)$$

Tc shows the culture period, while X_{in} and X_{fi} denote 'the initial and final concentrations of microalgae, respectively.

3.6.3 Nutrient removal analysis

Anodic wastewater was analyzed for ammonia nitrogen and chemical oxygen demand using the distillation and closed reflux methods, respectively. While in the cathode, the nutrients such as NH4+-N, TKN, PO43−P, and COD in synthetic treated domestic wastewater were measured by the standard method (APHA, 2017). For analysis, samples were collected daily from the cathode and were filtered through a 0.45 μm filter paper. Removal efficiency (%) for nutrients and salt was calculated using Eq. (2)

$$Removal\ \% = \frac{Cin - Cfi}{Cin}\ X\ 100 \qquad Eq.(2)$$

where, C_{in} and C_{fi} are initial and final concentrations of nutrient or salt.

3.6.4 Electrochemical analysis

Voltage was monitored with a data acquisition system (data logger-PicoLog 1000 series, USA) connected to the laptop and recorded per hour across 1000 ohms resistance. The current (I) and power output were calculated using ohms law by Eq.3 and Eq.4

$$I = \frac{v}{Rext} \qquad Eq.(3)$$

$$P = v \times I \qquad Eq.(4)$$

where I, V, R_{ext} and P represent electrical current (mA), cell voltage (mV), external resistance (Ω) and power output (mW), respectively. The power and current densities were determined in terms of electrode surface area and anode/cathode volume. A resistance box (Extech-380400) was used to obtain the polarization data by varying resistance from 4 to 100,000 Ω. Polarization data was used to find the maximum current and power densities, internal resistance, and open circuit voltage (OCV) for both MDCs.

Columbic efficiency (C.E) for batch and fed-batch experiment was calculated using Eq. 5

and Eq. 6

$$CE = \frac{M \int_0^{tb} I dt}{FbVan\Delta COD} \qquad Eq.(5)$$

$$CE = \frac{M\ I\ tb}{FbVan\Delta COD} \qquad Eq.(6)$$

Where M is molar weight of oxygen, F = Faraday's constant (96485 C/mol-e-), b = 4 (quantity of electrons substituted for every 1 mole of O_2), V_{an} = Volume of substrate in the anode chamber and ΔCOD = change in COD over time t.

CHAPTER 4

RESULTS AND DISCUSSION

This chapter describes the characteristics of wastewater used, voltage production, and organic and nutrient removal, dissolved oxygen levels and desalination when various concentrations of COD were applied to the anode chamber. Additionally, it demonstrates how different salt concentrations in a desalination chamber affect the performance of the MDCs in terms of COD removal, salt removal, voltage generation and algal biomass.

4.1 Anolyte, Catholyte, and Saline Water Characteristics

Anolyte and catholyte were categorized based on their organic and nutrient content, electrical conductivity, and pH. However, the salt solutions were categorized based on their electrical conductivity. The physicochemical characteristics of synthetic sugar industry wastewater (anolyte), secondary treated domestic wastewater, and PBS solution are depicted in the table. Synthetic sugar industry wastewater with COD ranging from 2,000-6000 mg/L was used as a carbon source to grow microorganisms in the anode chamber. The COD of wastewater from the sugar processing industry typically varies from 2300 to 8000 mg/L for cane processing, along with high ammonium content and up to 5,000 mg/L of TSS (Sahu & Chaudhari, 2015). The significant mineral, sugar, and carbohydrate content in these wastewaters makes them ideal substrates for bacterial cultivation, leading to the generation of valuable products such as organic acids, enzymes, biomass, and biogas (Wang et al., 2020). Moreover, sugar-processing wastewater typically contains significant amounts of organic and particulate matter that often surpass environmental discharge standards, exacerbating its detrimental impact (Sahu, 2018).

Wastewater used in the cathode chamber was synthesized to imitate secondary treated domestic wastewater and used as a growth medium for microalgae. Treated domestic wastewater has been shown to contain various essential nutrients important for microalgal growth and metabolism, including nitrogen and phosphorus (Cabanelas et al., 2013). Additionally, it exhibits lower toxicity levels compared to other wastewater sources, making it a favorable medium for cultivating microalgae (Wang et al., 2022). These

nutrients can be present in a range of forms, such as ammonia, nitrate, nitrite, and phosphate, and their availability can influence the rate and efficiency of microalgal growth. While microalgae are mostly cultured in a standard medium, this approach is unsuitable for large-scale production of microalgae products. Therefore, selecting secondary treated municipal domestic wastewater for microalgae cultivation can be a sustainable and efficient approach, benefiting both the environment and the production process. Additionally, domestic wastewater is freely available and easily accessible, making it a cost-effective option for microalgae cultivation.

Table 4.1 Characteristics of anolyte and catholyte.

Parameters	**Synthetic sugar wastewater**			**Treated domestic wastewater**	**PBS**
COD mg/L	2000	4000	6000	200	-
NH_4^+-N mg/L	29.4	60.2	85.4	39.2	-
$P0_4^{3-}$-P mg/L	7.8	16.3	25.5	23.2	8.9
pH	7.0 - 7.1			7.0 - 7.1	
EC mS/cm	5.2	5.4	5.6	12.32	12.13

Three concentrations of salt solutions were prepared to mimic the saline and brackish water. 35g/L NaCl was used to simulate seawater (saline), while 15 and 25 g/L represent brackish water characteristics; the latter was chosen to test the applicability of MDCs in regions without saline sources. Saline water has higher salinity with TDS concentration averaging at 35 g/L, while brackish water refers to groundwater with total dissolved solids (TDS) ranging from 1 to 25 g/L (Du et al., 2022).

Table 4.2 Characteristics of salt solutions.

Parameters	**Salt solutions (NaCl g/L)**		
	15	**25**	**35**
EC mS/cm	26.7	40.8	56.0

4.2 The Effect of Substrate Concentration on Performance of Cells

4.2.1 Voltage and polarization

The two systems, PMDC and MDC, were operated to determine the optimal substrate concentration to maximize power production. For all three substrate concentrations, PMDC generated higher voltage and power output in comparison to MDC. The voltage and power generation at the moderate substrate were higher both in PMDC and MDC (control). However, PMDC at 4000 mg/L yielded the highest value, as shown in Fig 4.1. At 2000 and 4000 mg/L concentrations, PMDC achieved an average maximum working voltage of 261.3 mV and 275.9 mV, respectively. However, the voltage decreased to 181.1 mV when a high substrate concentration of 6000 mg/L was used at the anode. PMDC exhibited 31.2%, 38.1%, and 39.9% higher voltages than the respective values obtained by MDC when supplied with 2000, 4000, and 6000 mg/L anodic substrate concentrations. This shows that raising the concentration from 2000 mg/L to 4000 mg/L resulted in a voltage increase; however, when the substrate concentration was raised to 6000 mg/L, the voltage generation was significantly decreased. This is because an increase in substrate concentration beyond the optimum level leads to most substrates remaining unconsumed at high concentrations, leading to increased mass transfer losses and a subsequent reduction in circuit voltage. Liu et al. (2019) observed a consistent pattern with the highest voltage of 555 mV recorded at anode influent COD 900 mg/L, compared to 400 mV and 500 mV at 400 and 1400 mg/L COD. In a parallel study on MFC, where various acetate concentrations of 0.4, 0.8, 1.2, and 1 g/L were used as a substrate in the anode of the maximum output voltage of 610 mV was attained at 0.8 g/L (Tan et al., 2020).

The relatively lower voltages observed at 6000 mg/L anodic substrate concentration both in MDC and PMDC may also be attributed to the acidic anolyte having (reduced pH) conditions resulting from bacterial substrate consumption, accumulation of hydrogen and/or ion migration from the desalination chamber deteriorating the voltage generation. Additionally, an increase in internal resistance further decreases the voltage. The ion migration from desalination to the anode increased the anolyte conductivity (mS/cm) from 5.2 to 6.2 and 6.0 at 2000 mg/L PMDC and MDC, from 5.4 to 7.2 and 6.9 at 4000 mg/L PMDC and MDC, from 5.6 to 6.4 and 5.9 at 6000 mg/L PMDC and MDC as shown in Fig

4.2.

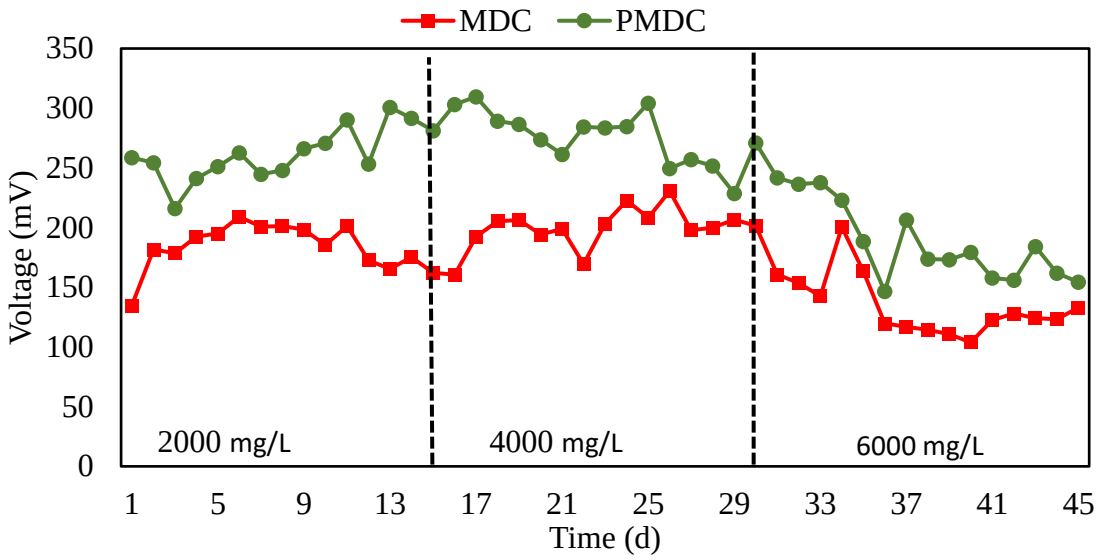


Figure 4.1 Voltage profile of MDC and PMDC at various substrate concentrations.

Additionally, the declining pH observed both in MDC and PMDC (Fig 4.3) anode, initially ranging from 7 to 7.1, indicated deteriorated microbial activity as the lowest pH values in PMDC were observed be 6.7 for 2000 mg/L, 6.6 for 4000 mg/L, and 6.2 for 6000 mg/L while MDC using 2000, 4000 and 6000 mg/L substrate concentration had lowest pH recorded values of 6.6, 6.4 and 5.9.

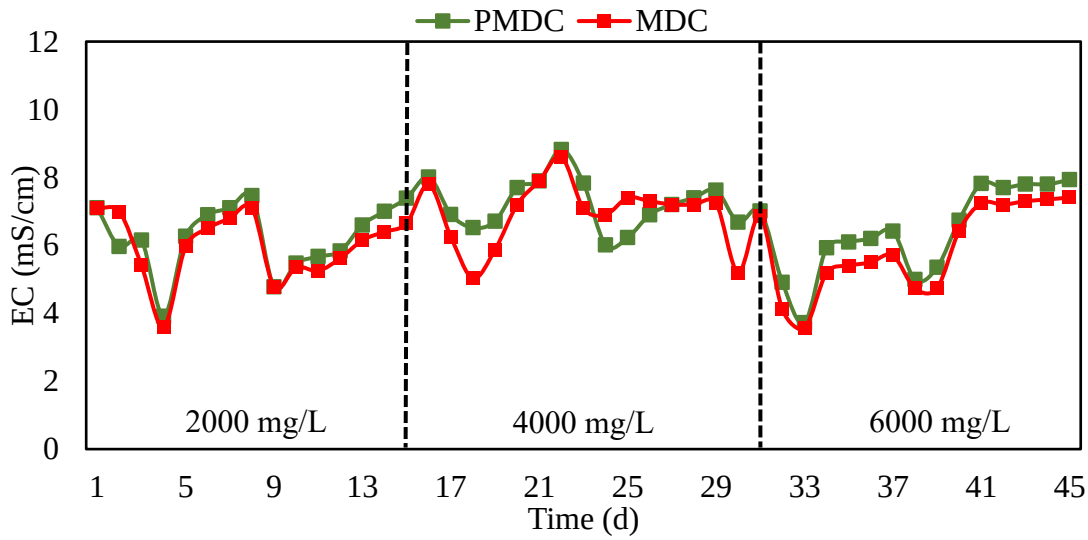


Figure 4.2 EC variations atanode of MDC and PMDC at various substrate concentrations.

The anode chamber experienced a negligible decrease in pH at low substrate concentrations due to biochemical reactions. However, anode acidification occurred at high concentrations due to biodegradation-induced organic acid accumulation. Therefore, due to the unfavorable conditions, microorganism activity was compromised, resulting in a decreased performance. According to the previous results, the highest current density and possible percentage of COD reduction can be achieved at a pH near neutral. To put it another way, at acidic pHs, the rate of microbial activity is relatively low (Rahmani et al., 2022).

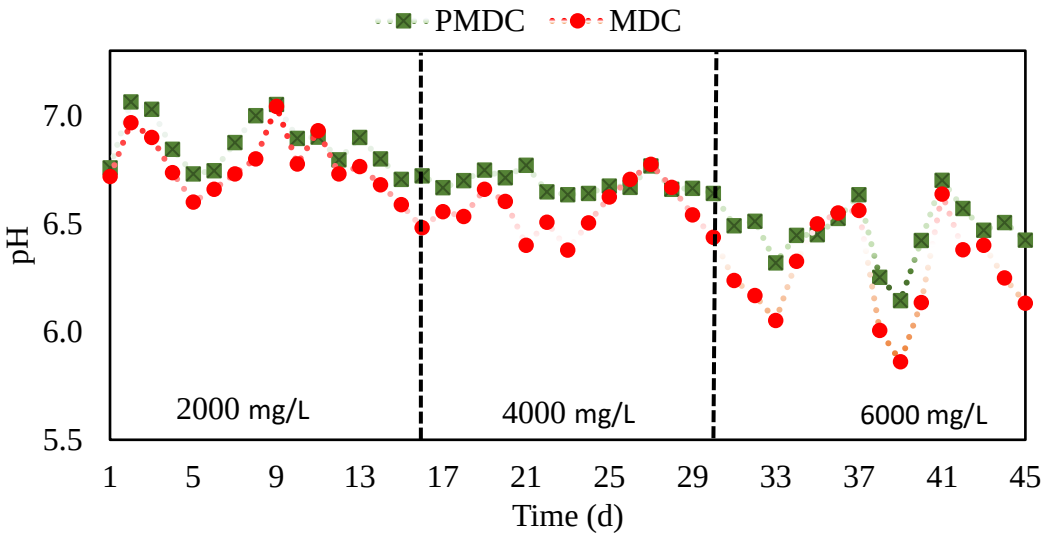


Figure 4.3 pH reduction anode of MDC and PMDC at various substrate concentrations.

The power density (P·D) calculated regarding anodic/cathodic volumes was also reported to be higher for PMDCs, as shown in Fig 4.4(a). P.D. of 7.7 mW/m², 11.9 mW/m², and 4.1 mW/m² were recorded for PMDC at 2000, 4000, and 6000 mg/L, respectively. Conversely, MDC exhibits lower power densities than PMDC, with reductions of 59.7%, 67.2%, and 26.8% at 2000, 4000, and 6000 mg/L, respectively. These power densities were much more significant than those previously reported, such as 1.7 mW/m^2 and 3.3 mW/m^2 for chemical and algal cathode using *Scenedesmus abundans* (Ashwaniy & Perumalsamy, 2017), using *Nannochloropsis Salina* reported 35.7 µW/m^2 (Girme, 2014). However, the recorded P.Ds of this study were lower than those reported earlier (Neethu et al., 2019; Bejjanki et al., 2021; Sadeq & Ismail, 2023), which may be attributed to the use of lower external resistors, which could enhance electrical performance by facilitating a more significant number of electrons transferred through electrogenic microorganisms to the anode at lower resistance

(Ragab et al., 2019a). However, these high-power densities cannot solely be attributed to variations in external resistors. Other factors, such as salt concentrations, algae species, and the mode of operation employed in their study, might have also played a role in achieving these results. Fig 4.4(a) depicts that PMDC generated about 200% higher power density than the MDC-Control when both operated at 4000 mg/L anodic substrate concentration. This study demonstrated that an algal biocathode could produce a higher current with the optimum substrate at the anode than the control. As the COD concentration was incremented from 2000 to 4000 mg/L, a corresponding rise in power density was observed, indicating that higher organic matter content in the MDC system boosts microbial metabolism and electron transfer activities, resulting in increased power generation. However, at a maximum COD concentration of 6000 mg/L, the power density was lower than the peak density achieved at 4000 mg/L. Thus, it can be concluded that MDCs produce the most efficient energy at 4000 mg/L. The same trend was observed in the MFC study across varying COD levels ranging from 1325 to 3825 mg/L in the anode chamber, with maximum power density recorded at 3325 mg/L (339.4 mW/m^2). The polarization curves also indicated that increased internal resistance could also be a reason for declined power density beyond 6000 mg/L COD. These curves offer a helpful way for the analysis and description of MDC performance, as presented in Fig 4.4(b). MDC exhibited an IR of 1260.8 Ω, 983.3 Ω, and 1270.9 Ω at 2000, 4000, and 6000 mg/L concentrations, respectively. In contrast, PMDC showed 56.5%, 69.2%, and 55.6% lower IR at the corresponding concentrations. The MDCs with higher substrate concentration have the highest IR in PMDC and MDC. Compared to oxygen produced by microalgae, higher internal resistance in the MDC control resulted in lower power densities, possibly due to fewer oxygen molecules serving as electron acceptors at the MDC cathode (Nadzri et al., 2023). The internal resistance for PMDC and MDC was relatively higher than those reported in the literature ranging from 130 Ω to 440 Ω (Neethu et al., 2019; Bejjanki et al., 2021; Kokabian & Gude, 2015; Saba et al., 2017; Sadeq & Ismail, 2023) one possible reason could be the use of sucrose based-sugar wastewater as a primary carbon source. Microbes utilize sucrose, a disaccharide, as a substrate by hydrolyzing it into glucose and fructose (Del-Campo et al., 2014), resulting in high IR and low PD (Sugumar &

Dharmalingam, 2022; Ullah et al., 2023).

