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Evaluating Power Performance Of Microbial Fuel Cells Undertaken Limited Variations of Ocean Salinity Levels

Chin-Tsan Wang[*,**], K Vasumathi[*,***], Bhanupriya Das[****], Jovanka Sheryn Tritanti[*], Jessica Renata Wijaya Tumboimbela[*] and Chi Wu[*****]

Keywords： microbial fuel cell, salinity, deep-sea sediment, microbial community, powering devices, electrochemical performance.

ABSTRACT

Ocean salinity varies from 33 to 37 g/L due to local geographic and climatic variations affecting ionic concentration, conductivity, microbial activity, and electron transfer rates. The impact of these natural salinity fluctuations on the performance of MFC remains underexplored. Hence, the present study investigates the influence of three salinity levels—lower (33 g/L, R33), optimum (35 g/L, R35), and higher (38 g/L, R38)—on power generation, bacterial viability, and biofilm formation in MFCs. Among the tested conditions, R35 exhibited the highest power and current density of 15.02 mW/m² and 103.59 mA/m², confirming that moderate salinity enhances microbial metabolism and electrochemical efficiency. At 160 mA/m², R33 displayed a second power peak with 16.5 mW/m², which indicates delayed concentration polarization and enhanced electron transport at higher current densities. Additionally, resulted in enhanced biofilm formation and the highest bacterial viability of 6.67 × 10^7 CFU/mL for R33. This indicates the gradual adaptation of Gram-negative bacteria and enhanced electron transfer rates. The power density varied from R33 and R35 by 17.3% and from R35 and R38 by 7.79%, which highlights the sensitivity of MFC performance to these narrow salinity changes. These results underscore the importance of salinity management during MFC operation in marine environments. Future work on microbial community analysis and adaptive salinity control strategies will help attain long-term stability and energy output in deep-sea environments.

INTRODUCTION

Powering underwater research stations and vehicles presents significant challenges due to the remote and extreme circumstances (Martínez de Alegría et al., 2024). Microbial fuel cells (MFCs) present a sustainable solution by employing electrogenic bacteria to oxidize organic matter, subsequently transferring electrons through biofilm formation and generating power (Kwofie et al., 2024). Deep-sea sediments are rich in bacteria and organic materials, resulting in electron transfer and thus facilitating power generation in marine environments (Song et al., 2024). Microbial viability and biofilm formation are greatly influenced by parameters such as salinity, pressure, temperature, and oxygen availability, which additionally affect MFC performance (Massaglia et al., 2018). Despite their potential, the performance of MFCs in deep-sea conditions remains underexplored, particularly for the narrow salinity fluctuations from 33 - 37 g/L. Understanding the effects of salinity variations promotes the widespread use of MFC technology in real-time marine environments.

Salinity significantly impacts MFC performance by altering electrolyte conductivity, microbial metabolism, and electron transfer efficiency. Guo et al. (2021) demonstrated that optimal salinity enhances power generation by increasing ionic conductivity and reducing internal resistance. Lefebvre et al. (2012) concluded that up to 20 g/L NaCl enhanced MFC performance by reducing internal resistance, but

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[*] *Department of Mechanical and Electro-Mechanical Engineering, National I Lan University, I-Lan, Taiwan.*
[**] *Department of Chemical Engineering, Indian Institute of Technology Guwahati, Assam, India*
[***] *Department of Biotechnology, Kalasalingam Academy of Research and Education, Virudhunagar, India*
[****] *Institute of Environmental Engineering and Management, National Taipei University of Technology, Taipei, Taiwan*
[*****] *Taiwan Ocean Research Institute, National Applied Research Laboratories, Kaohsiung, Taiwan*

higher concentrations lessened microbial activity. Similar observations are also reported by Monzon et al. (2015). Contrarily, Paul et al., 2014 and Li et al., 2024 reported that excessive salinity disrupts microbial electron transport and biofilm stability. For example, Miyahara et al. (2015) reported that power density increased with 5.8 g/L of NaCl concentration, whereas 17.5–105 g/L negatively impacted performance. The microbial community also varies. *Geobacteraceae* were dominant at low salinity, whereas *Desulfuromonas* and *Pseudomonas* thrived in higher-salinity conditions (Miyahara et al., 2016). These variations indicate that MFC efficiency depends on microbial adaptation and environmental conditions, reinforcing the need for targeted research on salinity effects in real-world marine settings.

