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Evaluating the effects of chloride on the performance of microbial fuel cells for wastewater treatment

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Abstract:

Up to 5% of wastewater generated worldwide is highly saline effluent. Microbial fuel cells (MFCs) have been considered as a promising saline wastewater treatment, which uses microorganisms to convert organic compounds into electrical energy. This study investigated lab-scale MFCs to evaluate the effect of chloride at different concentrations (0, 5, 10 and 20 g/l NaCl) on the performance of the MFC by examining its electrical generation and wastewater treatment efficiency. Increasing NaCl concentration led to a decrease in power generation with maximum power at 30, 18.7, 18.8, and 5.2 mW/m^2 corresponding to 0, 5, 10, and 20 g/l of NaCl, respectively. The COD removal efficiency of the four treatments did not significantly vary (p>0.05) and reached 65.9% to 78.7%. *Acidovorax* spp. was isolated from the treatment without NaCl while *Pseudomonas citronellolis* was isolated from the treatments containing 10 and 20 g/l NaCl. *Pseudomonas citronellolis* can be used as a potential inoculum for MFCs treating highly saline wastewater based on its high electrical generation ability (606.8 mW/m^2) based on inoculation of this pure strain into the mini MFC.

Keywords: ***Acidovorax*** **spp., chloride, microbial fuel cell (MFC),** ***Pseudomonas citronellolis*****, wastewater treatment.**

Classification number: **5.3**

Introduction

Water used in many industrial sectors such as food processing and agriculture farming contain high salt concentrations. Without prior treatment, effluent discharge containing high salinity and high organic content can have dangerous effects on aquatic life, water portability, and agriculture [1]. Therefore, the biological treatment of saline wastewater is an important problem that needs to be solved. In the past, saline effluents have been conventionally treated through physio-chemical treatments as many biological treatments are strongly inhibited by salts (mainly NaCl). However, with the cost of physio-chemical treatments being particularly high, alternative biological systems that reduce cost are increasingly becoming the focus of research.

MFCs are a promising new method due to a combination of effectiveness in wastewater treatment, electricity generation, and low cost in comparison with other methods of wastewater treatment [2]. An MFC is a device that directly converts the energy stored in the chemical bonds of substrates into electrical energy through catalytic reactions of microorganisms [3, 4]. The reaction occurs as a result of two processes in the MFC, as described by [5], and is given by Eqs. (1) through (3) below:

$$\text{Anode oxidation reaction: } (CH_2O)_n + nH_2O \xrightarrow{\text{Microorganisms}} nCO_2 + 4ne^- + 4nH^+ \quad (1)$$

$$\text{Cathode reduction reaction: } 4e^- + 4H^+ + O_2 \rightarrow 2H_2O \quad (2)$$

$$\text{Overall reaction: } (CH_2O)_n + nO_2 \rightarrow nCO_2 + nH_2O + \text{Electrical energy} \quad (3)$$

Added substrates are oxidized under anaerobic conditions by microbes in the anodic chamber of an MFC and release electrons, protons, and carbon dioxide. The electrons are transferred to the anode and transported to the cathode through an external circuit while the protons cross proton exchange membranes (PEMs) or a salt bridge to enter the cathodic chamber where they combine with oxygen to form water [6]. However, this technology is only in early research stages and more investigations are required before domestic MFCs can be made available for commercialization.

The ionic conductivity of the substrate is one of several factors that strongly influences the performance of an MFC [3, 7, 8]. Increasing the conductivity of a substrate could result in improved MFC electrical generation [7, 9]. However, increasing the ionic strength does not always increase MFC performance due to the capacity for salt tolerance of anodophilic bacteria, which are present at the anode of an MFC [10, 11]. Hence, it is important to understand the effect of conductivity and ionic strength on MFC performance in order to optimize bioelectricity production from wastewater treatment.

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Chloride, in the form of NaCl, was used to change the ionic conductivity in this study. NaCl was added into an MFC at different concentrations to evaluate the effects on performance in terms of electrical generation and wastewater treatment efficiency. The microorganisms in the anode at different salt concentrations were evaluated to see the impact to the microbial community.