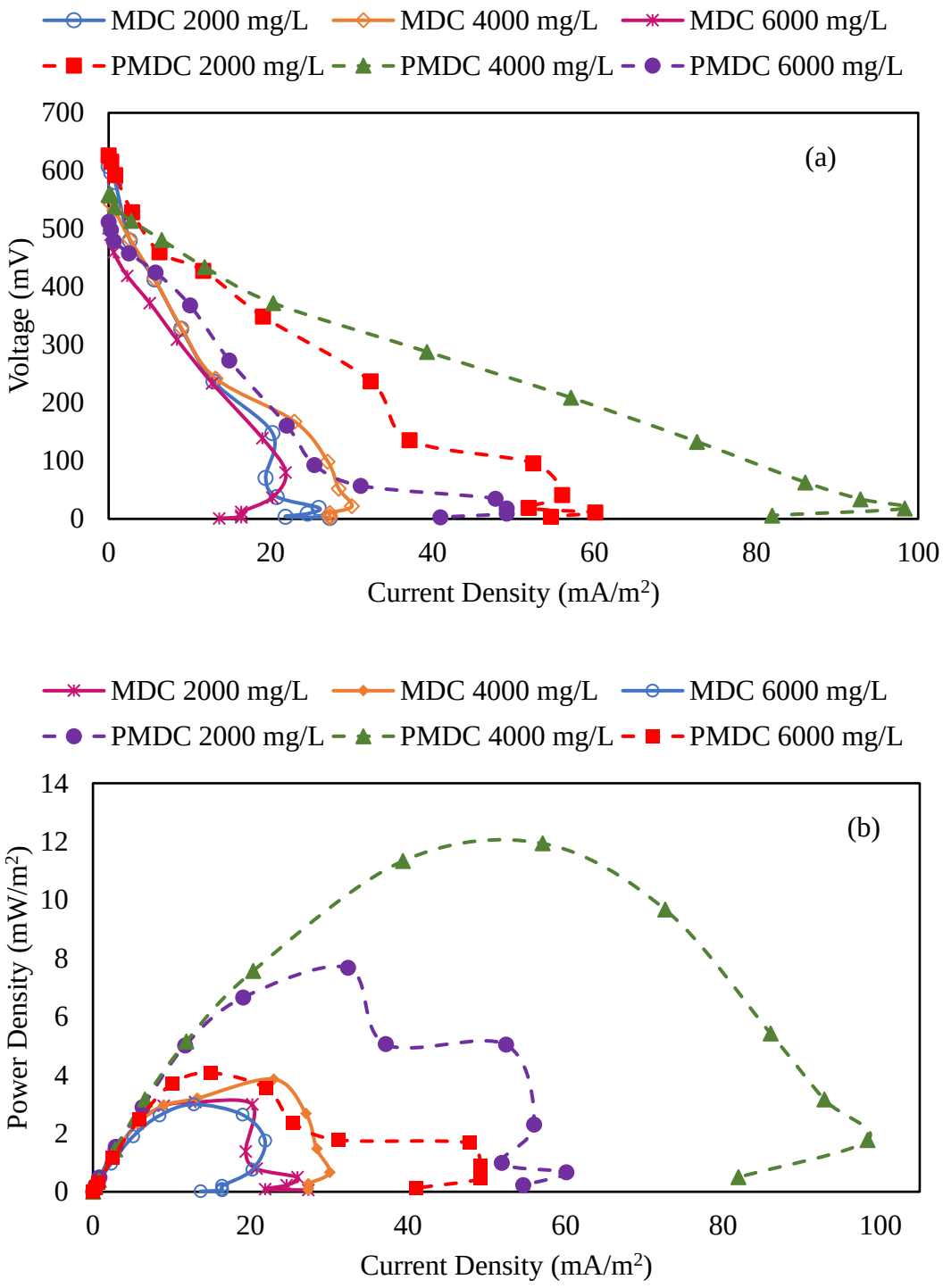


Figure 4.4 Power densities (A) and polarization behavior (B) of MDC and PMDC at various substrate concentrations.

4.2.2 Anode COD removal efficiency

The wastewater treatment efficiency of PMDC was evaluated at different substrate concentrations and compared with the control experiments. The highest COD removal was observed at 4000 mg/L, followed by 2000 mg/L and 6000 mg/L substrate concentration. Meanwhile, a sudden drop was observed initially when transitioning from low to high concentrations; it could indicate microbial adaptation or acclimatization to the higher substrate load, as depicted in Fig 4.5. The average removal efficiencies achieved by PMDC at concentrations of 2000, 4000, and 6000 mg/L were 72.1%, 82.9%, and 68.5%, respectively, which were around 11.1%, 6.1%, and 11.6% higher than those of MDC at the corresponding concentrations. This indicated that microalgae-cathode MDC exhibited high COD removal compared to the chemical cathode. Moreover, the results demonstrate that the system performed well at low to medium substrate concentrations due to the favorable conditions for microbial degradation and metabolic activity. At these concentrations, the microbial community efficiently decomposes organic pollutants, resulting in effective wastewater treatment without encountering inhibitory effects or excessive build-up of organic matter. At the same time, the best performance was achieved at medium concentrations, possibly because there is an optimal balance between substrate availability and microbial activity, which promotes more effective degradation of organic pollutants, and the microbial community reached a critical mass or metabolic activity level that enhanced the overall degradation process. Meanwhile, substrate saturation or inhibition effects at high concentrations may hinder the microbial community's ability to degrade organic pollutants effectively, as evidenced by the results.

A similar trend was observed by Ragab et al. (2019), where the average COD removal efficiencies were 90.0 ± 5.2, 92.3 ± 4.3, and 53.4 ± 7.2% at substrate concentrations of 500, 1500, and 3000 mg/L in MDC respectively. Liu et al. (2019) also reported that when the anode influent COD was 900 mg/L, the COD removal was higher than other CODs (400 and 1400), attributing it to the matured anode biofilm and appropriate COD concentration maintaining active microbial activity. Kokabian & Gude (2015) tested two substrate concentrations (500 & 1000 mg/L COD) in PMDC, and average COD removal of 76.1% ± 1.2 and 82.2% ± 1.3 was observed after the 1000h batch cycle, indicating the suitability of

PMDCs for treating low to moderate strength wastewaters. Higher COD removal was also reported in the literature using PMDCs such as Sadeq & Ismail (2023) reported that maximum and average COD removal up to 99.3 ± 0.5 % and 91.0 ± 0.8 %, respectively, for 5 days of operation using actual sewage (COD 550 ± 100) and with *Coelastrella* sp. and *Mariniradius saccharolyticus* algal cultures at the cathode. Hui et al. (2020) reported a 95% removal efficiency of COD in a *Chlorella vulgaris*-based biocathode of cylindrical PMDC for the treatment of landfill leachate. Significant lower COD removal of 49 % and 53 % were reported when using palm oil mill wastewater as an anolyte in biocathode MDCs with *Scenedesmus* sp. (UKM9) and *Chlamydomonas* sp. (UKM6), respectively (Nadzri et al., 2023).

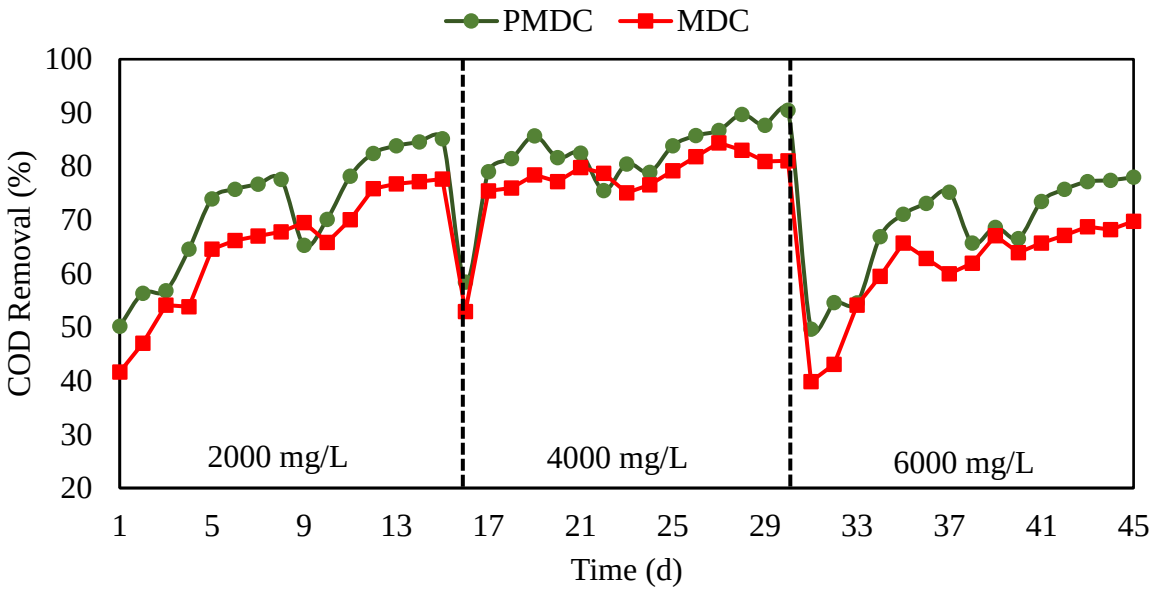


Figure 4.5 Anode COD removal efficiency of MDC and PMDC at varying substrate concentration.

Some possible explanations for the observed discrepancies in COD removal could be attributed but not limited to various factors such as differences in the anodic and cathodic biocatalysts, anodic substrate composition, and dimensional layout of PMDC. While COD concentration significantly influences MDC electrochemical performance, no conclusive evidence links the cathode chamber's aeration technique to the anode's COD removal. The primary factors affecting COD removal are organic matter and microbial activity. PMDCs

may exhibit slightly better performance due to enhanced electrochemical activity in a biocathode environment. For instance, in a study treating dairy effluent in a biocathode MDC, improved COD removal was observed, indicating that the synergy of both bio-sourced oxygen, acting as a terminal electron acceptor (TEA) and wastewater contributed to better COD removal (Bejjanki et al., 2021).

4.2.3 Desalination efficiency

Desalination efficiency is crucial in determining the MDC performance and can be impacted by substrate availability and resistance within the system (Ragab et al., 2019a). Electrical conductivity (EC) was used to evaluate desalination performance. The results for the desalination were tested over a batch of 4 days, subjected to various substrate concentrations at the anode of PMDC and MDC. The initial electrical conductivity of PMDC and MDC was 56 ± 0.2 mS/cm for the salt concentration of 35 g/L NaCl. The results revealed that PMDC performed better than MDC in terms of desalination. As shown in Fig 4.6, PMDC with 2000, 4000, and 6000 mg/L had better salt removal efficiency of 19%, 27%, and 21% than MDC at the respective concentrations, resulting in 16%, 23, and 17% desalination efficiency. Optimal desalination was observed at a moderate substrate concentration (4000 mg/L), potentially attributable to the lower internal resistance and increased electrical potential between the cathode and anode, facilitating a substantial amount of ion migration from the middle chamber. PMDC has approximately 17.4% higher desalination efficiency than MDC during 4 days at 4000 mg/L COD.

The better desalination at PMDCs could be because of the increased photosynthetic oxygen concentration in the cathode as a TEA, accelerating the redox reaction and improving efficiency (Neethu et al., 2019). Similar results are reported in studies where algal MDCs performed better than conventional (air or chemical) MDCs. For example, desalination of 44.8% and 32.4% for algal MDC using *Scenedesmus* sp. (UKM9) and *Chlamydomonas* sp. (UKM6) and 21.9% for the control setups was achieved, respectively, when using 35 g/L of NaCl (Nadzri et al., 2023). Saba et al. (2017) achieved salt removal of 41–45% using a chemical cathode and 43–45% using algae in a 24-hour cycle with an initial 35 g/LNaCl solution. The desalination performance was enhanced when the initial concentration was halved to 17.5 g/L, achieving 72% and 79% desalination for chemical and algal cathode,

respectively. The lower desalination efficiency in this study was possibly due to using higher salt concentration at the start-up, which might have slightly affected the microbes' activity in the anode and cathode chambers.

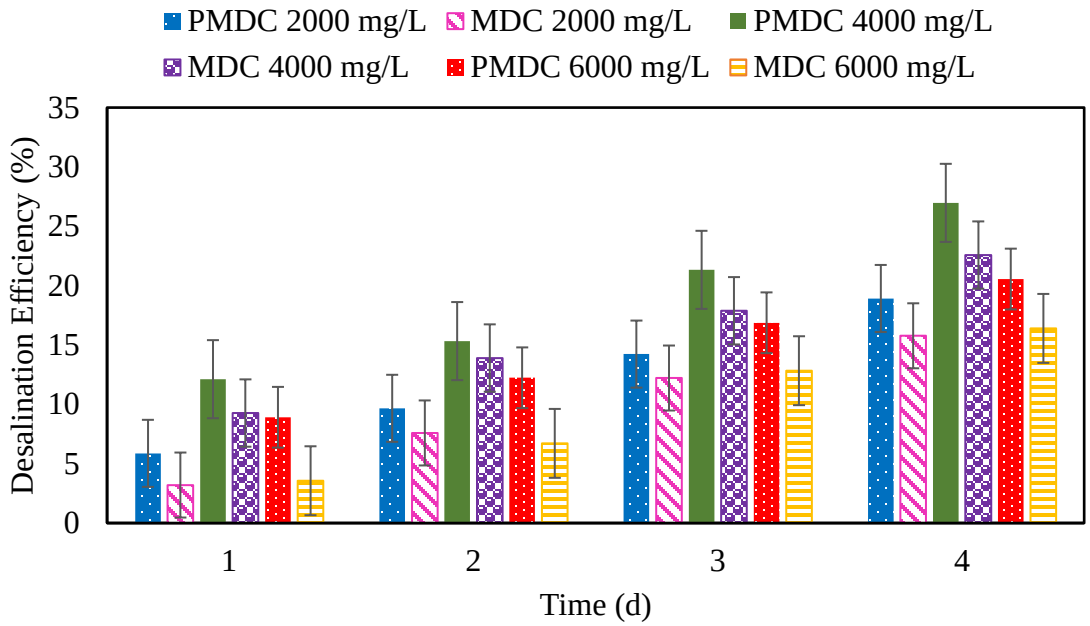


Figure 4.6 Desalination efficiency of MDC and PMDC in 4 days at various substrate concentrations.

For instance, Mahdi & Safi (2016) achieved 94.0% salt removal with a low salt concentration of 7.9 g/L at the startup, while desalination reached 65.8% when 20 g/L of NaCl was used (Bejjanki et al., 2021). Another factor impacting desalination efficiency could be the migration of competing ion species from both the anode and cathode chambers. Ammonia (in PMDC) and phosphates (in both MDC and PMDC) were observed migrating into the desalination chamber, diminishing the purity of the product for portable use. Nevertheless, the addition of nitrogen and phosphates and decreased salinity and electrical conductivity render the desalinated product suitable for agricultural purposes. Numerous variables, including the volume and concentration of wastewater and salt solution, the membrane surface area, oxidation-reduction reactions (ORR), and the hydraulic retention times (HRTs) impact desalination efficiency in microbial desalination cells (Kokabian & Gude, 2013). Therefore, a longer HRT was also tested for effective desalination to evaluate the performance of MDCs. The result showed that PMDC has

approximately 15.4% higher desalination efficiency in 15 days with a salt removal of 67. 6% for an initial 35 g/L NaCl compared to MDC, as shown in Fig 4.7. These findings not only align but surpass the results documented in the literature. For instance, Ashwaniy and Perumalsamy (2017) reported 55.3% desalination for the microalgae bio-cathode and 32.7% for the chemical cathode in the 20-day cycle.