MATERIALS AND METHODS

MFC setup

Three H-type dual-chamber MFCs (Figure 1) were constructed to evaluate the impact of salinity (33, 35, and 38 g/L) on microbial electricity generation. Each chamber had a working volume of 200 mL, with the anode and cathode separated by a Nafion® 117 proton exchange membrane (PEM) to facilitate ion exchange while preventing short circuits.

Deep-sea sediment samples collected from the South China Sea (22° 29’ 09.2"N, 120° 07’ 20.5"E, depth: 402 m) were used as the inoculum in the present study. The anode chamber was filled with deep-sea sediment, as depicted in Fig.1; the electrode was buried in the sediment to create an anaerobic environment. A flannel cloth was used to separate the solid and liquid. Artificial seawater (ASW) was used as the anolyte, with salinity adjusted to 33, 35, and 38 g/L (as reported in Table 1) (Nguyen, 2018). Additionally, 3 g/L of acetate was added as a carbon source. Potassium ferricyanide with PBS buffer was used as catholyte. Carbon felt of 3.5 cm × 3.5 cm dimensions were used as electrodes. The MFCs were connected to a 1 kΩ external resistance. The MFCs were operated in fed-batch mode for 23 days, replenishing the medium every 2 days. pH and salinity were regularly monitored to assess how salinity variations influenced MFC performance and microbial communities.

Electrochemical measurement and analysis

This study used electrochemical measurements and analysis to evaluate MFC performance at three different salinity concentrations (33 g/L, 35 g/L, and 38 g/L). DAS-5000 Data Acquisition System (Jiehan Technology, Taiwan) was used to record the voltage readings in a time interval of 5 minutes. Linear sweep voltammetry and electrochemical impedance spectroscopy analysis were performed to analyze the electrochemical behavior and internal resistance of the MFC. Before the analysis, the medium was refreshed with artificial seawater (ASW) and potassium ferricyanide solution and left in open-circuit conditions for 12 hours for stabilization.

Polarization curves were obtained from LSV measurements recorded using a JIEHAN 5640 electrochemical analyzer with a sweep rate of 0.005 V/sec. The current and power density were calculated using the ohms law and based on the cathode's surface area. EIS analysis, performed using a HIOKY 35522-50 LCR HiTESTER (Japan) at a frequency range of 100,000 to 0.1 Hz, examined internal resistances. Nyquist plots were generated to visually represent impedance variations and assess the impact of salinity on MFC performance.

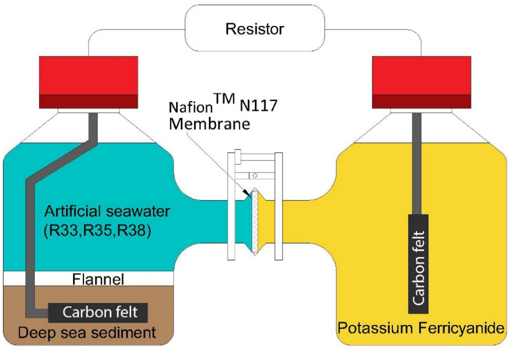

Figure 1. Schematic Diagram of the microbial fuel cell utilized in this study.

Table 1. Media composition of Artificial Seawater (ASW) with salinity Concentrations of R33, R35, and R38 (Nguyen, 2018)

Ingredients	**Amount (g/l)**		
	R33	**R35**	**R38**
NaCl	26.70	28.32	30.74
$MgCl_2 \cdot 6\ H_2O$	5.16	5.48	5.95
$MgSO_4$	3.39	3.60	3.91
$CaCl_2 \cdot 2\ H_2O$	1.05	1.11	1.20
KCl	0.73	0.77	0.84
$NaHCO_3$	0.19	0.20	0.22
Acetate	3	3	3

Viable Cell Enumeration

The agar plating method (Miles et al., 1938) was used to evaluate cell viability and to determine microbial abundance at different salinity levels (33, 35, and 38 g/L). The anode surface was swabbed, placed in a 0.009 g/L NaCl solution, and vortexed to remove microorganisms. The samples were serially diluted, and 10 μL of each dilution was plated on the agar plate. After incubation, the colonies in the range of 3–30 CFU were counted. The microbial variation at different salinity conditions was observed by calculating and analyzing using GraphPad Prism 9.