Materials and methods

Materials

Synthesis wastewater preparation: the microbial fuel cells were fed with artificial wastewater as a substrate following the formula of [12] i.e., glucose 445 mg/l, $NaHCO_3$ 750 mg/l, NH_4Cl 159 mg/l, K_2HPO_4 13.5 mg/l, KH_2PO_4 4.5 mg/l, $CaCl_2.2H_2O$ 125 mg/l, and $MgSO_4.7H_2O$ 32 mg/l. Trace elements supplemented in the synthetic wastewater followed the formula of [13]. The electrolyte solution was comprised of KCl 0.1 mg/l, NH_4Cl 0.2 mg/l, NaH_2PO_4 0.6 mg/l, and $NaHCO_3$ 2 mg/l [14].

Reactor configuration:

- Lab-scale microbial fuel cells (MFCs): microbial fuel cells were constructed according to the design of [15] with a minor modification using easily available materials to reduce cost (Fig. 1A). Each cell was constructed by using polyvinyl chloride (PVC) pipe. Electrodes were made of round-shaped carbon cloths that were 10 cm in diameter and 5-mm thick sandwiched in between two pieces of stainless steel mesh. The carbon cloth, which had a density of 160±10 g/m^2, a specific surface area of 1200 to 1500 m^2/g, and pore volume of 0.7 to 0.8 ml/g, was purchased from Mien Nam Tec Company, Viet Nam. These components of the electrode were connected together by nuts and bolts that connect to copper wire on the top of the bolt. The disk-shaped anode was located 25 cm from the bottom of the cell and the cathode at the top, which was separated by layers of glass wool and glass beads 30 cm high. The electrodes were filled with granular graphite. The aeration was located just below the cathode chamber to create the aerobic environment. The port for sludge injection was 27 cm from the bottom, just above the electrode. Gravel was placed at the bottom and the top of each cell to prevent the movement of the cell. The anode and cathode were connected by copper wire and to a resistor (1000 Ohm) in order to consume the electricity generation continuously.

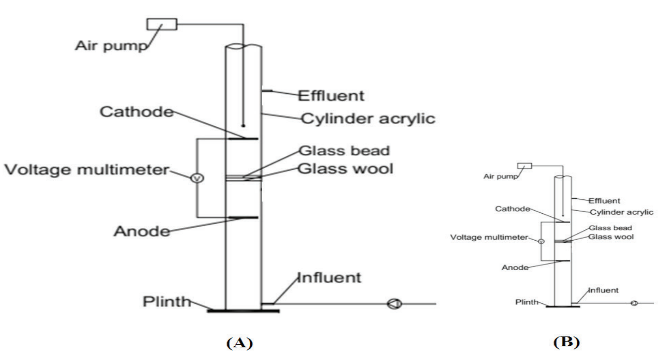

Fig. 1. Schematic of the two MFC models used in the study: (A) the lab-scale MFC (diameter of 10 cm; height of 75 cm) and (B) the mini MFC (diameter of 5 cm; height of 10 cm).

- Small-scale microbial fuel cells (mini MFCs): mini MFCs were made by scaling down the lab-scale MFC design (Fig. 1B). Its body was also made of PVC in a cylinder shape with 10-cm height. The electrodes were also made of round-shaped carbon cloth with a diameter of 50 mm.

- MFC inoculum, the source of microorganisms: anaerobic sludge, which was collected from the Brewery Company in district 12, Ho Chi Minh city, was used as an inoculum for all the MFCs. The pH of the inoculum was found to be 7.

Experimental design

The experiments were comprised of four MFC treatments with different NaCl concentrations (0, 5, 10, 20 g/l) so-called Treatment 1, Treatment 2, Treatment 3, and Treatment 4, respectively. Each treatment was triplicated.