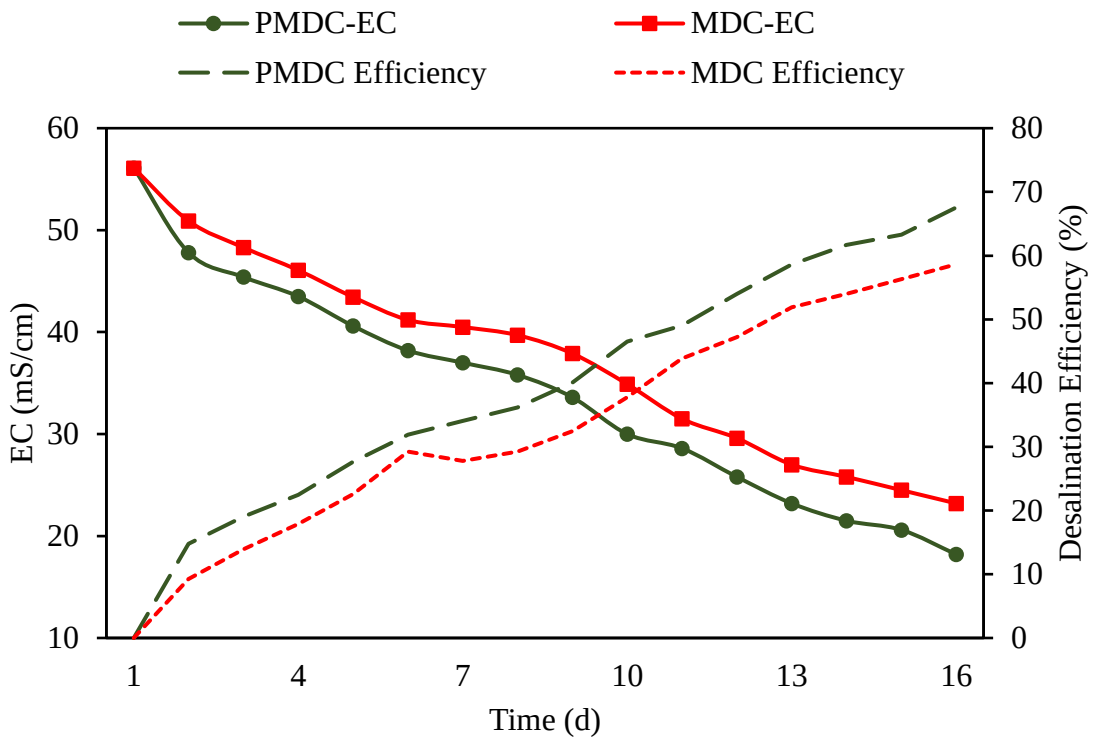


Figure 4.7 Desalination efficiency of MDC and PMDC in 15 days at 4000 mg/L concentration.

4.2.4 Algal growth, nutrient removal, and dissolved oxygen at the cathode

The nutrient removal and algal concentration of the cathodic chamber of PMDC for different analyte concentrations are shown in Fig 4.8. Although the catholyte remained unchanged through the experiment, the PMDC cathode showed slightly varied removal efficiencies corresponding to the varied concentrations of anolyte. In contrast to NH_4^+-N and PO_4^{3-} P, the average removal efficiency of COD was higher in PMDCs. The COD removal of 63.8%, 71.9%, and 64.0%, NH_4^+-N removal of 60.6%, 62.4%, and 61.6% while and PO_4^{3-}-P removal of 35.6%, 42.9%, and 41.8% were achieved for 2000, 4000, and 6000 mg/L respectively. The nutrient removal in terms of NH_4^+-N removal was higher due to

increased photosynthetic activity in microalgal cells. However, this relationship did not follow a linear trend with algal growth. This discrepancy may be attributed to potential migrations of NH_4^+-N across the cation exchange membrane from the cathode to the desalination chamber; moreover, volatilization and oxidation of ammonia might also have led to the concentration losses in the catholyte (Hou et al., 2020). Furthermore, a lower phosphate removal (35%- 43%) was probably due to the higher phosphate concentration in this test, as phosphorus removal mechanisms include assimilation by microalgae and precipitation at high pH. As the pH was below 8, as shown in Fig 4.9, it is assumed that microalgae assimilation was the primary method for phosphate removal. Only a few studies exist that have treated wastewater at the PMDC cathode, such as Jaroo et al. (2019), treated oil refinery wastewater at the cathode and achieved COD removal of 79.2%, respectively, during the cycle of three days. In another study, 90% of nitrate and approximately 20% phosphates removal was observed in 50h during continuous cathode operation, and approximately 42% of nitrate and 16% phosphate were removed during 7 days in Photo-bioreactor MDC (Kokabian et al., 2018a; Kokabian et al., 2018b).

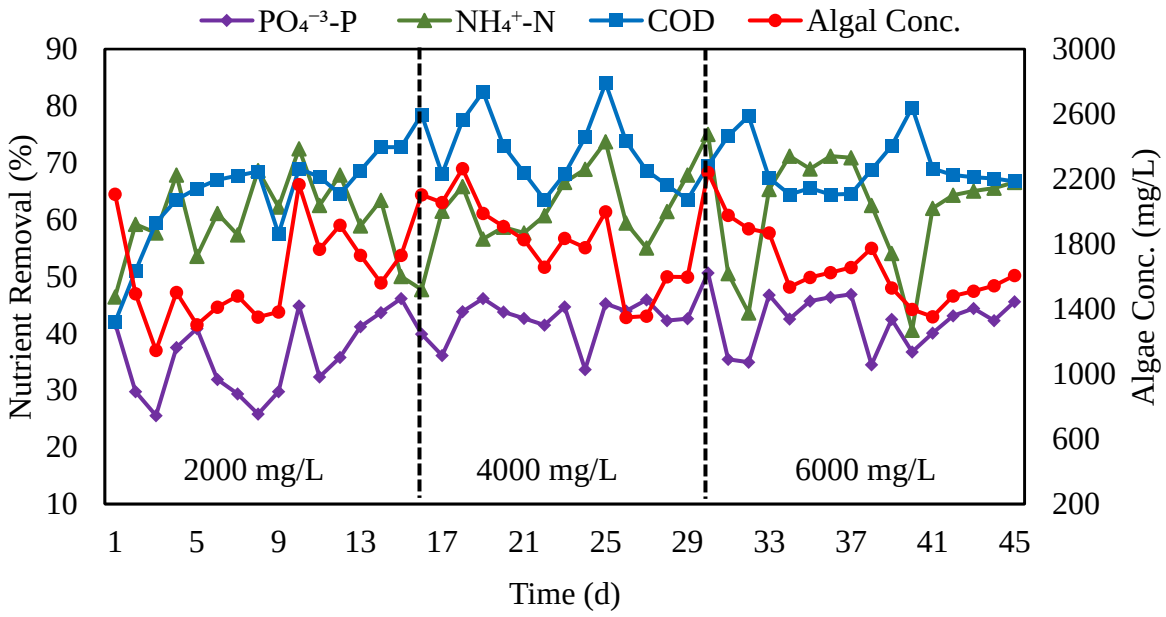


Figure 4.8 Nutrient removal and algal growth in PMDC cathode in response to varying anode substrate concentrations.

Algal growth concentrations also varied in accordance with anolyte concentrations despite having the same catholyte. A higher microalgae concentration was achieved in PMDC with

4000 mg/L anodic substrate with an average growth rate of 418 mg/L/d, as shown in Figure 7, with an initial concentration of 1000 mg/L, which was about 14.6% and 13.2% higher than PMDC at 2000 and 6000 mg/L anodic substrate concentration, respectively. Observing higher algae growth and current generation in PMDC at 4000 mg/L proved that more electrons transferred to the cathode chamber positively affected algae growth. Since algae demand electrons for reproduction through their metabolism, access to more electrons can enhance their growth (Zamanpour et al., 2017), as shown in our results. Moreover, from the cathode, a daily algal suspension was collected along with the effluent. This algal suspension holds significant potential due to Scenedesmus obliquus's versatile commercial applications (Duan et al., 2020). For instance, it can be used for biofuel production (Oliveira et al., 2020). Therefore, the collected algal suspension presents an opportunity for further exploration and utilization in various industries, making the system a green and renewable source of bio-energy in water treatment technologies.

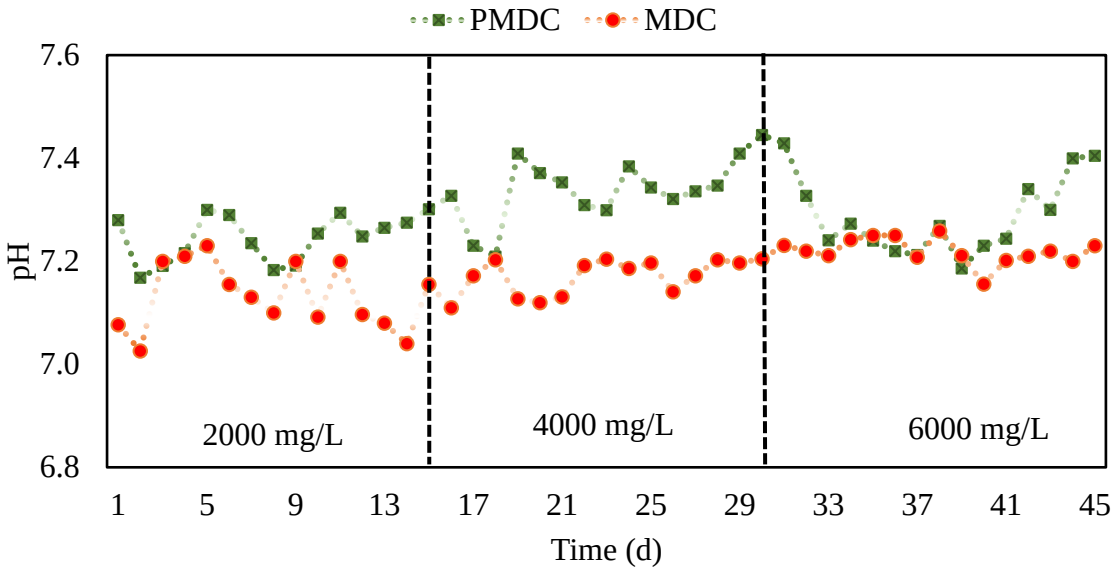


Figure 4.9 pH variation at the cathode of MDC and PMDC at various substrate concentrations.

The cathode chamber was supplied with algae medium in a fed-batch mode retaining the existing algae from the batch phase. This resulted in persistent dissolved oxygen and algal growth until the agal concentration dropped below 1000 mg/L. The fresh algal culture was then introduced into the system to maintain the initial algal of 1000 mg/L. The introduction

of fresh algae reinitiated algae reproduction, resulting in increased algae growth and DO concentrations. The photosynthetic MDC has shown higher DO concentration than the control MDC, as shown in Fig 4.10.

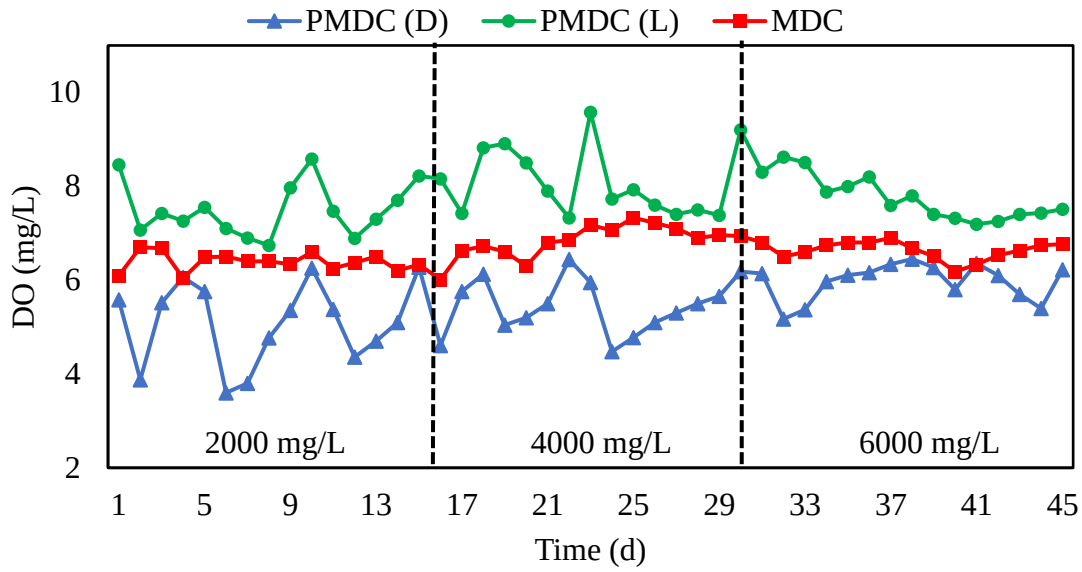


Figure 4.10 Dissolved oxygen levels of PMDC and MDC in response to varying anode substrate concentrations (D and L represent dark and light photoperiod).

MDC has shown almost consistent DO with slight variation in all three experiments, which might be because the same amount of oxygen was provided daily, and there was no passive source of oxygen, such as algae in the cathode of PMDC. The maximum DO was attained utilizing 4000 mg/L in both PMDC and MDC, with concentrations of 9.5 mg/L and 7.3 mg/L, respectively. Previous studies have also reported high DO using algal biocathode in MDC compared to abiotic MDC. For instance, Neethu et al. (2019) reported a DO of 7.6 mg/L using PMDC and around 5 mg/L in MDC. The lower DO concentration in MDC using PBS could be the lower solubility of oxygen in PBS, which is attributed to the higher saline concentration in PBS, which reduces the ability of oxygen to dissolve in the solution (Danaee et al., 2023). Another factor contributing to this lower O_2 concentration could be the complex oxygen transport process. Before oxygen can reach the cathode surface, it must pass through several resistance obstacles. After diffusing from the primary gas to the gas-liquid junction through a relatively still liquid area near the bubble, it flows through the bulk liquid toward the static zone around the cathode. Eventually, it reaches the cathode

surface, where the reduction reaction occurs. Because of the mechanically aerated catholyte, MDC exhibits a combination of all these resistances. In contrast, in PMDC, where microalgae are cultivated in suspension, and photosynthesis produces pure oxygen, the first resistance (Bulk Gas to Gas-Liquid Interface) is removed. Additionally, algal biofilm may build on the cathode surface. Here, the diffusing oxygen reaches the cathode surface by passing a single stagnant area devoid of bulk liquid (Ullah et al., 2023).

4.3 The Effect of Salt Concentration on Performance of Cells

4.3.1 Voltage and polarization

The two systems, PMDC and MDC, were operated to determine the optimal salt concentration at the desalination chamber to maximize desalination efficiency and power production. For all three salt concentrations of 15, 25 and 35 g/L, PMDC generated higher voltage and power output in comparison to MDC. The voltage and power generation at the moderate to high salt concentrations were higher both in PMDC and MDC (control). However, PMDC yielded the highest value at 25 g/L salt, as shown in Fig 4.11. At 25 and 35 mg/L concentrations, PMDC achieved an average maximum working voltage of 283.3 mV and 282.7 mV, respectively. However, the voltage decreased to 225.2 mV when a low salt concentration of 15 g/L was used in the desalination compartment. PMDC exhibited 53.1%, 39.2%, and 39.9% higher voltages than the respective values obtained by MDC when supplied with 15, 25, and 35 g/L salt concentrations. This shows that raising the concentration from 15 g/L to 25 g/L resulted in a voltage increase; further raising the salt concentration to 35 g/L did not significantly affect the voltage generation. This is because that a low concentration of salts can impede voltage and power generation by reducing conductivity. Nevertheless, a salt concentration of 20-25 g/L appears to be effective in averting this hindrance. Our findings align with Safwat et al. (2023), indicating that a salt concentration of up to 20 g/L is essential for improvements in voltage and power generation. It is important to highlight that increasing the salt concentration does not consistently lead to a proportional increase in voltage generation. The performance of cells is influenced by complex relationships and various interconnections. One important factor to consider is the pH variation caused by the movement of ions in each chamber. This can lead to an imbalance in the electrolytes' pH, which in turn affects the electrogenic

microorganisms and their ability to generate power. Additionally, when chlorine ions are present in higher concentrations in the anode chamber, it negatively impacts the activity of exoelectrogenic microbes, resulting in a decrease in current generation in the MDC. (Bejjanki et al., 2021, 2021).