Gram staining studies

Gram staining was performed to differentiate bacterial types and observe morphology using a rapid Gram stain kit (BaSO Biotech, Taiwan). A bacterial colony was mixed with deionized water (DI) on a clean slide, heat-fixed, and sequentially stained with crystal violet, iodine, decolorizer, and Fuchsin dye. The slide was air-dried and examined under a DVM6 Digital Microscope (Leica, Germany) at 1,651–1,660X magnification to confirm bacterial presence and structure.

Biofilm analysis

After MFC operation, the anode was carefully removed, rinsed with 0.9% NaCl, and examined under scanning electron microscopy (SEM) for biofilm formation. The biofilm was fixed in 2.5% glutaraldehyde for 4 hours to maintain structural integrity and then dehydrated with a gradient ethanol solution from 25% to 100% (Jayashree et al., 2019). The samples were subsequently dried and subjected to SEM analysis.

RESULT AND DISCUSSION

Influence of salinity on electrochemical performance of MFC in marine conditions

Salinity is one of the crucial components influencing the performance of MFC by altering ionic conductivity, microbial viability, biofilm development, and charge transfer resistance. Three distinct salinity conditions—R33 (33 g/L), R35 (35 g/L), and R38 (38 g/L)—were used for this study to assess their effects on MFC performance that mimic the salinity fluctuations in real marine environments. Electrochemical performance has been evaluated by open circuit voltage (OCV), polarization behavior, and internal resistance, providing insights into the effects of salinity changes on MFC operation in real-time.

The OCV revealed a trend of increasing voltage readings, which corresponds to increased salinity concentrations, with measurements of 0.281 V (R33), 0.491 V (R35), and 0.502 V (R38). This enhancement is due to the enhanced ionic conductivity of the electrolyte. Najafgholi et al. (2015) reported that increased salinity enhances ion mobility, thus enhancing the voltage performance.

Polarization curve analysis is performed to identify activation, ohmic, and concentration losses, which indicate the overall MFC efficiency. The power density results revealed that R35 exhibited the highest current and power density of 103.59 mA/m² and 15.02 mW/m², followed by R38 (84.38 mA/m² and 13.85 mW/m²), and R33 (70.54 mA/m² and 12.80 mW/m²) (Figure 2). The higher power output of R35 is due to improving charge transfer and microbial electron transport mechanisms. The results align with the observations of Nikhil et al. (2018) and Lefebvre et al. (2018) reported that power density increases up to an optimum point, beyond which it declines due to ohmic losses and electrode overpotentials. Excessive salinity hinders power density by limiting microbial metabolic functions (Guo et al., 2021).

R33 also demonstrated a second peak power of 16.5 mW/m² at 160 mA/m², indicating that the delayed concentration polarization effect and subsequent biofilm maturation enhance charge transfer and sustain power production. Koseoglu et al. (2018) reported similar behavior, stating that delayed concentration polarization effects could extend power production in bioelectrochemical systems. Thus, R33 provides a more stable long-term power supply compared to R35 and R38 despite its initially lower power density.

The charge transfer rates, ionic concentrations, and electron flow are influenced by internal resistance developed in the system. The characteristics of the electrolyte, cell design, temperature fluctuations, and electrode material are the important factors that influence the resistances (Chen et al., 2021; Mian et al., 2019). R33 and R35 displayed flatter slopes, which indicates lowered ohmic losses and slightly higher activation losses, whereas R38 exhibited a steeper slope, indicating increased ohmic losses (Şen-Doğan et al., 2021; Sangeetha et al., 2021), enhanced ionic resistance, and decreased microbial viability, which impedes overall electron transfer (Chen et al., 2018).
Furthermore, the drop in OCV at high current densities indicates that concentration losses occurred in all reactors. According to Zhang et al. (2019) and Kim et al. (2021), mixed culture will lead to concentration losses due to substrate limitations or inadequate biofilm enrichment. Furthermore, Watson and Logan (2011) proposed that microbial adaptation to electrochemical circumstances occurs gradually, producing constant power. These results are consistent with R33's second power peak, which could result from microbes adapting to the rising electrochemical environment.