MFCs operation: firstly, the MFCs were rinsed with electrolyte solution. Then, anaerobic sludge inoculum was injected into the anodic zone of all four treatments with mixed liquor suspended solids (MLSS) 4 g/l. Finally, artificial wastewater with different concentrations of NaCl (0, 5, 10, and 20 g/l) were injected to the anodic zone of the MFCs for Treatments 1, 2, 3, and 4, respectively. All MFC systems were operated in batch mode under room temperature and aerated continuously for 15 days.

Electrogenic bacteria isolation: microorganisms in the sludge samples of each treatment were withdrawn from the anode after stabilization of the performance. After 15 days, a 1-cm^2 piece of carbon cloth from the anode of each MFC was removed to isolate the electrogenesis bacteria. The carbon cloth samples were placed into a culture bottle (25 ml) containing 20 ml of sterilized GA medium [16]. The suspension of each medium was collected and diluted in 25-ml sterile tubes. Six gradients in triplicate were carried out for the bacteria enrichment. The petri dishes were prepared with agar medium to inoculate bacteria liquids of 10^3 to 10^5 gradients (1 ml). Then, the inoculated bottles were erectly cultivated for 5 days until various single colonies were grown in the medium. Based on the colony's characteristics, target strains were picked out with a sterile needle. Colonies forming on the plates were picked and further purified by quadrant streaking on fresh plates.

Isolated electrogenesis bacteria evaluated with mini MFCs: after isolation, the bacteria strains were enriched in GA medium to densities of 10^6 to 10^8 CFU and then inoculated to the mini MFCs. The mini MFCs were made of PVC cylinders of 10-cm length. The electrodes were made of round-shaped carbon cloth with a diameter of 50 mm. Artificial wastewater was used as the feed substrate. The voltage and current across each pure strain of bacteria was measured after 1 day by a digital multimeter.

Analytical methods

The pH was measured by a pH Extech 407228. Turbidity was measured by a UV-Vis spectrophotometer (GENESYS 10 UV-Vis, Thermo Fisher Scientific, Inc., USA) at 450 nm using the nephelometric turbidity unit (NTU) as a standard solution. Turbidity removal efficiency was calculated by the difference between outflow and inflow. Chemical oxygen demand (COD) was determined using a closed reflux titrimetric method [17]. Mercuric sulphate was used to eliminate the interference of chlorides at a 10:1 weight ratio of mercuric sulphate to chloride. COD removal rates were calculated by the difference between COD outlet and inlet. Electric current and voltage were measured daily for 15 days. The circuit voltage was measured using a digital multimeter (KYORITSU Model 1009, Japan) with an external resistance of 200 Ohm. The power density (in mW/m^2) was calculated by dividing power by the anode surface area (m^2).

After isolation, the bacteria were gram stained and observed at 100x under microscope to identify gram staining and cell morphology. DNA from the pure bacteria species that generated the highest electricity (verified by inoculating the mini MFCs) was extracted using a DNA kit and subsequent sequencing for specie identification.

Statistical analysis

The results were statistically analysed by comparing the mean values of the effect of chloride at different concentrations on the performances of the MFCs as a single factor using one-way ANOVA. The statistics were expressed as mean ± standard deviation (SD) with $p<0.05$ as significant. These statistical analyses were performed using the SPSS program (Version 20.0, IBM Corp., USA).

Results and discussion

pH value

Figure 2 shows the pH value of the four treatments during the 15 days of experiment. The pH value of all four treatments were stable with values around 8.5 to 9.0. Statistical analysis showed that the pH value of Treatment 4 was not significantly ($p>0.05$) different with each other. In other words, the change in chloride concentration did not cause much change to the pH value.

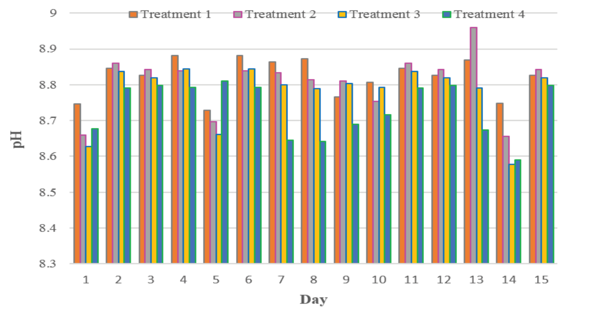

Fig. 2. The pH value of MFCs at different NaCl concentrations over the 15 days of experiments.