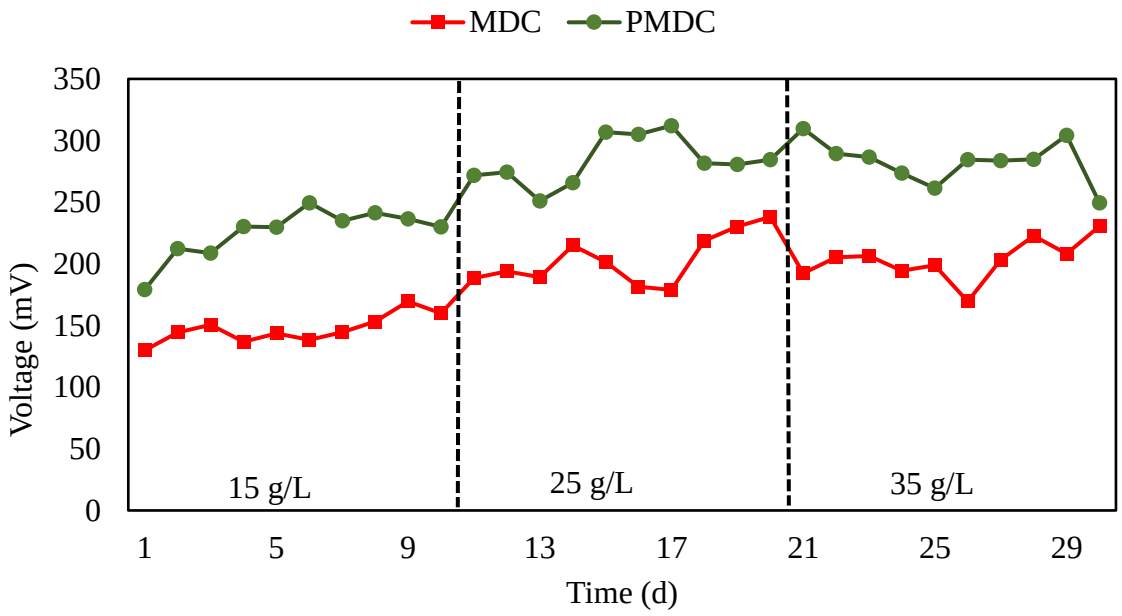


Figure 4.11 Voltage profile of MDC and PMDC at various salt concentrations.

Moreover, a lower voltage attained at low salt concentration indicates that the reduced salt content in the middle chamber hinders current generation, which is vital for desalination. Hence, the internal resistance (IR) resulting from the salt concentration in desalination is a critical factor that impacts the performance of the MDC (Balushi et al., 2022). The IR, derived from polarization curves as depicted in Fig 4.12, shows that, as the salt concentration decreased from 25 g/L to 15 g/L, the increment of ohmic resistance was quite profound at lower salt concentrations both in MDC and PMDC. At the same time, a slight increase in IR was observed when salt concentration was increased from 25 to 35 g/L. MDC exhibited an IR of 1446.9 Ω, 423.3 Ω, and 983.3 Ω at 15, 25, and 35 g/L salt concentrations, respectively. In contrast, PMDC showed 68.3%, 45.0%, and 69.2% lower IR at the corresponding concentrations. The MDCs with lower salt concentrations have the highest IR in PMDC and MDC. A study revealed a similar trend when examining the effect of salt concentration on ohmic resistance. At lower salt concentrations of 15, 10, 5, and 1

g/L, the ohmic resistance values were measured at 68, 182, 194, and 332 Ω, respectively. Ohmic losses refer to the resistance encountered during the transfer of electrons between the electrodes and the electrolytes. Consequently, the conductivity of the electrolyte, particularly in the middle chamber, significantly influences these losses (Balushi et al., 2022). Compared to the literature, this study resulted in higher internal resistance for PMDC and MDC, which might be because of the use of sucrose-based-sugar wastewater as a primary carbon source. Microbes utilize sucrose, a disaccharide, as a substrate by hydrolyzing it into glucose and fructose (Del-Campo et al., 2014), resulting in high IR and low PD (Sugumar & Dharmalingam, 2022; Ullah et al., 2023).

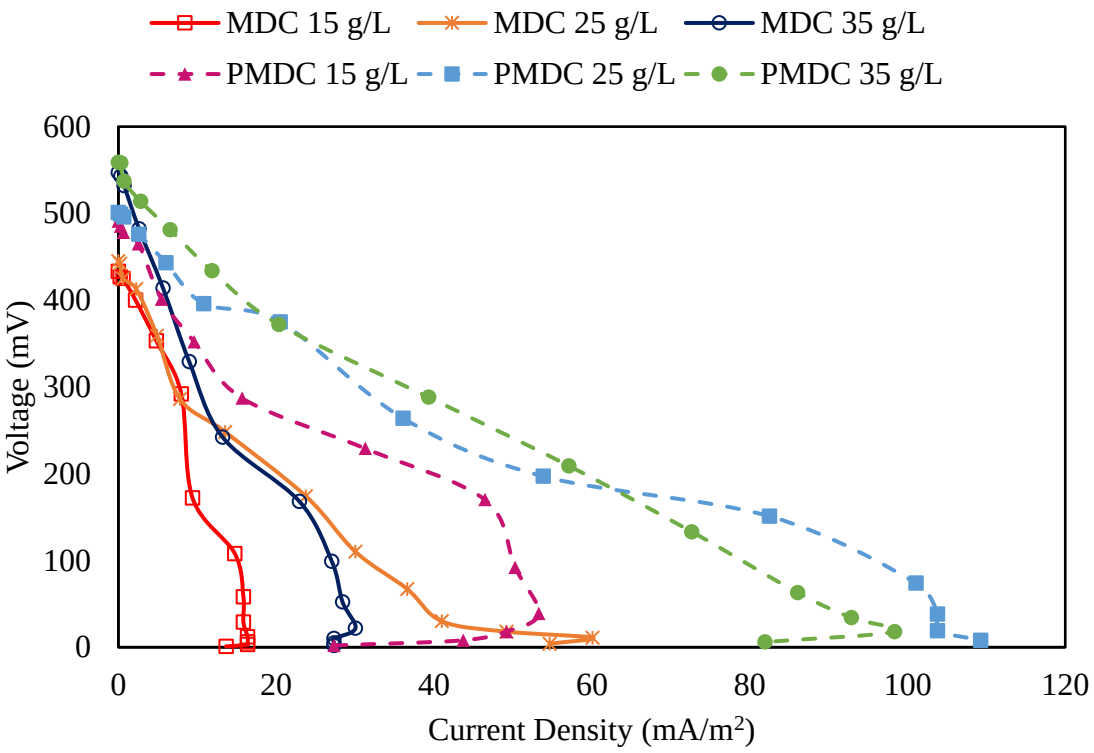


Figure 4.12 Polarization behavior of MDC and PMDC at various salt concentrations.

This increased IR correlates with power density as systems having higher IR result in lower power density levels. The power density (P·D) calculated regarding anodic/cathodic volumes for MDC and PMDC are shown in Fig 4.13. P.D. of 7.9 mW/m², 12.5 mW/m², and 11.9 mW/m² were recorded for PMDC at 15, 25, and 35 g/L salt concentrations, respectively. Conversely, MDC exhibits lower power densities than PMDC, with

reductions of 70.8%, 67.2%, and 67.2% at 15, 25, and 35 g/L, respectively. Our study complements the results of Bejjanki et al. (2021), where at 10, 20, and 30 g/L salt concentration, the highest power density of 44.1 ± 1.0 mW/m^2 was observed for the middle salt concentration (20 g/L). The power densities of 32.8 ± 1.0, 31.1 ± 1.0, and 34.6 ± 1.5 mW/m^2 were obtained for 35 and 10 g/L salt concentrations and control experiments with PBS.

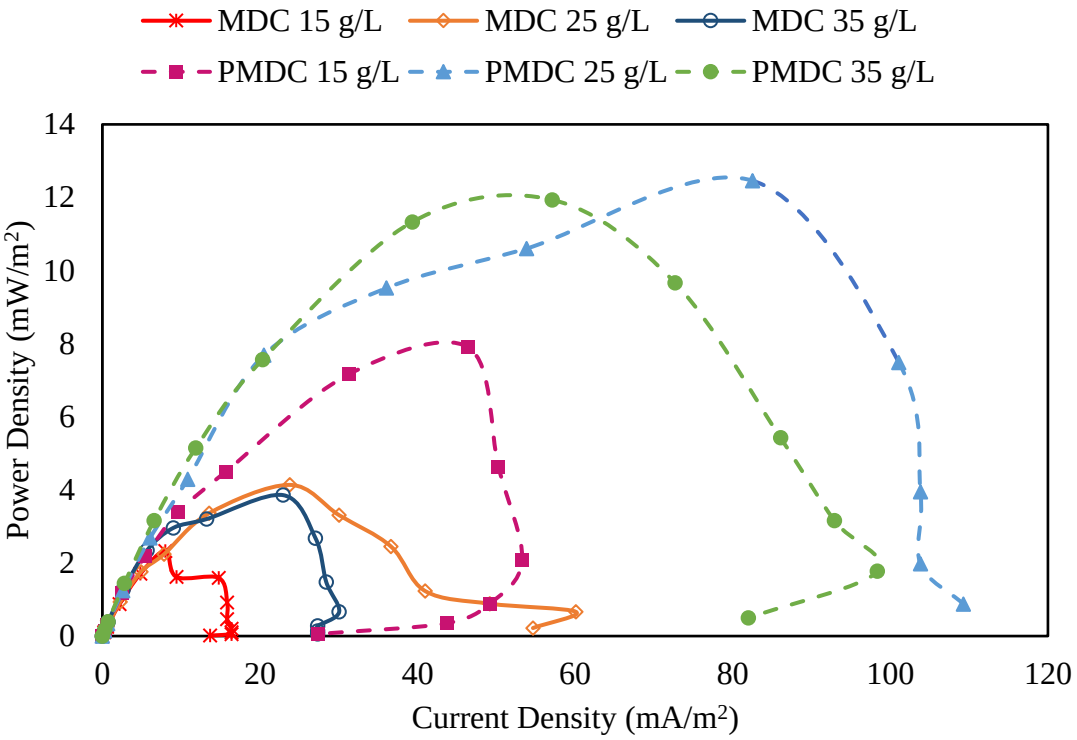


Figure 4.13 Power densities of MDC and PMDC at various salt concentrations.

4.3.2 Anode COD removal efficiency

The wastewater treatment efficiency of PMDC was evaluated at different salt concentrations and compared with the control experiments, as depicted in Fig 4.14. The highest COD removal was observed at 25 g/L, followed by 35 g/L and 15 g/L salt concentration, respectively. Interestingly, salt concentrations of 25 g/L and 35 g/L exhibited nearly identical removal rates, with 25 g/L achieving 77.8% and 35 g/L achieving 77.5% COD removal, while a concentration of 15 g/Lresulted in 74.7% COD removal over 2 desalination cycles for MDC. PMDC outperformed MDC, consistently showing higher COD removal rates. Specifically, at salt concentrations of 15, 25, and 35

g/L, PMDC achieved 3.9, 3.6, and 6.9% higher COD removal compared to MDC under identical salt conditions. Enhanced performance in biocathode MDC can be attributed to various factors. Initially, the presence of bacteria leads to an increased production of electrons by accelerating the biodegradation of COD in the anode chamber. Furthermore, the combination of oxygen and wastewater as the electron acceptor in the biocathode facilitates a higher COD elimination rate. This is due to the strong oxidizing nature of oxygen, which rapidly consumes all available electrons in the cathode chamber, creating a strong pull on electrons from the anode in a closed circuit configuration (Ebrahimi et al., 2018).

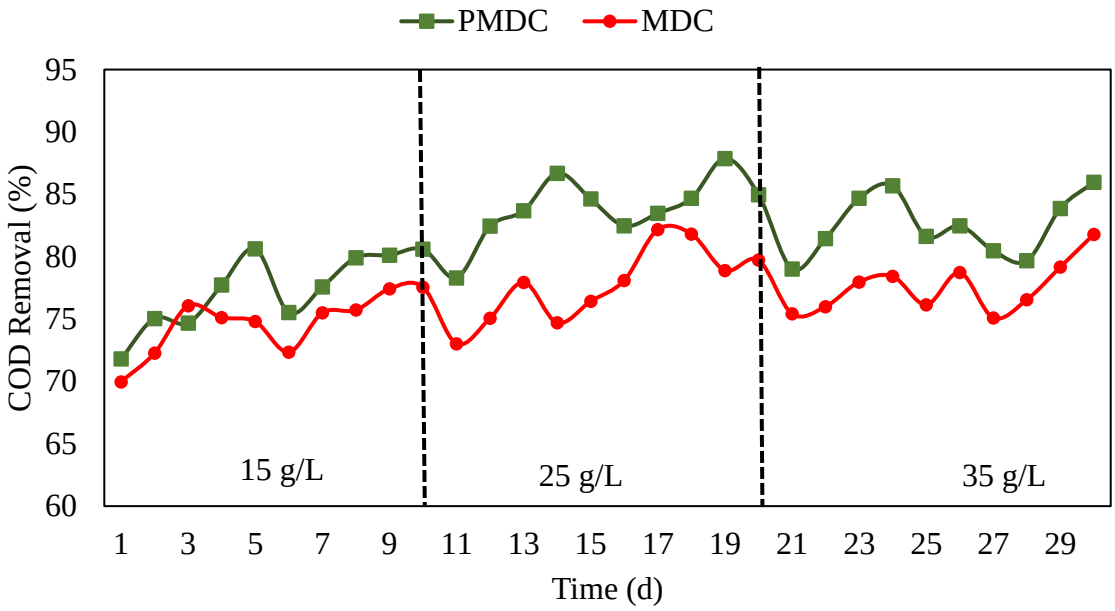


Figure 4.14 Anode COD removal efficiency of MDC and PMDC at various salt concentrations.

Higher salt concentrations were found to enhance the removal of COD due to the increase in osmotic pressure and reduction in internal resistance, thereby influencing the behavior of the bacteria (Liaquat et al., 2021). A similar trend was observed by Abd-almost et al. (2022), where a higher TDS concentration of 25 g/L resulted in higher COD removal of 40%, and 25% maximum COD removal was achieved at 15 g/L TDS concentration in batch mode with 48h HRT using pure oxygen at the cathode of MDC. For Using *Oscillatoria* sp. as biocathode in MDC resulted in COD removal efficiency of 75.6 ± 1.0, 78.2 ± 1.0, and 80.2 ± 1.0%, respectively, for the salt concentrations of 10, 20, and 30 g/L

(Bejjanki et al., 2021). In literature, utilizing salt concentrations ranging from 30-35 g/L typically resulted in higher COD removal rates. However, this study observed lower efficiency at 35 g/L compared to the literature, with relatively similar results to those obtained at 25 g/L salt concentration. This discrepancy could be attributed to increased IR and reduction in pH, as our study noted a more profound reduction in pH at this higher concentration might be resulting from bacterial substrate consumption, accumulation of hydrogen, and/or ion migration from the desalination chamber. Fig 4.15 shows the pH for MDC and PMDC anode, initially ranging from 7 to 7.1; average pH in MDC for 15, 25, and 35 g/L salt were observed to be 6.8, 6.7, 6.5, and PMDC had recorded on average pH values of 6.8, 6.7 and 6.6. Ashwaniy & Perumalsamy,(2017) reported the similar trend where anolyte pH decreased to 24% with 35 g/L NaCl and 10% with 20 g/L Nacl over the fed batch period.

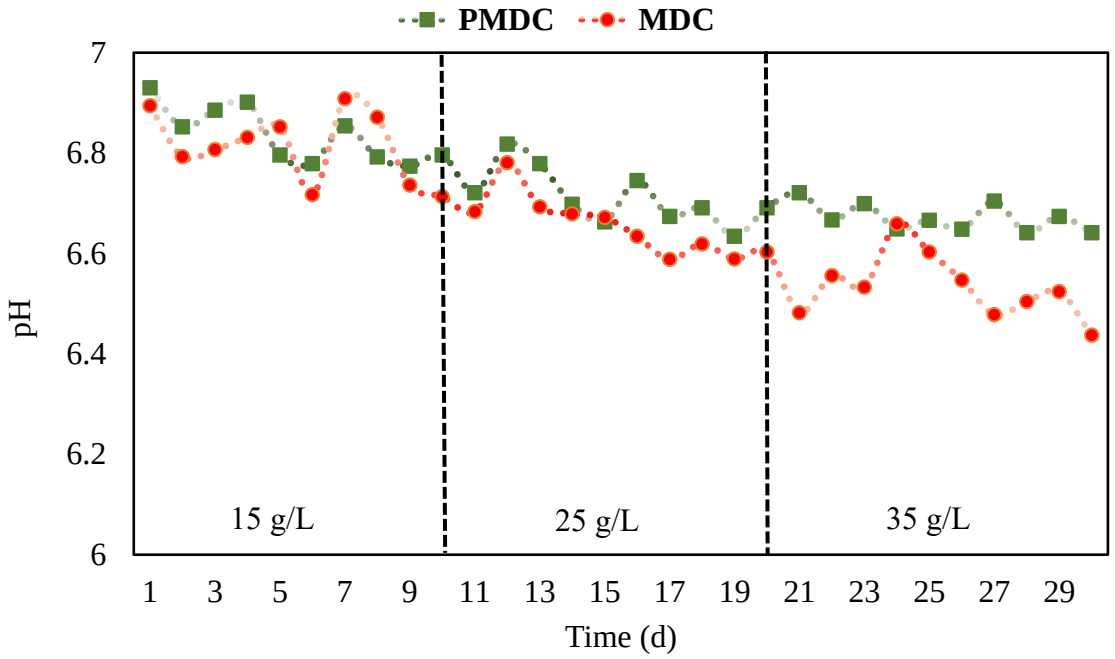


Figure 4.15 pH reduction at the anode of MDC and PMDC at various salt concentrations.