In conclusion, the power density varied by 17.3% between R33 and R35 and 7.79% between R35 and R38. R33 significantly extends the power output by delaying the onset of concentration polarization and microbial adaptation. According to these findings, precise salinity control methods or the integration of real-time monitoring systems are essential for optimizing the performance and efficiency of microbial fuel cells in maritime

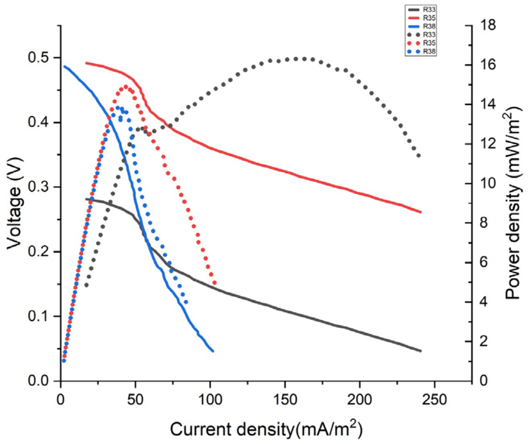

Figure 2. Polarization Curve of MFCs at Various Salinity Levels (Color Indication: Black - R33, Red - R35, Blue - R38)

Assessment of internal resistance in MFC under varying salinity conditions

Electrochemical impedance spectroscopy (EIS) was performed to analyze internal resistance developed in the MFC operation across different salinity levels (R33, R35, and R38). Figure 3 depicts the Nyquist plot of varying salinity levels, which includes the solution resistance, charge transfer resistance, double-layer capacitance, and Warburg resistance. The equivalent circuits are depicted in Fig. 3a and 3b, following the model proposed by Kumar et al. (2020). The findings reveal that R33 exhibited the lowest overall internal resistance, while R35 and R38 displayed the highest resistance, reducing electron transfer efficiency and power output.

The ohmic resistance represents the solution resistance (Rs) and electrode conductivity, which varied significantly among the reactors. R35 exhibited the lowest ohmic resistance (29.06 Ω), followed by R38 (37.79 Ω) and R33 (38.67 Ω) (Table 2). The results indicate that the slightly higher ion concentration in R35 improved electrolyte conductivity, reducing resistance and enabling efficient charge transport. However, R38 indicates that excessive salinity leads to ion saturation, reduced ion mobility, and higher resistance to charge flow (Guo et al., 2021). This also aligns with the observations of Lefebvre et al. (2012), who reported that increasing NaCl concentrations beyond 20 g/L negatively impacted power output due to electrolyte saturation and microbial inhibition. Hence, balancing ion availability and conductivity is crucial for optimizing MFC performance.

Charge transfer resistance (Rct) plays a crucial role in determining electron transport efficiency from the microbial biofilm to the anode. R33 exhibited the lowest charge transfer resistance (253.3 Ω at the anode), which indicates efficient electron transfer and enhanced microbial biofilm activity. In contrast, R35 and R38 exhibited much higher charge transfer resistances (1544 Ω and 1712 Ω, respectively), suggesting that increased salinity hindered electron flow, slowed bacterial metabolism, and negatively impacted power generation. Higher salt concentrations can lead to cell lysis and biofilm detachment (Jang et al., 2013). Thus, in R35 and R38, initially, the power density increased and decreased due to inhibited bacterial activity and disrupted biofilm integrity (Bassin et al., 2012; Wang et al., 2013).

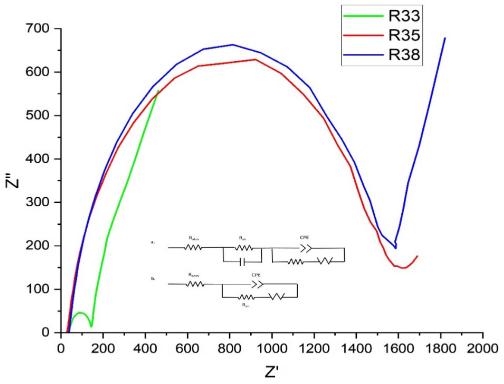

Figure 3. Nyquist plots and equivalent circuits of MFCs at varying salinity levels: (a) R33 and R38, (b) R35

Table 2. Circuit component values of MFCs at varying salinity levels

Reactor	Rohm	Anode charge transfer losses	Cathode charge transfer losses	Warburg resistance
R33	38.67	253.3	107.3	115.5
R35	29.06	1544	-	149.9
R38	37.79	1712	1553	226.6

Warburg resistance indicates ion diffusion limitations within the electrolyte, resulting in a distinct increasing trend with rising salinity. R33 exhibited the lowest diffusion resistance (115.5 Ω), followed by R35 (149.9 Ω) and R38 (226.6 Ω). This demonstrates that excessive salinity increases diffusion resistance and thus reduces the efficiency of charge transfer processes. Kumar et al. (2020) also reported that Warburg resistance increased significantly at higher NaCl concentrations due to ion crowding effects, limiting charge mobility. Additionally, diffusion resistance in R35 and R38 may be attributed to the buildup of excess cations near the proton exchange membrane (PEM), which impairs proton transfer efficiency and increases internal resistance (Borole et al., 2010).

