Understanding the Effect of Fe(II) and Fe(III) in Generating Electricity from Real Waste Sludge in Microbial Fuel Cells

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Understanding the Effect of Fe(II) and Fe(III) in Generating Electricity from Real Waste Sludge in Microbial Fuel Cells

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Abstract: *Microbial fuel cells (MFCs) are a versatile technology for power generation from biodegradable solid wastes. This study aimed to investigate Fe(II) and Fe(III) effects on MFC's power generation and wastewater treatment performance. All MFCs were operated in a batch mode and incubated at a constant temperature (40 °C) for 45 days of operation. The experimental procedure went through MFCs construction, collection and characterization of waste sludge samples, and bacterial growth counting. Results showed that the Fe(III) addition exhibited a higher power output by 295 % and a shorter start-up time. The microbial growth increased by 92.18 %, and the anolyte's resistivity decreased with an increase in the organic matter digestion by 52.78 %. However, the amount of power generated in these MFCs was limited by the biological Fe(III) reduction rate high enough to restrict the produced current.*

Keywords: Anaerobic Digestion; Bacterial Growth; Electricity Generation; Iron Addition; Microbial Fuel Cell.

1. INTRODUCTION

According to the United Nations Report, climate change is projected to increase water-stressed regions and exacerbate shortages in already water-stressed regions. Moreover, water depletion is emerging as a vital issue. Around 2 billion people are living in countries experiencing water stress. Also, according to the shortage of global energy, the energy consumption of excess sludge treatment is too high. Abundant organics cannot be utilized effectively in sludge, causing waste of resources. Globally, it is likely that over 80% of wastewater is released into the environment without adequate treatment. Therefore, it becomes crucial to define sustainable water conservation and wastewater treatment techniques to limit carbon-based energy resources and restrain greenhouse gas emissions and environmental pollution [1]–[5] .

In this context, bio-electrochemical systems, and microbial fuel cells (MFCs) in particular, have been gaining popularity over the past decades to utilize the chemical energy contained in wastewater as this substance contains more energy potential than what is currently used for trading it for our manufacture and consumer energy needs [6],[7]. MFCs create a sustainable way to produce energy and power. In other words, if MFC delivers on its promise, wastewater would be waste no longer. A primary developing application of MFCs is their sustainable bioenergy, organic wastewater treatment, and electricity. An MFC represents an electrochemical device for the direct conversion of chemical compounds into electrical energy. In the anode chamber, the sludge is utilized as fuel, and electrons are generated via oxidizing organics under anaerobic conditions. Electrons flow through a circuit, so we extract that electrical current. The electrons end up on the other side of the MFC called the cathode chamber and combine with oxygen [8], [9]. The reduction of oxygen ions results in high redox potential. The high difference in redox potentials drives the electrons to flow, and therefore, bioelectricity is generated.

The theory is simple but putting it into practice is challenging. Many challenges have been facing the MFC technology. The electron transfer mechanisms, the low system performance, high resistivity, and low Coulombic efficiency are the main weaknesses of this technology. Many studies have highlighted the main advancements to improve MFC technology's performance. MFC reactor size, operating mode, electrode material, external resistance, inoculum culture source, proton exchange membrane (PEM), and substrate type are crucial to define the MFC's output. Further technical developments are necessary for its practical use. The limitation to widespread MFC utilization is that the power densities are too low for envisioned applications. Substantial improvements will be required for the uses of microbial fuel cells, such as the large-scale conversion of organic wastes and biomass to electricity or powering vehicles, mobile electronic devices, or households with suitably scaled microbial fuel cells will be possible.

Iron is one of the most frequently used metals for the treatment of contaminants present in water. It is the fourth most abundant element after oxygen, silicon, and aluminum are present in its crust. Iron is a reactive metal with standard redox potential E_o= -0.44V, making it an effective reductant when reacting with oxidized contaminants. It is a valuable and versatile tool for the purification of waters and soils owing to its: (i) environmental friendliness, (ii) high reactivity, and (iii) cost-effectiveness [10], [11].
Fe particles offer a grand promise for the environmental treatment of various contaminants and wastewater treatment [12]–[15]. In addition, Fe facilitated the anaerobic digestion process and helped buffer pH in a suitable range for exoelectrogen growth. It was found that the response of the MFC is positively correlated with the bacterial composition, in particular electroactive bacteria. Specific microbial strains have been exploited in the literature due to their high sensitivity and vulnerability to Fe [16], [17].
Referring to the literature, microbial fuel cells (MFCs) offer high reliability for wastewater treatment and energy generation. This is mainly due to the high organic matter content in waste sludge. With this said and considering the wastewater treatment costs, MFCs have cost advantages over other wastewater treatment techniques. Several research projects are currently focusing on the development of cost-effective techniques to make it fulfill the required performances. The investigation of microbial fuel cells is carried out. The considered