Fig 4.16 shows the anolyte conductivity (mS/cm), which is attributed to ion migration from desalination to the anode, showing an increase from 3.9 to 4.9, 5.2, and 5.4 for MDC with 15, 25, and 35 g/L salt in desalination chamber while PMDC recorded average EC values of 5.3, 5.7 and 6.4 at the corresponding salt concentration, respectively. The anode

chamber experienced a negligible decrease in pH at low substrate concentrations due to biochemical reactions. However, anode acidification occurred at high concentrations due to biodegradation-induced organic acid accumulation. Therefore, due to the unfavorable conditions, microorganism activity was compromised, resulting in a decreased performance. According to the previous results, the highest current density and possible percentage of COD reduction can be achieved at a pH near neutral. To put it another way, at acidic pHs, the rate of microbial activity is relatively low (Rahmani et al., 2022).

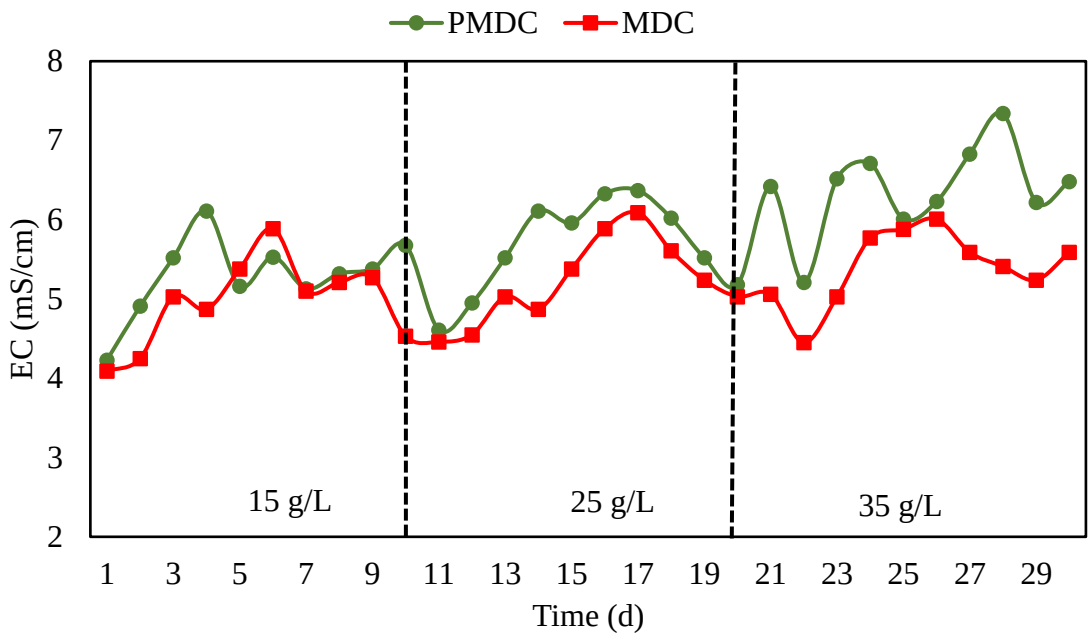


Figure 4.16 EC profile of MDC and PMDC anode at various salt concentrations.

4.3.4 Desalination efficiency

Desalination efficiency is crucial in determining the MDC performance and can be impacted by salt concentrations. Electrical conductivity (EC) was used to evaluate desalination performance. The desalination results for three salt concentrations of 15, 25, and 35 g/L were tested over a batch of 4 days in MDC and PMDC. The initial conductivity of PMDC and MDC for 15, 25, and 35 g/L in the middle chamber was 26.7±0.2, 40.8±0.2, and 56 ± 0.2 mS/cm. The results revealed that using high salt concentration resulted in higher desalination efficiency, as shown in Fig 4.17. MDC with 15, 25, and 35 g/L had salt removal efficiency of 14.6%, 25.4%, and 22.7% respectively for two desalination cycles. However, PMDC performed better than MDC in terms of desalination by achieving 30.8,

17.7, and 19.4% higher salt removal efficiency than MDC. This shows that MDCs are suitable for high salt content desalination because high salinity boosts bioelectricity, speeding up desalination by reducing resistance and increasing concentration gradient (Ashwaniy & Perumalsamy, 2017). Bioelectricity produced in the system and the difference in salt concentration are considered the two main factors that drive the spontaneous removal of salt in the MDC systems. These factors are particularly beneficial for desalination when dealing with higher salt concentrations (Jafary et al., 2018). Kokabian, et al. (2018a) observed a similar trend in their study where high salt concentrations resulted in higher salt removal percent, such as using 5, 20, and 35 g/L NaCl, resulted in 58%, 63%, and 64% desalination efficiency. In another study, using 35, 17.5, and 8.25 g/L NaCl in both MDC and PMDC systems; MDC system achieved 41–45% desalination while PMDC achieved desalination efficiency of 43–45% with 35 g/L NaCl. By reducing the salt content to half (17.5 g/L), higher desalination of 72% and 79% by MDC and PMDC was observed . However, both systems showed <20% desalination when the salt concentration was further reduced to 8.25 g/L (Saba et al., 2017). The lower desalination efficiency in this study was possibly due to using higher salt concentration at the start-up, which might have slightly affected the microbe's activity in the anode and cathode chambers. For instance, Mahdi & Safi (2016) achieved 94.03% salt removal with a low salt concentration of 7.9 g/L at the startup, while desalination reached 65.8% when 20 g/L of NaCl was used (Bejjanki et al., 2021). Another factor impacting desalination efficiency could be the migration of competing ion species from both the anode and cathode chambers. Ammonia (in PMDC) and phosphates (in both MDC and PMDC) were observed migrating into the desalination chamber, diminishing the purity of the product for portable use. Nevertheless, the addition of nitrogen and phosphates and decreased salinity and electrical conductivity render the desalinated product suitable for agricultural purposes. Therefore, assessing the MDC as a stand-alone desalination system in the current technological context with limited performance capabilities may not be practical. Nevertheless, it is advisable to consider it as a sustainable and cost-efficient technology for the preliminary treatment phase of established desalination systems, like RO. This approach offers notable advantages in terms of energy and cost savings, as well as a

reduction in the burden and cost associated with the conventional desalination process (Balushi et al. (2022).

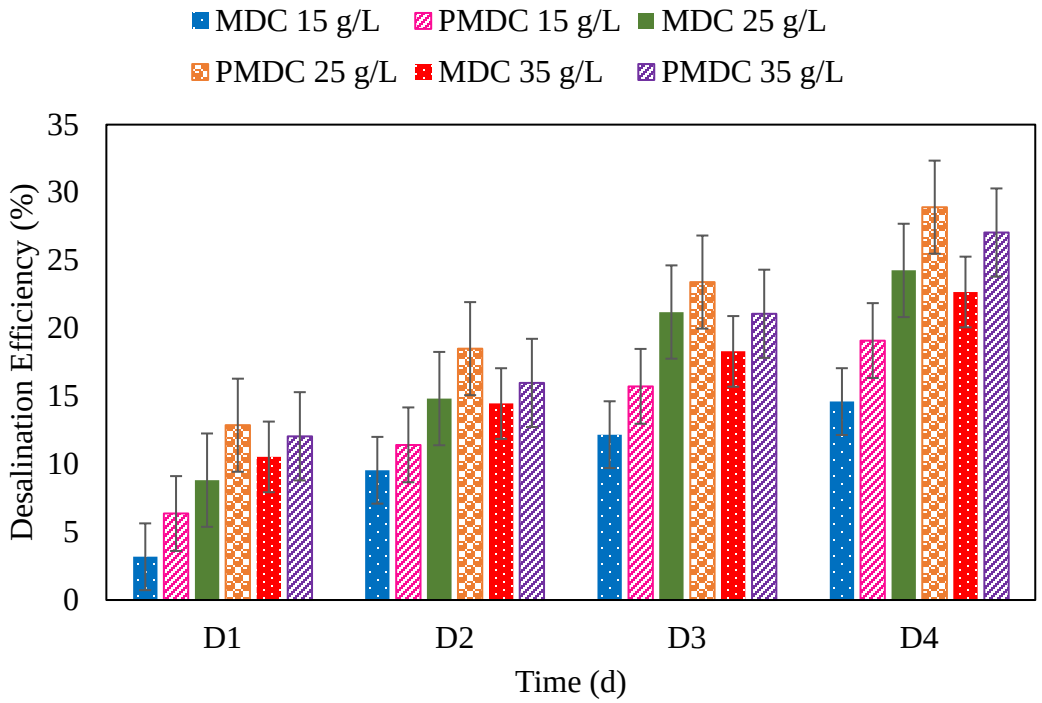


Figure 4.17 Desalination efficiency of MDC and PMDC at various salt concentrations.

4.4.4 Algal growth, nutrient removal, and dissolved oxygen at the cathode

The nutrient removal and algal concentration of the cathodic chamber of PMDC for different salt concentrations are shown in Figure 4.18. Although the catholyte remained unchanged throughout the experiment, the PMDC cathode showed slightly varied removal efficiencies and algal growth corresponding to the salt concentration in the middle chamber. In contrast to NH_4^+-N and PO_4^{3-} P, the average removal efficiency of COD was higher in PMDC. The COD removal of 66.7%, 76.5%, and 73.5%, NH_4^+-N removal of 56.2%, 66.4%, and 65.8% while and PO_4^{3-}-P removal of 38.2%, 43.0%, and 42.9% were achieved for 15, 25, and 35 g/L respectively. The nutrient removal in terms of NH_4^+-N removal was higher due to increased photosynthetic activity in microalgal cells. However, this relationship did not follow a linear trend with algal growth. This discrepancy may be attributed to potential migrations of NH_4^+-N across the cation exchange membrane from the cathode to the desalination chamber; moreover, volatilization and oxidation of

ammonia might also have led to the concentration losses in the catholyte (Hou et al., 2020). Furthermore, a lower phosphate removal (38%- 43%) was probably due to the higher phosphate concentration in this test, as phosphorus removal mechanisms include assimilation by microalgae and precipitation at high pH. As the pH was below 8, as shown in Fig 4.19, it is assumed that microalgae assimilation was the primary method for phosphate removal. Only a few studies exist that have treated wastewater at the PMDC cathode, such as Jaroo et al. (2019), treated oil refinery wastewater at the cathode and achieved COD removal of 79.2%, respectively, during the cycle of three days. In another study, 90% of nitrate and approximately 20% phosphates removal was observed in 50 h during continuous cathode operation, and approximately 42% of nitrate and 16% of phosphate were removed during 7 days in Photo-bioreactor MDC (Kokabian et al., 2018a; Kokabian.

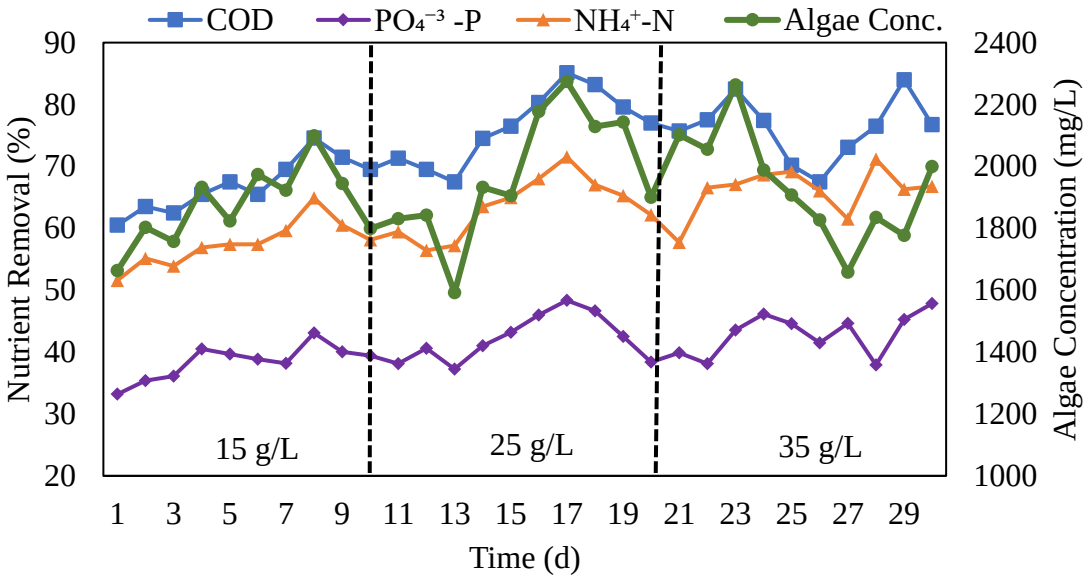


Figure 4.18 Algal growth and nutrient removal at PMDC cathode subjected to various salt concentrations.

Algae growth concentrations also varied in accordance with salt concentrations of the middle despite having the same catholyte, but the impact was not significant. A higher microalgae concentration was observed at a higher salt concentration in the middle chamber, as shown in Fig 4.18. PMDC resulted in an average growth rate of 470.5 and 486.5 mg/L/d for 35 and 25 g/L, however at 15 g/L the growth rate was 436.0 mg/L/d

respectively, with an initial concentration of 1000 mg/L. Observing higher algae growth and current generation in PMDC at 25 g/L proved that more electrons transferred to the cathode chamber positively affected algae growth. Since algae demand electrons for reproduction through their metabolism, access to more electrons can enhance their growth (Zamanpour et al., 2017), as shown in our results. Evidently, the algal chamber remains relatively unaffected by fluctuations in salt concentration in the desalination chamber. As a result, the increased algal growth is linked to the increased availability of CO_2 in the cathodic chamber, which is facilitated by more efficient substrate degradation in the MDC. (Neetu. et al., 2019).

Moreover, from the cathode, a daily algal suspension was collected along with the effluent. This algal suspension holds significant potential due to *Scenedesmus obliquus*'s versatile commercial applications (Duan et al., 2020). For instance, it can be used for biofuel production (Kokabian et al., 2018a; Oliveira et al., 2020). Therefore, the collected algal suspension presents an opportunity for further exploration and utilization in various industries, making the system a green and renewable source of bio-energy in water treatment technologies.

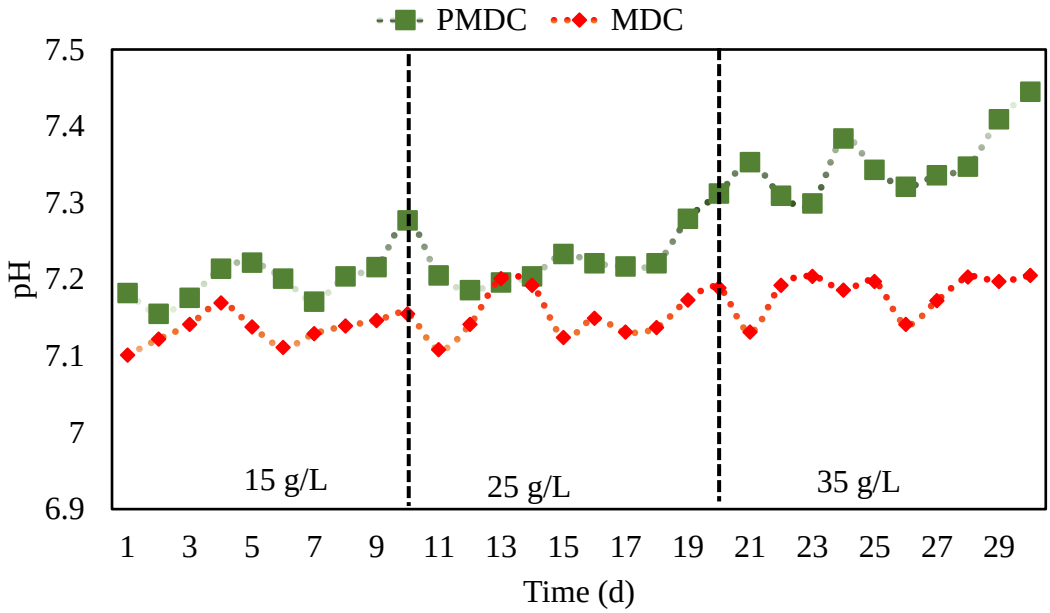


Figure 4.19 pH variation of MDC and PMDC cathode at various salt concentrations.