The combined effect of ohmic, charge transfer and diffusion resistances significantly influenced power density trends across different salinity conditions. R35 demonstrated peak power density at lower current densities (~100 mA/m²), suggesting efficient performance under moderate ionic conditions. However, R38 exhibited a sharp decline in power density due to elevated charge transfer resistance, limiting sustained power output. Notably, R33 showed a delayed peak in power density (16.5 mW/m² at 160 mA/m²), indicating that biofilm adaptation gradually improved electron transfer, reducing resistance over time. This aligns with the findings of Guo et al. (2021), who noted that microbial adaptation to salinity fluctuations influences power stability in MFCs.

Impact of salinity on microbial viability, Community Structure, and biofilm formation in MFC

Microbial viability is fundamental to microbial fuel cell (MFC) performance. This study used the Miles et al. (1938) method to assess how different salinity levels (R33, R35, and R38) affected microbial growth. The results, presented in Figure 4, revealed notable variations in bacterial viability in the reactors. The highest bacterial viability was recorded in R33 (6.67×10^7 CFU/mL), followed by R38 (5.33×10^7 CFU/mL) and R35 (4.67×10^7 CFU/mL). This trend suggests that R33 provided the most favorable conditions for microbial activity, while R38 and R35 experienced osmotic stress, limiting bacterial growth and biofilm development. The microbial viability trend observed is consistent with the EIS data (Fig. 3), which showed that R33 had the lowest charge transfer resistance. The findings align with Danovaro et al. (2005), who reported that optimal salinity enhances microbial survival and biofilm adhesion, thereby improving MFC efficiency.

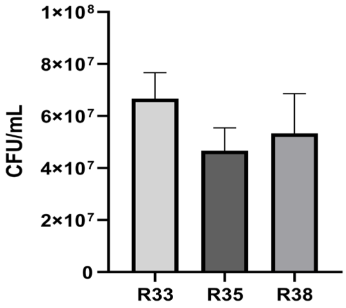

Figure 4. Colony-Forming Units (CFU/mL) at varying salinity levels in MFCs.

Gram staining was performed on bacterial samples from all the reactors to analyze microbial diversity further (Figure 5). The results indicated that Gram-negative bacteria dominated all three MFCs, highlighting a shared microbial composition despite variations in biofilm formation. The predominance of Gram-negative bacteria suggests that these microbes have adapted to the saline conditions of the MFC anolyte. Similar observations were made by Nicholas et al. (2000), who found that Shewanella species adjust their membrane fatty acid composition to maintain stability and cell function under hyperosmotic conditions. Additionally, Or et al. (2007) reported that microbes exposed to fluctuating salinity allocate more energy to maintaining membrane permeability, which affects biofilm integrity and electron transfer efficiency.

Although Gram staining patterns were similar across all reactors, noticeable differences were observed in colony morphology and bacterial arrangement (Fig. 5: A1, A2, B1, B2, C1, C2). The bacillus-shaped bacteria were predominant in all samples, but variations in bacterial arrangement suggest subtle differences in microbial adaptation to salinity. In contrast, studies by Zhao et al. (2016a) found variations in Gram-positive bacterial communities (e.g., *Actinobacteria, Proteobacteria, and TM7*) across different salinity conditions. Similarly, Bassin et al. (2012) reported that salinity changes in aerobic ecosystems led to shifts in bacterial composition, favoring *Proteobacteria, Bacteroidetes, and Acidobacteria*. However, since this study focused on anaerobic conditions, the dominance of Gram-negative bacteria highlights their adaptability to MFC environments, particularly in saline conditions.
Microbial communities exhibit diverse salt tolerance mechanisms, influencing their ability to form biofilms in MFCs. Some bacteria, such as *Microbulbifer*, thrive in saline environments by degrading complex carbohydrates (Jeong et al., 2013). Similarly, *Aeromonas hydrophila* grows at low to moderate salinity (0–3% NaCl) but fails to survive at higher concentrations (Jahid et al., 2015; Ulkhaq et al., 2020). In contrast, *Halomonas venusta* can tolerate salinity levels ranging from 1% to 20% NaCl (Berlanga et al., 2012). Studies by Zhao et al. (2016b) found that *Actinobacteria* abundance increased from 3.22% to 39.52% as salinity rose from 0% to 3%, while other bacterial species, such as *Myxococcales*, declined at higher salinity levels. These findings suggest that microbial community shifts directly impact MFC functionality, and identifying specific bacterial species in future research could help optimize bioelectrochemical systems.