Turbidity

Figure 3 shows the turbidity change over the 15 days of experiments for the four treatments. There was a significant difference ($p<0.05$) between Treatment 1 (without NaCl) and Treatments 2, 3, and 4 (5, 10, and 20 g/l NaCl, respectively). While the turbidity of Treatment 1 increased over 15 days, Treatments 2, 3, and 4, which contain NaCl in artificial wastewater, had reduced turbidity with time during the 15 days.

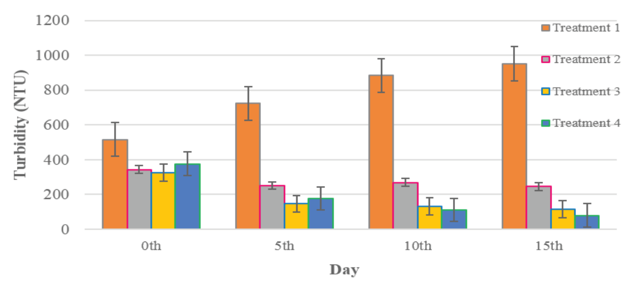

Fig. 3. The turbidity of the MFCs at different NaCl concentrations over the 15 days of experiments. Vertical bars are the standard deviation.

The value of turbidity typically depends on the residue of organic matter inside the feed. After 15 days, the turbidity removal efficiencies were 28.1, 64.7, and 78.7% for Treatments 2, 3, and 4, respectively, which contain 5, 10, and 20 g/l NaCl, respectively. On the other hand, the turbidity of Treatment 1 (without NaCl) increased over the time. The addition of NaCl, as reported by [18], causes decreased turbidity in water during the electrocoagulation process. Hence, the MFC was found as a self-powered electrocoagulation reactor [19]. The increase in turbidity removal with increase of salt concentration in the MFC could occur due to the electrocoagulation process, which might take place inside MFC compartments.

Electricity generation

Voltage output: Fig. 4 indicates the change of voltage output of the four treatments during the 15 days of experiment. There was a significant difference in voltage output between Treatment 1 (without NaCl) and Treatments 2, 3, and 4 ($p<0.05$), as well as between each treatment. The results showed that increasing NaCl concentration led to a decrease of voltage output. From highest to lowest, the voltage output of all treatments was 0.32 V (Treatment 1), 0.21 V (Treatment 2), 0.12 V (Treatment 3), and 0.11 V (Treatment 4). The maximum voltage recorded for the experiment was approximately 0.32 V, which is lower than 0.53 V obtained in the study of [15] (similar MFC design, but without the addition of NaCl).

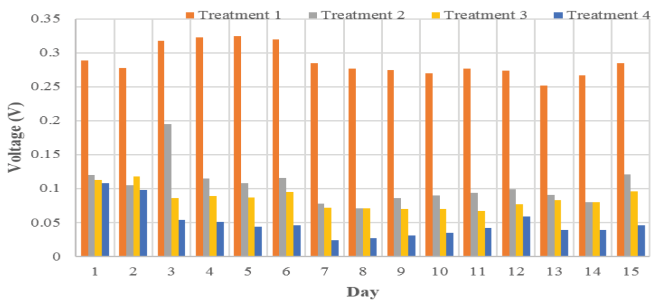

Fig. 4. The voltage output of the MFCs at different NaCl concentrations over the 15 days of experiments.

Power generation: figure 5 provides the power generated by the four treatments over 15 days. There was a remarkable change in power generation (mW/m^2) from day 1 to day 4. The maximum value of power was attained around day 6 for Treatments 1, 2, and 3, then decreased in the later days. Treatment 4 with 20 g/l of NaCl peaked at day 2 then decreased. There were significant differences ($p<0.05$) in power generation between the treatments, except for Treatments 2 and 3. It was found that the higher concentration of NaCl, the lower power generation attained by the MFCs.