The cathode chamber was supplied with algae medium in a fed-batch mode retaining the

existing algae from the batch phase. This resulted in persistent dissolved oxygen and algal growth until the agal concentration dropped below 1000 mg/L. The fresh algal culture was then introduced into the system to maintain the initial algal of 1000 mg/L. The introduction of fresh algae reinitiated algae reproduction, resulting in increased algae growth and DO concentrations. The photosynthetic MDC has shown higher DO concentration than the control MDC, as shown in Fig 4.20. MDC has shown almost consistent DO with slight variation in all three experiments, which might be because the same amount of oxygen was provided daily, and there was no passive source of oxygen, such as algae in the cathode of PMDC. The maximum DO was attained at a salt concentration of 25 and in both PMDC and MDC, with DO concentrations of 9.1 mg/L and 7.3 mg/L, respectively. Previous studies have also reported high DO using algal biocathode in MDC compared to abiotic MDC. For instance, Neethu et al. (2019) reported a DO of 7.6 mg/L using PMDC and around 5 mg/L in MDC. The lower DO concentration in MDC using PBS could be the lower solubility of oxygen in PBS, which is attributed to the higher saline concentration in PBS, which reduces the ability of oxygen to dissolve in the solution (Danaee et al., 2023). Another factor contributing to this lower O_2 concentration could be the complex oxygen transport process. Before oxygen can reach the cathode surface, it must pass through several resistance obstacles. After diffusing from the primary gas to the gas-liquid junction through a relatively still liquid area near the bubble, it flows through the bulk liquid toward the static zone around the cathode. Eventually, it reaches the cathode surface, where the reduction reaction occurs. Because of the mechanically aerated catholyte, MDC exhibits a combination of all these resistances. In contrast, in PMDC, where microalgae are cultivated in suspension, and photosynthesis produces pure oxygen, the first resistance (Bulk Gas to Gas-Liquid Interface) is removed. Additionally, algal biofilm may build on the cathode surface. Here, the diffusing oxygen reaches the cathode surface by passing a single stagnant area devoid of bulk liquid (Ullah et al., 2023). In this study, as aeration in MDC control was provided without using any catalyst at the cathode electrodes (graphite rods), it might have led to hydrogen peroxide (H_2O_2) formation as an intermediate. This may have limited the Oxygen Reduction Reaction (ORR) at the cathode, as hydrogen peroxide can be produced through a two-electron pathway instead of the more efficient four-electron

pathway. Although hydrogen peroxide can be further reduced to water, its formation indicates a less efficient oxygen production process (Rizo et al., 2021). In contrast, algal biocathodes serve as an excellent source of oxygen, outperforming conventional aeration due to their unique capability of in situ oxygen production through algal photosynthesis, bypassing the need for intermediate production steps and ensuring a more reliable and efficient oxygen supply.

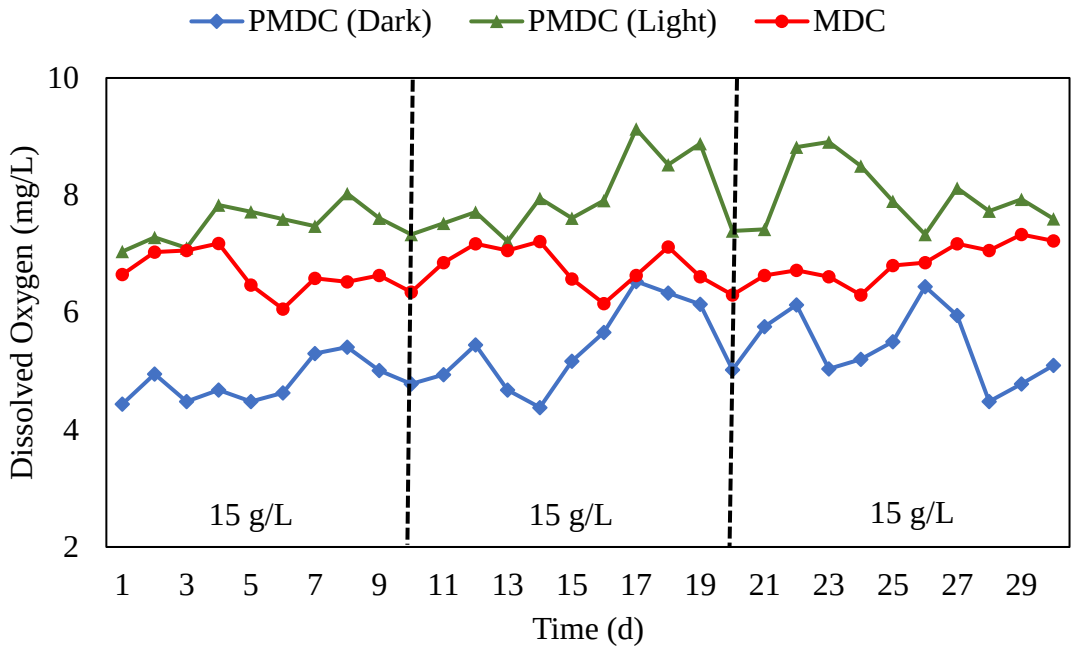


Figure 4.20 Dissolved oxygen levels of MDC and PMDC at various salt concentrations(D and L represent dark and light photoperiod).

4.4 Biofilm Formation and Membrane Fouling

The SEM images of the AEM, CEM, and anode displayed a smooth and highly defined surface prior to the experiment. Upon further examination of the anode surface post-experiment, it revealed dense clusters of rod-shaped bacteria, indicating the presence of biofilm formation , as shown in Fig 4.21 and 4.22.

Cracked surfaces were detected in unused ion exchange membranes in both MDCs, whereas sediment layers were observed in used membranes. The surfaces of anion exchange membranes (AEMs) exhibited biofouling, primarily caused by rod-shaped bacteria. Additionally, inorganic crystal scale, resulting from the deposition of inorganic

compounds within the artificial waste, was identified on the AEMs. The development of biofilm on the AEM near the anode, where microbial growth is active, is anticipated and unavoidable(Pandit et al., 2018). The presence of a thick biofilm on the membrane surface adjacent to the anode side can impede effective ionic transfer across the membrane, leading to issues such as membrane fouling, increased operational expenses, and membrane deterioration.

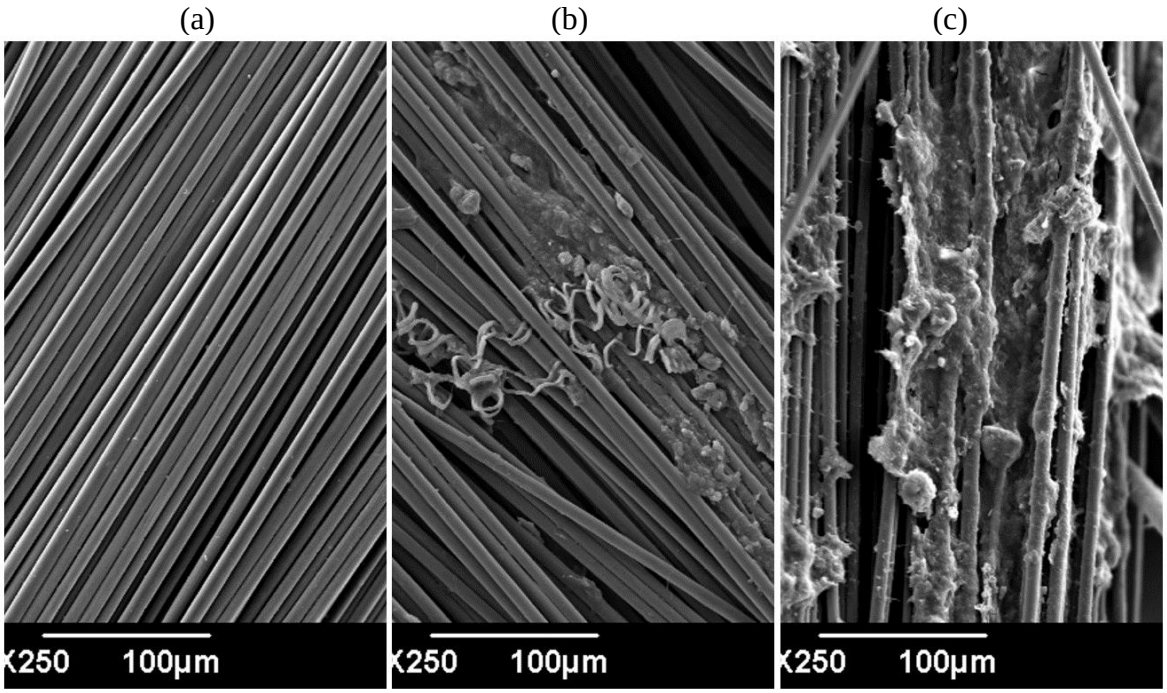
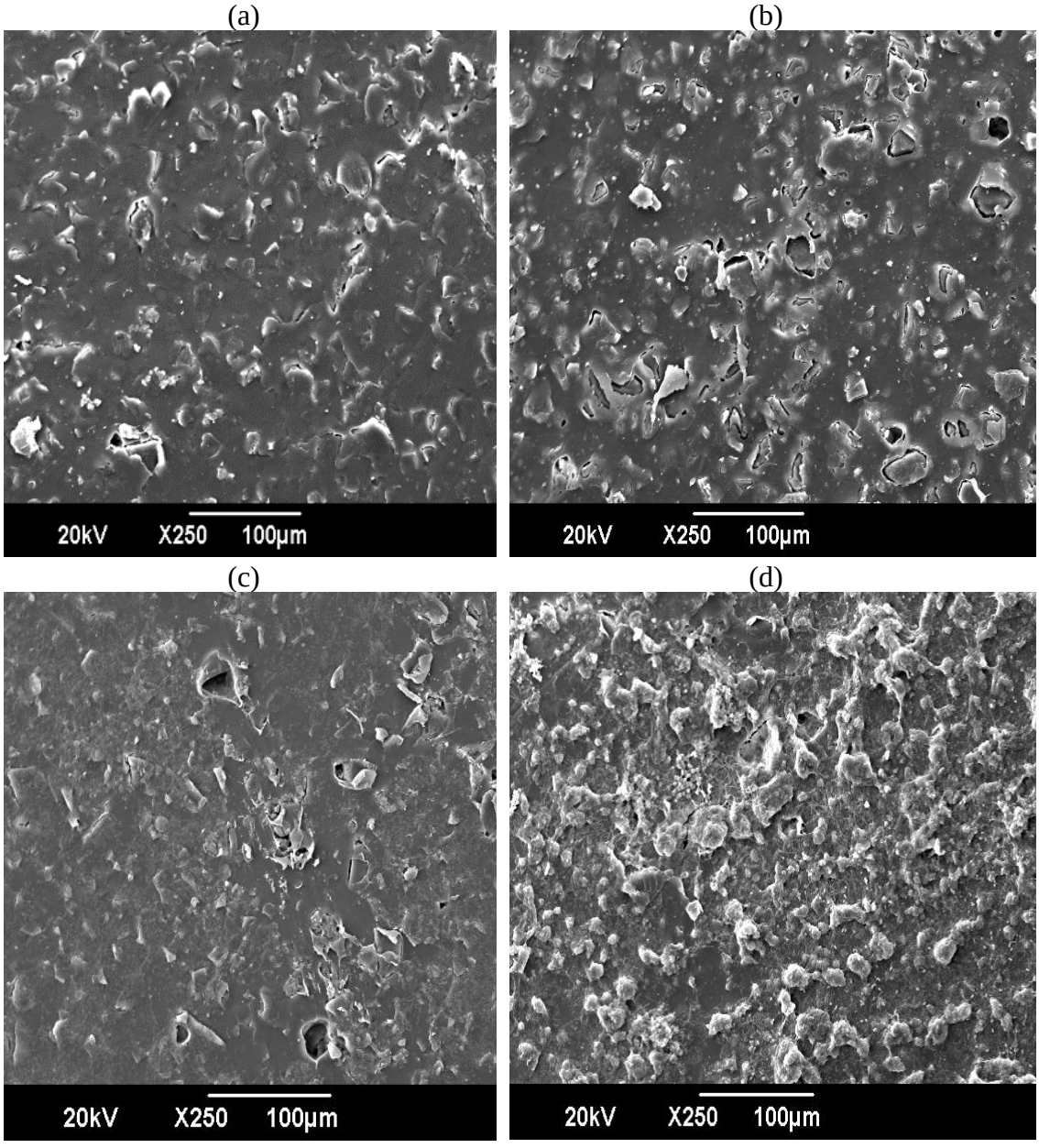


Figure 4.21 SEM images of anode electrode before the experiment (a) and after the experiment of MDC (b) and PMDC (c).

In contrast, the cathode chamber in the MDC did not exhibit biofilm formation on the CEM-facing side, but some visible scaling was observed, resulting in the formation of a flaky inorganic fouling layer compared to the original SEM images. This scaling may be attributed to cathodic reduction reactions. In the PMDC, where microalgae were cultivated within the cathode chamber, microalgae cells accumulated on the surface of the cationic membrane. The deposition of microalgae can lead to deactivation of certain areas on the membrane and electrode surface, thereby introducing additional resistance to the internal resistance of the system. This played a significant role in halting the desalination process

at the end of the experimental phase (Barahoei & Hatamipour, 2023). Moreover, examination of the AEM and CEM surfaces exposed to the desalination sides revealed the presence of blade-shaped salt crystals. Moreover, the scaling layer on these membranes facing the desalination chamber could contribute to the transfer of organic ions, leading to impurities in the water intended for portable use (Ghasemi et al., 2022; Hui et al., 2020; Kokabian & Gude, 2015).



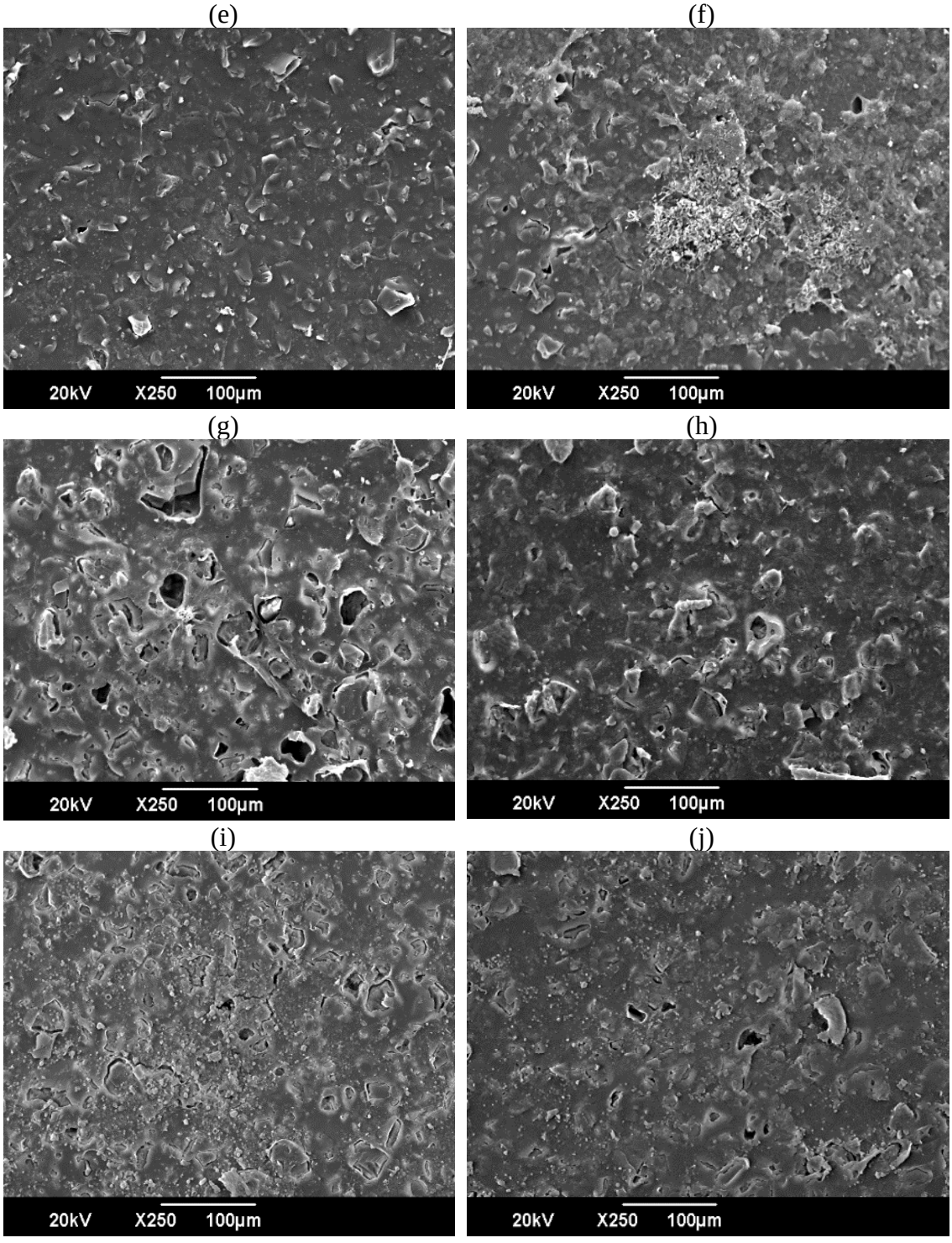


Figure 4.22 Unsed AEM (a), unused CEM (b), used AEM desalination side-MDC (c), used AEM Anode side-MDC (d), used AEM desalination side-PMDC (e), used AEM Anode side-PMDC (f), used CEM desalination side-MDC (g), used CEM cathode side-MDC (h), used CEM desalination side-PMDC (i), used CEM cathode side-PMDC (j).