To further investigate biofilm morphology, SEM was performed (Figure 6). The results showed distinct differences in biofilm thickness and adhesion across reactors. R33 exhibited the thickest biofilm, followed by R35, while R38 had the thinnest biofilm layer. The SEM images (Fig. 6: A1, A2, B1, B2, C1, C2) revealed that microbial cells infiltrated the carbon felt more effectively in R33 and R35, leading to stronger biofilm adhesion. In contrast, R38 displayed weaker biofilm attachment, possibly due to osmotic stress hindering bacterial aggregation.

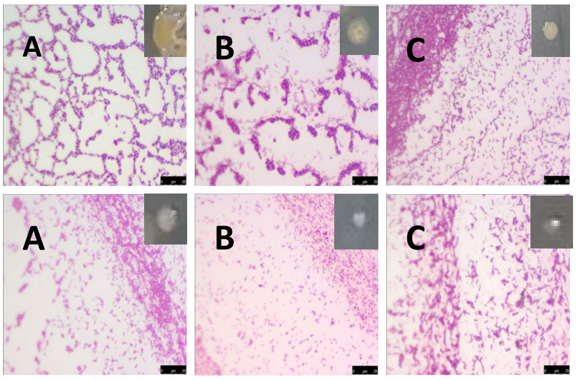

Figure 5. Gram Staining Results for Bacterial Colonies in MFCs at Varying Salinity Levels: (A1) R33, Yellowish Colony; (A2) R33, Small White Colony; (B1) R35, Yellowish Colony; (B2) R35, Small White Colony; (C1) R38, Yellowish Colony; (C2) R38, Small White Colony.

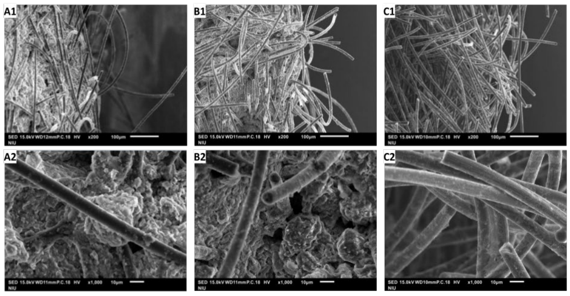

Figure 6. Surface Morphology of Anode Electrodes in MFCs at Varying Salinity Levels and Magnifications A1) R33, 200x; A2) R33, 1000x; B1) R35, 200x; A2) R35, 1000x; C1) R38, 200x; C2) R38, 1000x.

Microbes adapt to high salinity stress through osmoadaptation mechanisms, which include accumulating potassium ions and producing extracellular polymeric substances (EPS) to protect against osmotic damage (Kempf and Bremer, 1998; Sleator and Hill, 2002). This study found that low salinity levels (R33) promoted bacterial reproduction and increased EPS production, enhancing biofilm formation. Kim and Chong (2017) reported similar findings, where low salinity stimulated bacterial growth and EPS secretion, facilitating strong biofilm adhesion. However, EPS production decreased at higher salinity (R35 and R38), leading to thinner biofilms and reduced microbial viability. Despite this, bacteria remained metabolically active in R38, suggesting that they compensated for high salinity stress by overproducing EPS, which in turn hindered biofilm formation (Reid et al., 2006; Joghee and Jayaraman, 2016).

Salinity also influences concentration polarization, which occurs when solutes accumulate near the membrane surface, impeding ion transport and lowering MFC efficiency (Kim and Chong, 2017). This study found that R33 maintained stable biofilm integrity, allowing for sustained power generation, while R35 and R38 exhibited faster polarization effects, reducing overall performance. The high salt concentration at the biofilm-membrane interface in R35 and R38 likely contributed to localized concentration polarization, restricting electron transfer and lowering power output.