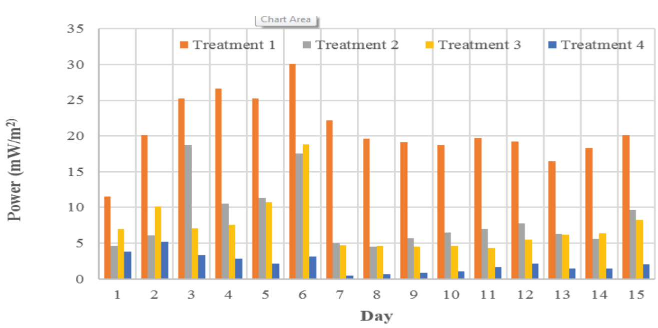

Fig. 5. The current output of MFCs at different NaCl concentrations operated in 15 days of experiments.

The power density widely varied in previous studies and was highly dependent on the configurations of the MFC systems (Table 1). However, the power density obtained in this study was comparable and even in the higher range compared to reports of other studies. For example, the study of [20] that evaluated the effect of NaCl on the anodic bacterial community of MFC reported a reduction in power generation when the salt concentration was higher than 0.1 M (equivalent with 5.85 g/l NaCl). In this study, we also found that power reduced with a salt concentration of 5 g/l.

Table 1. Comparison of MFC performance between different studies.

MFC configuration	Inoculum	e-donor	Anode material	Cathode material	Membrane/ Separation material	Power density (mW.m^{-2})	References
Single chambered	Mixed culture (Anaerobic sludge)	Petroleum sludge	Graphite plates	Graphite plates	Nafion-117	53.11	[21]
Dual chambered	Mixed culture (activated sludge)	Acetate	Toray carbon paper (20% Teflon)	Toray carbon paper (10% Teflon)/Pt coated	Sterion membrane	1.01	[22]
Single chambered	Mixed culture (domestic wastewater)	Acetate	Carbon cloth	Carbon cloth/Pt	Non-woven fabric	1200	[23]
Single chambered	Mixed culture (MFC effluent)	Fermented primary effluent	Graphite fibre brushes	Carbon cloth/Pt	PTFEa	320	[24]
Dual chambered	Mixed culture (Anaerobic sludge)	Acetate	Graphite rod	Graphite rod	Nafion-117	13.59	[25]
Single chambered	Mixed culture (Anaerobic sludge)	Acetate	Carbon cloth	Carbon cloth/Pt	PVA-STAa	58.8	[26]
Single chambered	Mixed culture (Anaerobic sludge)	Glucose	Carbon cloth	Carbon cloth	Gloss wool as separator	30	This study

COD: in order to evaluate the wastewater treatment ability of MFCs, COD was measured at the end of the 15 days of experiment. The COD remarkably decreased over the 15-d period for all treatments. The COD removal efficiency of Treatments 1, 2, 3, and 4 were 68.4, 77.1, 78.7, and 70.5 %, respectively. The results showed that increasing NaCl concentration did not cause any reduction in COD removal efficiency. The statistical analysis showed that there were no significant differences ($p>0.05$) in COD value between the four treatments after 15 days. However, the results of COD removal efficiency in this study are quite low compared to the 85-90% COD removal in the experiment using an MFC in [27]. The difference in COD removal could be caused by different configurations and/or operation conditions (such as influent COD concentration, HRT, specific organic loading rate, and reactor volume) of the MFC models [28], but it does not come from the change in salt concentration.

Electrogenic microorganism isolation

Anode carbon cloth samples from treatments with the addition of NaCl (Treatments 1, 2, 3, 4) were collected for electrogenesis bacterial isolation. After the isolation process using GA medium, 5 isolates were obtained from the anode of the MFCs and named No.1 through No.5. There was one isolate from Treatments 1, 3, and 4, while two isolates were extracted from Treatment 2. All isolates were gram negative and had a rod shape. The morphology of isolate No.1 was yellow-brown raised circular, while isolate No. 2 to isolate No.5 were yellow-brown raised circular with slightly wrinkled margins. Microscopic photos were taken for the gram-stained isolates observed at 100x and are presented in Fig. 6.