4.5 Practical Applications of the Current Study

This study offers numerous practical applications of MDC technology;

- This study's comparison between traditional and photosynthetic MDCs, especially in treating high-strength wastewater, offers practical insights crucial for optimizing wastewater treatment processes. By focusing on varied anode substrate concentrations, it addresses a key gap in existing research, enhancing the effectiveness of MDC technology in real-world applications. These findings provide valuable guidance for industries and wastewater treatment facilities aiming to improve their wastewater treatment efficiency.
- Incorporating algae into the cathode chamber of Microbial Desalination Cells (MDCs) offers numerous advantages, particularly in wastewater treatment and bioenergy production. Algae serve as a secondary treatment for domestic wastewater by utilizing and assimilating nutrients like nitrogen and phosphorus present in the wastewater. Additionally, algae growth within MDCs results in algal biomass production, which can be utilized for various practical applications, including biodiesel production. Algal biomass is rich in lipids, making it suitable for extraction and conversion into biodiesel, even if the electricity generated by the MDC is not substantial.
- This study extends its scope beyond traditional saline water desalination and examines the applicability of Microbial Desalination Cells (MDCs) in treating sea and brackish water. The study offers valuable guidance for areas facing water scarcity without access to traditional saline water sources by providing practical insights into MDC technology in non-saline environments. This expanded application of MDCs underscores their versatility and potential in addressing diverse water treatment challenges, contributing to sustainable water management practices in regions with varying environmental conditions.
- This study's holistic approach to treating both domestic and industrial wastewater in Microbial Desalination Cells (MDCs) offers practical benefits for wastewater treatment facilities, especially in industrial parks. By enabling simultaneous treatment of diverse wastewater streams within a single unit, the study demonstrates

the versatility and effectiveness of MDC technology in addressing complex wastewater treatment scenarios. These findings provide valuable guidance for industries seeking efficient and integrated solutions for wastewater management, ultimately enhancing sustainability and operational efficiency

4.6 Future Research Directions

Microbial Desalination Cells (MDCs) have great potential for generating sustainable energy and treating water and wastewater. However, there are still many challenges to overcome before they can be used on a large scale. Factors like cost and operating conditions are important considerations for making MDCs practical and economically viable.

- Operating the desalination chamber in continuous or semi-continuous mode prevents adverse ion accumulation effects by ensuring consistent water flow and periodic flushing to remove accumulated ions, optimizing desalination efficiency.
- Experimenting with varied algae concentrations, light intensities, and wastewater sources at MDC cathodes optimizes nutrient uptake and biomass production for efficient wastewater treatment, which is crucial for sustainable water management.
- A deeper analysis of the various reactor components, such as electrode types and membranes, is essential for understanding their influence on power generation in AMFC systems
- The application of genetic engineering techniques holds promise for enhancing power generation in AMFC systems. By leveraging genetic modifications, researchers can optimize the performance of AMFC components, such as catalysts or microorganisms, to increase energy conversion efficiency and overall power output

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

This chapter summarizes the the findings of the research and presents actionalable recommendations based on the outcomes.

5.1 Conclusions

Simultaneous electric current, wastewater, and desalination were explored at different Substrate concentrations in PMDC against a controlled experiment MDC. The substrate concentration that gives maximum efficiency in energy production, water, and wastewater treatment was chosen to evaluate the appropriate concentration of saline feed for the desalination chamber. The following conclusions can be drawn from the current study.

- The findings revealed that MDCs were most effective when supplied with medium substrate concentration (4000 mg/L), exhibiting optimal COD removal, desalination, and power production, benefiting from favorable conditions for microbial growth alongside optimum substrate concentration and lowermost internal resistance. Conversely, low and high substrate concentrations (2000 and 6000 mg/L) resulted in lower MDC performance due to higher internal resistance and substrate inhibition. Notably, the results also proved that PMDC outperformed MDC in all power generation and wastewater treatment experiments. For instance, PMDC achieved an average maximum working voltage of 354 mV, 90.5 % anode COD removal, and 27% desalination, surpassing MDC by 38.09%, 7.52%, and 17.39% at corresponding voltage, anode COD removal and desalination.
- The concentration of saline feed in the desalination chamber also significantly affected the performance of MDCs. High salt concentrations, i.e., 25 and 35 g/L, favored more salt removal, higher power production, and better wastewater treatment, possibly due to lower internal resistance. However, 25 g/L proved the optimum concentration, while lower salt concentration resulted in lower MDC performance. The PMDC outperformed the control MDC, achieving 39.2% higher voltage, 17.2% desalination, and 3.6% COD removal at 25 g/L NaCl.
- SEM analysis revealed that operating MDCs for longer durations can lower their

performance due to membrane clogging and fouling.

- The best PMDC performance makes it a sustainable water treatment alternative in wastewater treatment facilities, owing to its added benefit of nutrient removal and biomass production at the cathode chamber besides water and wastewater treatment and energy generation. This involves treating a diverse range of wastewater at anode, cathode, and desalination chambers simultaneously, ultimately improving the efficiency and cost-effectiveness of the traditional or post-treatment methods.

5.2 Recommendations

The following recommendations are noteworthy for further study.

- The integrated PMDC and RO or FO system presents a promising approach to desalinating saline water and treating wastewater by reducing the toxic byproduct, e.g., brine disposal. Hence, further research is needed to understand its process mechanism fully and optimize its performance. Specifically, research should focus on how the integrated system can effectively control pH and EC variations at the anode and cathode. This will pave the way for developing more effective and sustainable water and wastewater treatment systems in the future.
- A dedicated study on ion recovery and migration is crucial for optimizing desalination efficiency. By understanding the dynamics of ion movement within the desalination process, researchers can develop targeted strategies to enhance recovery rates and minimize energy consumption, ultimately improving the overall effectiveness of desalination technologies. Furthermore, it is crucial to investigate the relationship between methanogens and exo-electrogens to gain a deeper understanding of the potential applications of this technology.
- To better understand the economic viability of algae-assisted MDC, it is necessary to optimize the process by providing outdoor conditions to the PMDC system. Such investigation would yield valuable insights into this technology's feasibility and potential benefits and enable us to explore and identify its commercial applications.

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APPENDIX

RESULTS OF BATCH MODE

- **Anode COD removal efficiency**

During a four-day batch operation, PMDC achieved an average COD removal of 73.8%, surpassing MDC's 62.3%. In the two-day batch operation, PMDC also outperformed MDC with a COD removal of 65.1% compared to MDC's 61.4%. Specifically, PMDC achieved 18.5% higher COD removal than MDC in the four-day batch and approximately 6% improvement in the two-day batcho peration.

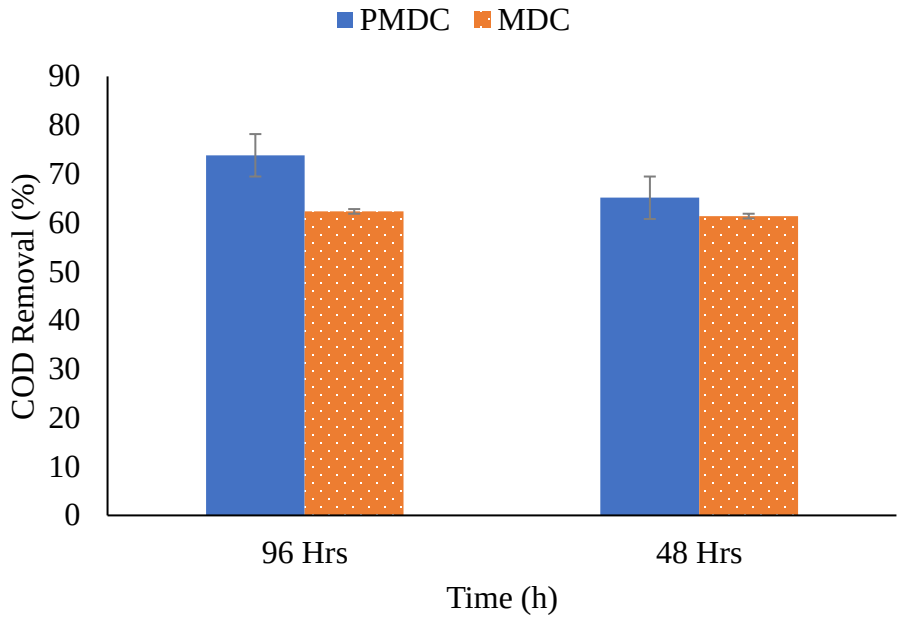

Figure 1-A Anode COD removal efficiency in 4 and 2 days batch HRT.

- **EC variations at anode**

From an initial EC of 4.17 mS/cm, PMDC showed an increase in EC of 6.73 mS/cm, while MDC had an EC of 6.29 mS/cm in the four-day batch operation. This reflects 7% higher increase in EC for PMDC compared to MDC in this duration. Similarly, in the two-day batch operation, PMDC exhibited an EC of 6.39 mS/cm from an initial EC of 4.17 mS/cm, whereas MDC had an EC of 6.11 mS/cm, indicating 4.6% higher increase in EC for PMDC compared to MDC.

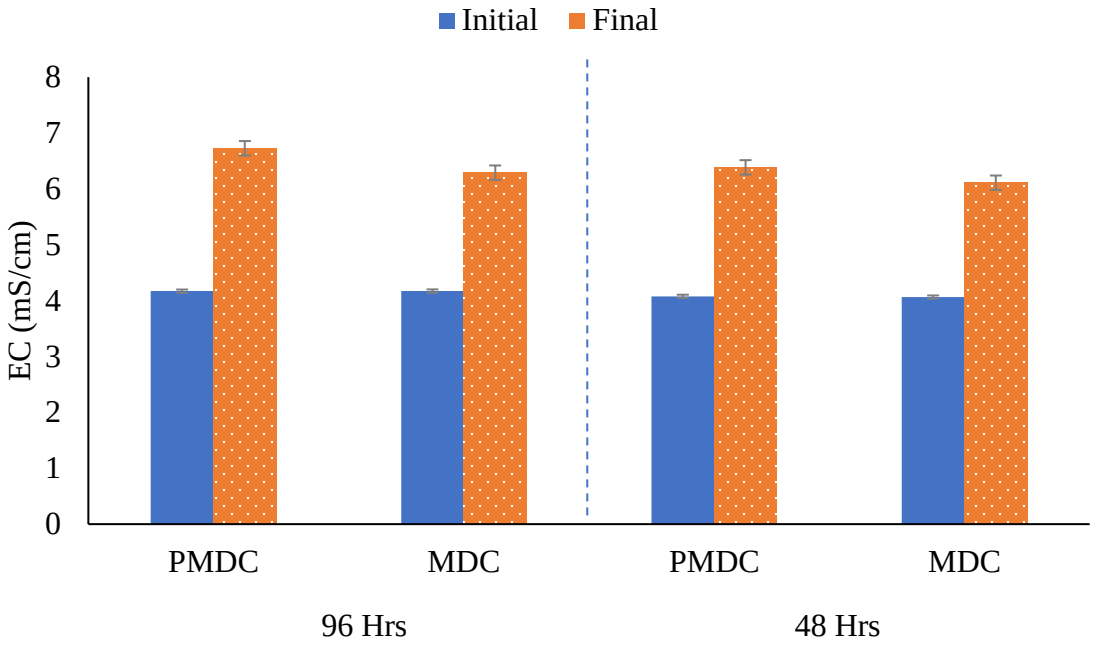

Figure 2-A EC variations at anode in 4 and 2 days batch HRT.

- **pH reductions at anode**

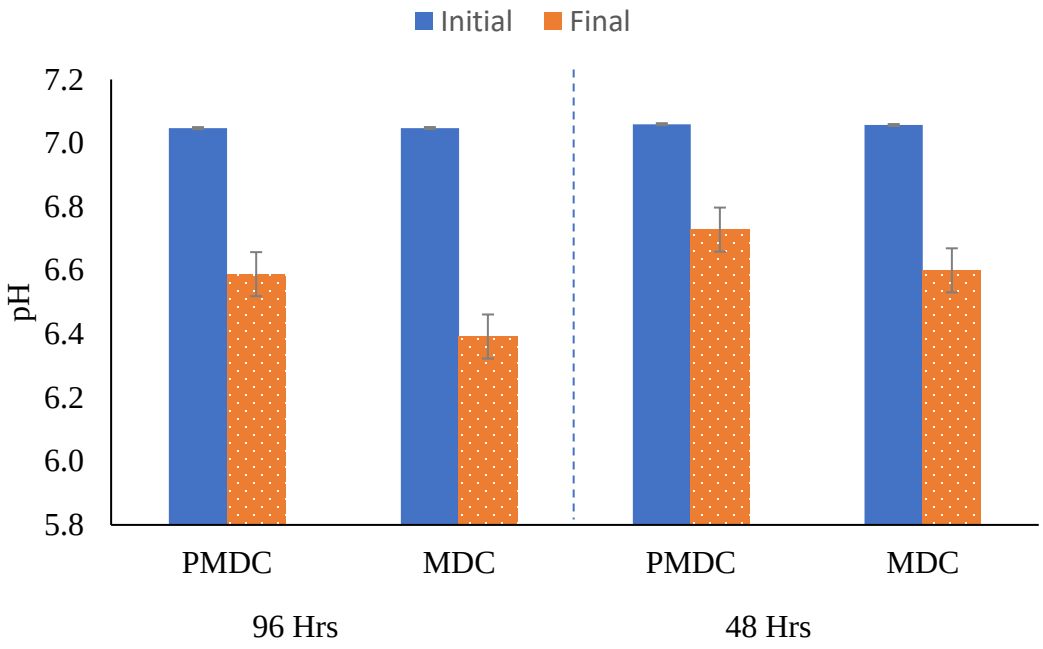

Figure 3-A pH reduction at anode in 4 and 2 days batch HRT.

In the four-day batch cycle, PMDC exhibited a pH decrease from 7.0 to 6.59, while MDC showed a decrease from 7.0 to 6.39. This indicates that PMDC had approximately 3.13% less pH decrease compared to MDC in this duration. In the two-day batch cycle, PMDC's pH decreased from 7.0 to 6.73, and MDC's pH decreased from 7.0 to 6.60. Here, PMDC had approximately 1.97% less pH decrease compared to MDC.

- **pH variations at cathode**

In the four-day batch cycle, PMDC showed a pH increase from 7.04 to 7.53, while MDC exhibited an increase from 7.04 to 7.31. This indicates that PMDC had 3.00% higher pH increase compared to MDC. In the two-day batch cycle, PMDC's pH increased from 7.05 to 7.30, whereas MDC's pH increased from 7.05 to 7.21. Here, PMDC had 1.25% higher pH increase compared to MDC.

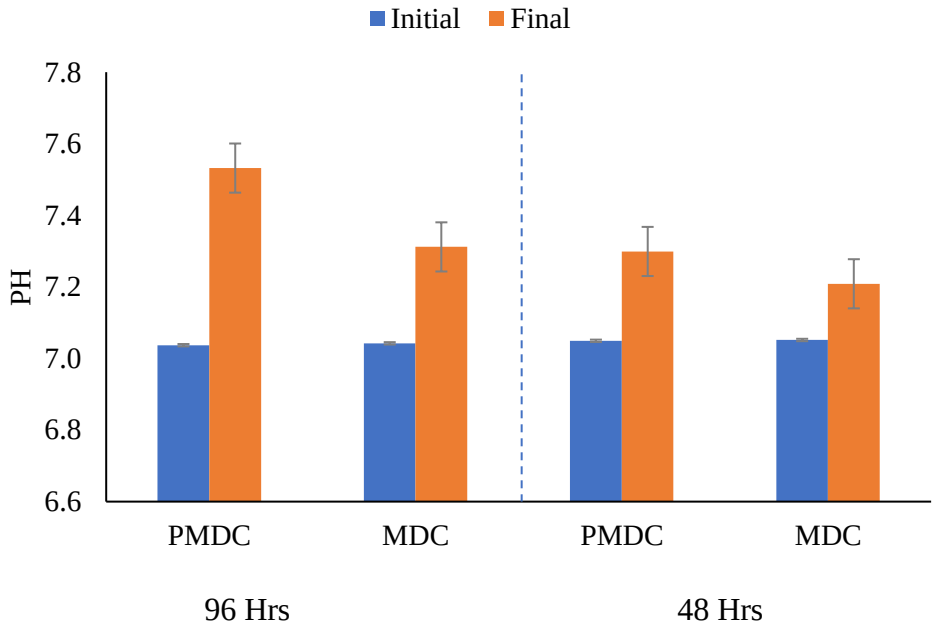

Figure 4-A pH variations at cathode in 4 and 2 days batch HRT.

- **EC variations at cathode**

In the four-day batch cycle, PMDC exhibited an increase in an average EC of 13.71 mS/cm from 12.9 mS/cm, while MDC had an average EC rise of 13.44 mS/cm. This indicated that PMDC had 2.00% higher increase in EC compared to MDC. In the two-day batch cycle, PMDC's average EC rise was 13.42 mS/cm from 11.7 mS/cm, whereas MDC's average EC

was 12.54 mS/cm. Here, PMDC had 7.01% higher increase in EC compared to MDC.