Impact of pH and salinity concentration on the performance of MFCs

The performance of microbial fuel cells (MFCs) is significantly influenced by pH and salinity, which affect microbial power generation. This study examined pH and salinity variations in R33, R35, and R38 by measuring their values at the beginning and end of each 2-day operational cycle. Unlike salinity, pH was an uncontrolled variable since no stabilizers like phosphate-buffered saline (PBS) were used in the anode chamber. Consequently, pH levels fluctuated between 7.7 and 8.9 (Table 3), reflecting natural changes in microbial metabolism and ionic exchange. According to Margarita et al. (2017), denser biofilm formation and enhanced power density were obtained in the optimal pH range of 8–10. And pH levels below 5.5 or above 10 significantly affect MFC performance by influencing microbial activity and biofilm formation. On comparing with the results, pH fluctuations remained within the optimal range, thus resulting in biofilm stability and further electron transfer efficiency.

Patil et al. (2011) observed that changes in pH affect microbial diversity and microbial community, thus impacting power output. His findings include that under neutral to slightly alkaline pH conditions, the biofilms are dominated by *Geobacter sulfurreducens*, and extreme pH environments result in lower performance with diverse microbial consortia. Microbial consortia are impacted by pH, which lowers power performance.

Salinity increases proton transport and electrolyte conductivity, which boosts MFC performance. Although, the medium was replenished every two days. However, each cycle decreased salinity steadily, averaging 1.5 g/L in R33, 2.5 g/L in R35, and 2.9 g/L in R38 (Table 3). This decline may be due to the ion diffusion. Lefebvre et al. (2012) claim that raising salinity first reduces internal resistance, which enhances power generation and proton transfer efficiency. His results align with those of R35, which showed that moderate salinity produced the highest power production.

Excessive salt concentrations influence microbial metabolism. According to Yan et al. (2015), acidophilic bacteria are negatively impacted by high salinity concentrations, which reduces microbial viability, thus resulting in limited power output. Similar observations were reported by Khudzari et al. (2016), who reported that electrogenic bacteria were hindered by high salinity (over 10 g/L NaCl), which in turn reduced MFC power generation. Gul et al. (2021) also found that microbial activity near the anode dropped when salinity exceeded the system's optimum threshold. This further supports the idea that persistent biofilm growth and effective electron transfer depend on a precise salinity balance.

This study highlights the critical role of pH and salinity in regulating microbial activity and biofilm stability, thereby influencing MFC performance. The study did not control pH, but it remained within a reasonable range, likely promoting microbial growth and electricity generation. Future research should focus on adaptive biofilm engineering, electrolyte management strategies, and real-time monitoring of pH and salinity fluctuations to optimize MFC performance in marine environments.

Table 3. Average pH and Salinity Variations of R33, R35, and R38 in DS-SMFCs

Parameter (Anode chamber)	**R33**	**R35**	**R38**
pH range	7.7 - 8.9	7.7 - 8.9	7.7 - 8.9
Average Salinity variation	↓ 1.5 g/L	↓ 2.5 g/L	↓ 2.9 g/L

CONCLUSIONS

This study investigated the effects of ocean salinity fluctuations by choosing three salinity concentrations, R33, R35, and R38 g/L, on MFC performance. R33 had resulted in prolonged power output due to the onset of concentration polarization, higher bacterial viability, and robust biofilm formation. Although the OCV values are high for R35 (0.491 V) and R38 (0.502 V) compared to R33 (0.281 V), R35, due to the increased ionic concentration, exhibited higher internal resistance, which limits the electron transfer and thus power output. At a lower current density of 103.59 mA/m², R35 exhibited the highest power density (15.02 mW/m²). In comparison, R33 exhibited a second peak power at 16.5 mW/m² at 160 mA/m², indicating stable power due to its delayed concentration polarization effect and microbial adaptations.

Furthermore, R33 resulted in the highest bacterial viability of 6.67×10^7 CFU/mL, which helps thicker biofilm formation and thus enhanced electron transfer efficiency. R38 and R35 showed reduced microbial activity due to osmotic stress and cell lysis. The biofilm structure and microbial community composition varied with salinity, influencing power generation.

Overall, R33 offered the most advantageous circumstances for prolonged MFC performance, with enhanced power density, bacterial viability, and reduced internal resistance. These results highlight the importance of salinity control in real-time MFC implementation. Future research should focus on adaptive salinity management and microbial community analysis to improve MFC efficiency for deep-sea energy applications and aquatic ecosystem monitoring.

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