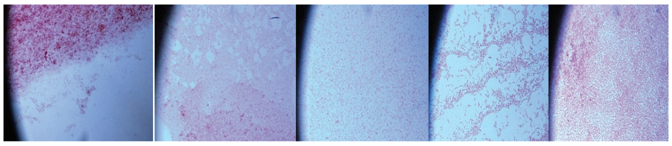

Fig. 6. Microscopic photos taken for the gram-stained isolates observed at 100x. No.1 through No.5 denote the five isolates from four treatments.

The isolates were tested for the electrochemical abilities by inoculating each isolate into the anode of the mini MFC. Artificial wastewater was used as the substrate and electrical generation was measured after the one-day operation.

All five isolates evaluated in the mini MFCs showed higher values of the maximum power generation, which were 10 to 20 times higher than the maximum power generated in the larger scale that was inoculated with anaerobic sludge (Table 2). The maximum electricity generation was obtained from days 3 through 6. This could be due to the different configuration of the MFC, which comes with a different internal resistance. This result points out the potential for using those isolates for treating saline wastewater in MFCs.

Table 2. Power generation with mini MFCs inoculated with isolates obtained from different treatments.

Name	Maximum voltage (V)	Maximum current (mA)	Maximum power (mW/m^2)
No. 1-Treatment 1	0.33	1.48	248.87
No. 2 -Treatment 2	0.48	2.02	494.06
No. 3 -Treatment 3	0.39	1.97	391.49
No. 4 -Treatment 3	0.33	1.49	250.55
No. 5 -Treatment 4	0.52	2.29	606.78

Out of the five isolates, three were selected for molecular identification including isolates No. 1 - Treatment 1 (0 g/l NaCl added), No. 2 - Treatment 2 (10 g/l NaCl), and No. 5 - Treatment 4 (20 g/l NaCl). Isolate No.1 was applicable for no addition of NaCl. Isolate No. 2 and No. 5 showed the highest power output with the addition of NaCl. After sequencing, the results indicated that No. 1 was *Acidovorax* spp. while No. 2 and No. 5 were the same strain, which was *Pseudomonas citronellolis*. *Acidovorax* spp. is the non-pathogenic genus of proteobacteria found in the soil and environment. In this study, Treatment 1 (without NaCl), where the presence of *Acidovorax* spp. was dominant, showed the high power generation (249 mW/m^2). It was a gram-negative rod, which usually occurs singly but occasionally in pairs. *Pseudomonas citronellolis* grows anaerobically in the presence of nitrates over a range of temperature from 25 to 37^{o}C [29]. The wild type of *Pseudomonas aeruginosa* was studied by inoculation into microbial fuel cells by [30]. In their study, the wild type *Pseudomonas aeruginosa* was found to use different electron shuttles that resulted in the enhancement of the maximum current of the MFC. *Pseudomonas citronellolis* was isolated from Treatment 2 and Treatment 4, which contained 5 and 20 g/l NaCl, respectively, and demonstrated that this bacterium had high salt tolerance ability. Therefore, *Pseudomonas citronellolis* can be used as a potential inoculum for MFCs treating highly saline wastewater. This result showed a change in the electrogenesis bacteria due to the change in NaCl concentration. Even *Pseudomonas citronellolis,* which was dominant at higher salt concentrations, possessed good electricity generation and the shift in bacterial community caused a reduction in power at higher NaCl concentration. Along with the study of [20], it is confirmed that a significant change of anodic bacterial community comes with a change of salt concentration.

Conclusions

The results from this study showed that chloride had a large impact to the performance of the MFCs. An increasing chloride concentration led to a decrease in power generation. However, the wastewater treatment performance of MFCs are still feasible even at high chloride concentration. The isolated bacteria from the high saline MFC, *Pseudomonas citronellolis*, gave high power density when inoculated into the MFC, which offers this specie as a potential type of inoculum for MFCs treating highly saline wastewater based on its saline tolerant ability and electrical generation.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

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