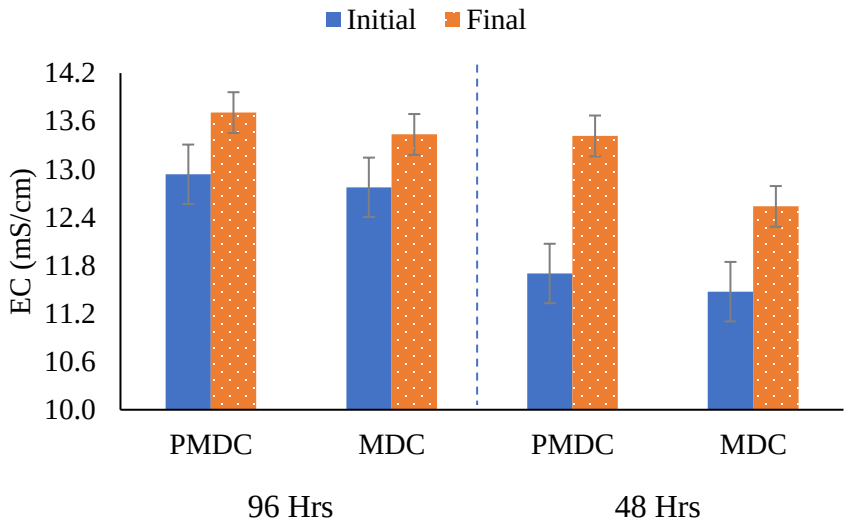

Figure 5-A EC variations at cathode in 4 and 2 days batch HRT.

- **DO levels of cathode**

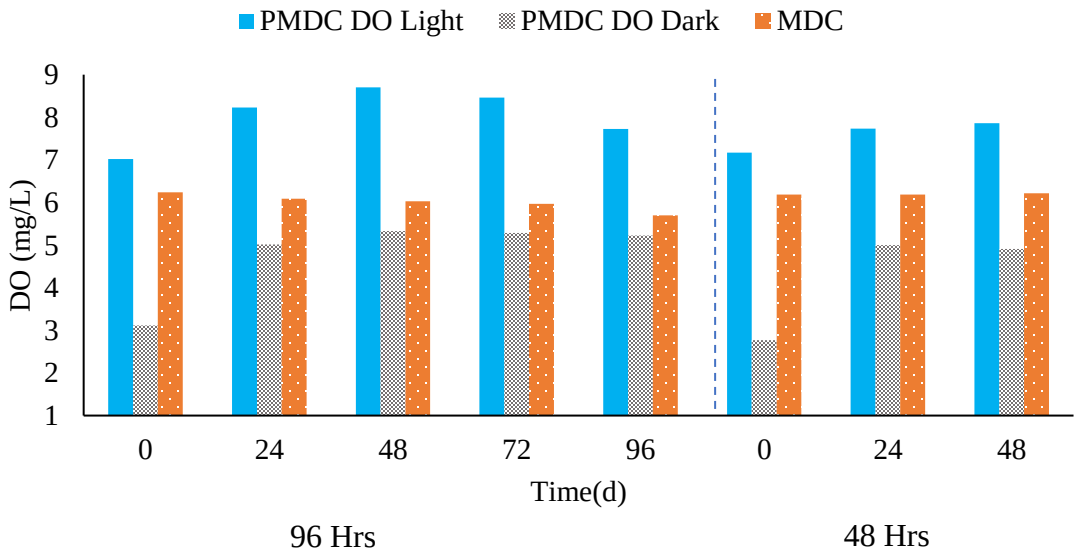

Figure 6-A DO levels in MDC and PMDC in 4 and 2 days batch HRT.

The dissolved oxygen (DO) levels in the four-day batch cycle were 8.03 mg/L for PMDC under light conditions, 4.79 mg/L for PMDC under dark conditions, and 6.01 mg/L for

MDC. In the two-day batch cycle, the DO levels were 7.59 mg/L for PMDC under light conditions, 4.23 mg/L for PMDC under dark conditions, and 6.20 mg/L for MDC.

- **Nutrient removal and Algae growth at PMDC cathode**

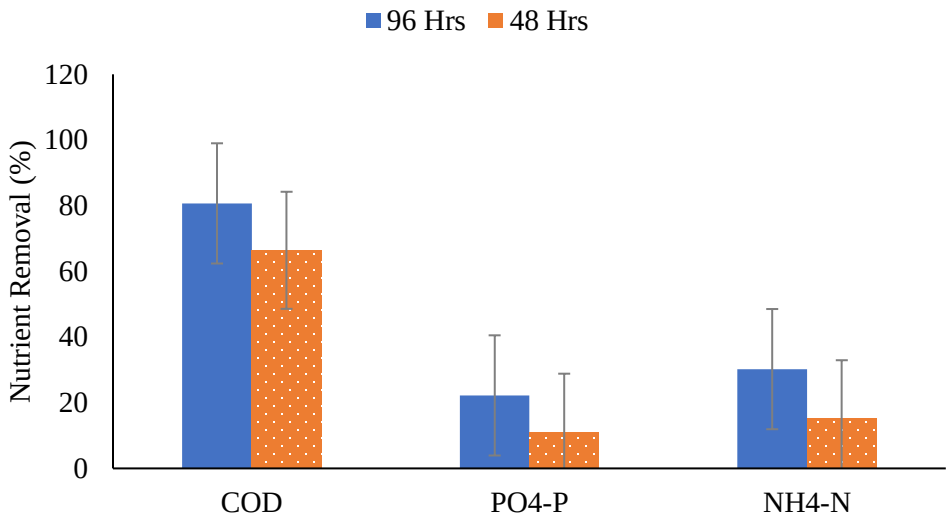

Figure 7-A Nutrient removal at PMDC cathode in 4 and 2 days HRT.

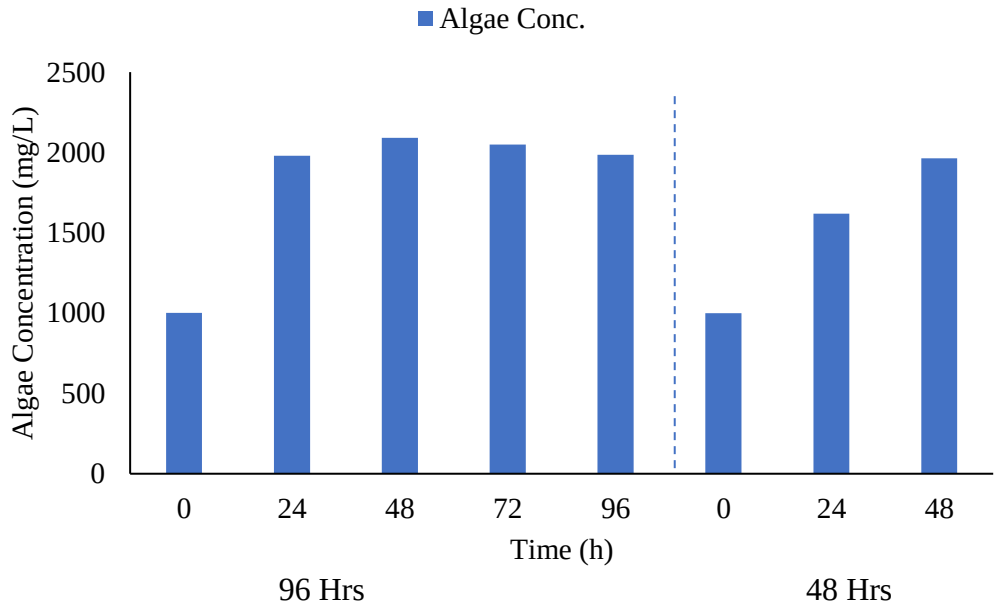

Figure 8-A Algal growth at PMDC cathode in 4 and 2 days batch HRT.

In the four-day batch cycle, there was an average COD removal of 80.7%, PO_4^{3-}P removal of 22.2%, and NH_4^+-N removal of 30.2%. For the two-day batch cycle, the average nutrient

removal was 66.4% for COD, 11.0% for PO_4^{3-}P, and 15.1% for NH_4^+-N. Moreover, in the four-day batch cycle, the algal growth was 1821 mg/L, while in the two-day batch cycle, it was 1528 mg/l. The growth rate of algae in the four-day batch cycle was 205.25 mg/L/d, and in the two-day batch cycle, it was 264 mg/L/d. This indicated that PMDC had 19.2% higher algal growth in 2 days compared to the four-days batch cycle.

- **Desalination efficiency**

In the four-day batch cycle, PMDC achieved an average desalination efficiency of 21.58%, while MDC had a desalination efficiency of 16.19%, resulting in MDC being 6.88% less efficient than PMDC in desalination. Similarly, in the two-day batch cycle, PMDC's desalination efficiency was 15%, whereas MDC achieved 10.10%, making MDC 6.92% less efficient in desalination compared to PMDC.

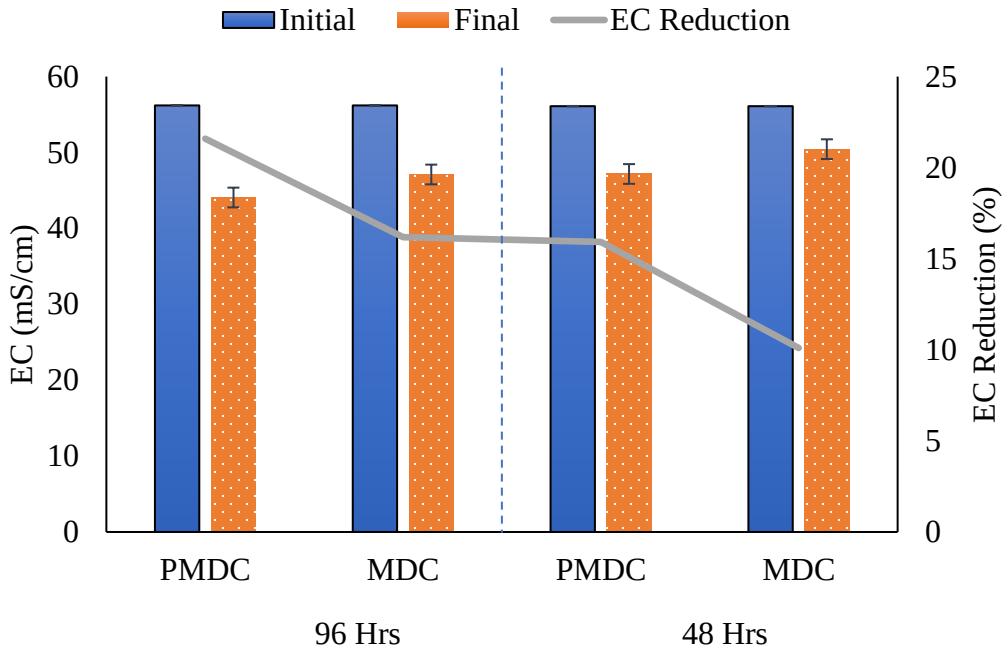

Figure 9-A Desalination effciency of MDC and PMDC at 4 and 2 days batch HRT.

- **Voltage generation and Polarization**

In the four-day batch cycle, PMDC exhibited an average voltage of 336.9 mV, while MDC showed 292.7 mV, indicating that PMDC had 15.1% higher average voltage than MDC. In the two-day batch cycle, PMDC had an average voltage of 237.9 mV, significantly higher

than MDC's 192.3 mV, resulting in 23.71% higher average voltage in PMDC compared to MDC.

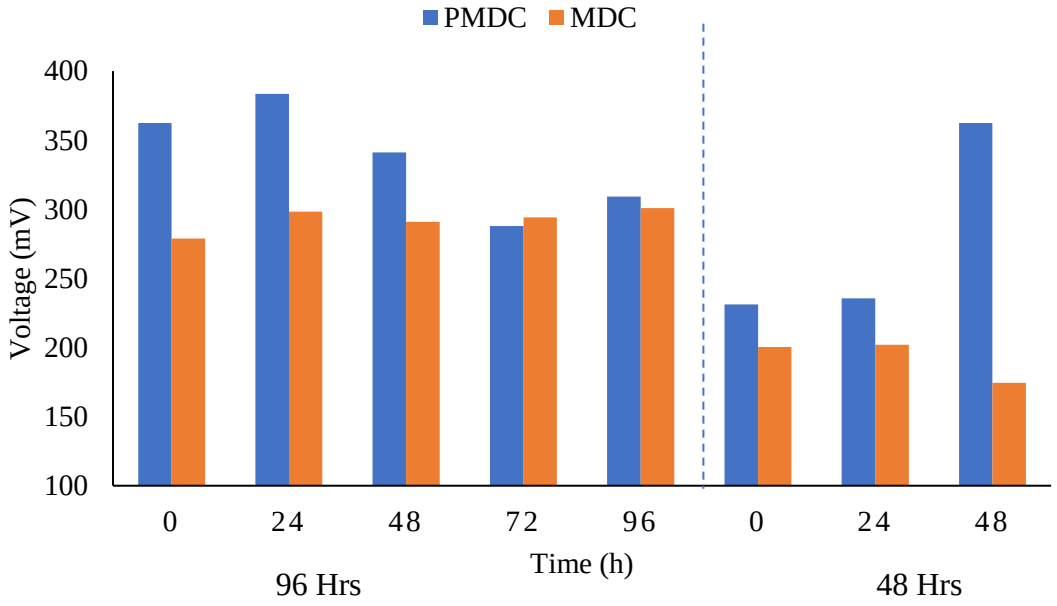

Figure 10-A Voltage generation in PMDC and MDC in 4 and 2 days batch HRT.

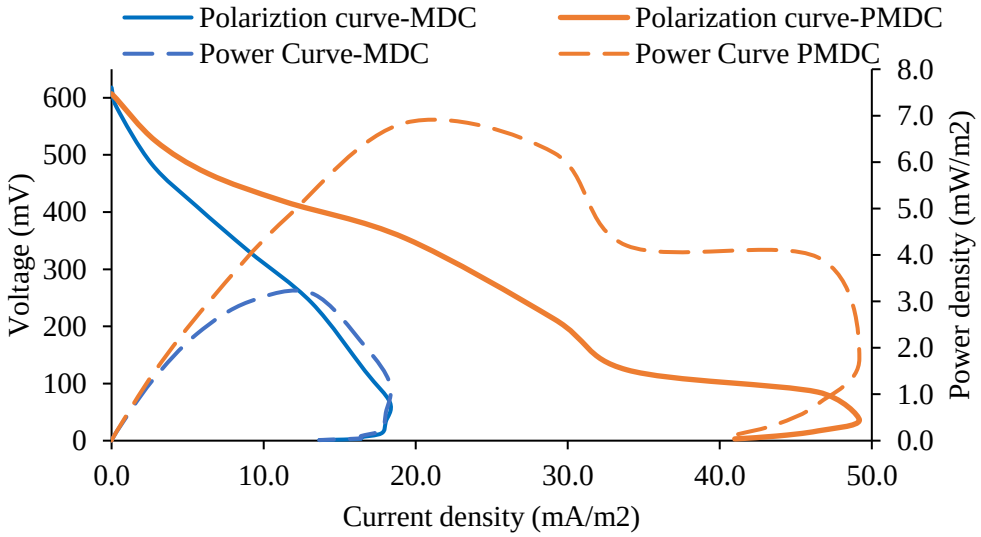

Figure 11-A Polarization behavior of MDC and PMDC in batch mode.

In the four-day batch cycle, PMDC exhibited a maximum P.D of 6.8 mW/m², while MDC showed 3.2 mW/m², indicating that PMDC had approximately 52.9% higher maximum

P.D than MDC. Additionally, in terms of Internal Resistance, PMDC had an internal resistance of 657.6 Ω, approximately 62.74% lower than MDC's 1768.3 Ω, indicating that PMDC had significantly lower internal resistance compared to MDC in the four-day batch cycle.

- **Coulombic efficiencies (CE) for batch mode**

For a 4-day HRT, the CE for PMDC was 0.41%, whereas for MDC, it was 0.38%. In the case of a 2-day HRT, PMDC had a CE of 0.64%, while MDC had a CE of 0.57%.

COULOMBIC EFFICIENCIES FOR FED BATCH MODE

- **For Objective 1 (using different substrate concentration)**

The Coulombic Efficiency (CE) for PMDC over 15 days using a substrate concentration of 2000 mg/L was approximately 1.85%, while for MDC, it was about 1.69%. When the substrate concentration was increased to 4000 mg/L, PMDC showed a CE of 0.91%, whereas MDC exhibited a CE of 0.74% over the 15-day fed batch operation period. Finally, with a substrate concentration of 6000 mg/L, PMDC's CE was 0.83%, while MDC's CE was at 0.57% in 15-day fed batch cycle.

- **For Objective 2 (using different salt concentration)**

At a salt concentration of 15 g/L, MDC exhibited a CE of 0.57%, while PMDC showed an approximate CE of 0.83%. Increasing the concentration to 25 g/L resulted in higher CEs, with PMDC reaching around 1.10% and MDC achieving about 0.76%. Further increasing the concentration to 35 g/L resulted in PMDC with a CE of 0. 98% and MDC with a CE of 0.74%.

The lower Coulombic efficiency could indicate unconverted substrate, possibly due to competition between methanogens and exoelectrogens. Methanogens might have suppressed exoelectrogens, resulting in lower CE. This competition may lead to electron loss, potentially affecting electricity production.