technology is characterized by: Power density output, Total volatile solids analysis, Coulombic efficiency (CE). The investigation was focused on the anode chamber's performance as it is one of the critical factors that determine power generation in MFCs. The experimental studies were carried out in lab-scale microbial fuel cells. Series of strategies were executed to improve overall system performance. The experimental procedure went through the construction of lab-scale MFCs (MFC's type, electrodes material), the collection and characterization of waste sludge, and estimating bacterial growth improvement.

2. MATERIALS & METHODS

2.1 Sludge collection and characterization

The sludge used in this study was obtained from the Mikasagawa domestic wastewater purification center located in Fukuoka, Japan. The sludge was kept at a temperature under 40 ºC to keep its freshness and inhibit bacterial growth throughout experiments. Waste sludge samples were collected at different periods and analyzed. Fe(II) concentration, the total dissolved Fe were measured using a UV spectrophotometer (Hach DR 3900, USA). pH and ORP were controlled using a pH meter. Conductivity, resistivity, salinity, and total dissolved solids (TDS) were monitored using a conductivity meter. Chemical Oxygen Demand (COD) values were controlled using the chemical acids that oxidize organic and inorganic substances in the waste sludge sample. Analyses of COD are essential since it reflects the removal of the contaminants (biologically or chemically). COD represents the total measurement of all oxidized chemicals in the water. However, COD values are less specific since they measure everything that can be chemically oxidized rather than just biodegradable organic matter. 2 mL of the tested waste sludge sample is injected in the vial then heated at 150 C for 2 hours using a UV spectrophotometer (Hach DR 3900, USA).

TS and TVS were determined. For solids composition characterization, TVS values were measured considering suspended and dissolved solids. The amount of TVS is essential since it reflects the strength of the waste. The more TVS, the stronger the waste sludge is. Besides, suppose the TVS/TS ratio reflects whether the waste sludge is primarily organic or not. In that case, the MFC operation's impact will be more significant if TS is primarily organic. The following table summarizes the initial values of the sludge characteristics. Figure 1 presents the COD and TVS analysis.

Oxidative Reductive Potential (ORP) measurements can also indicate the degree to which a substance can oxidize or reduce another sense. ORP values can be: (i) positive when the essence is an oxidizing agent, or (ii) negative when the substance is a reducing agent.

Table 1. Sludge characterization

Parameter	Unit	Value
pH	-	7.17
ORP	mV	-38
Conductivity	µS/cm	*794.60*
Resistivity	Ω.cm^2	*1000*
Salinity	ppt	*0.5*
TDS	mg/L	397
TS	g/L	4.37
TVS	g/L	3.60
COD	mg/L	5849
Fe^{2+}	mg/L	21.68
Fe	mg/L	42.89

Fig. 1: (A) UV spectrophotometer (Hach DR 3900, USA, (B) Various vials testing the COD values in different waste sludge samples. The vials change the color from orange to green based on the amount of oxidation. (C) Drying the mixed samples of waste sludge in the oven at 105 ºC for 24 hours.

2.2 Construction of microbial fuel cells (MFCs)

The used MFCs consisted of "H" type MFCs, containing two bottles with a total capacity of 500 mL, and disconnected by a Nafion 117 proton exchange membrane (PEM). Carbon felt (surface area 24 cm^2) was used for both anodic and cathodic electrodes, and a copper wire, connected via a resistance of 200 Ω, was applied to close the electrical circuit. These MFCs are considerably inexpensive and applicable for basic research, such as examining power production using new materials or types of sludge. However, they typically produce low power owing to the reduced surface of the proton exchange membrane. Figure 2 presents the schematic design, whereas Figure 3 shows the real H-type MFCs used for the experiments.

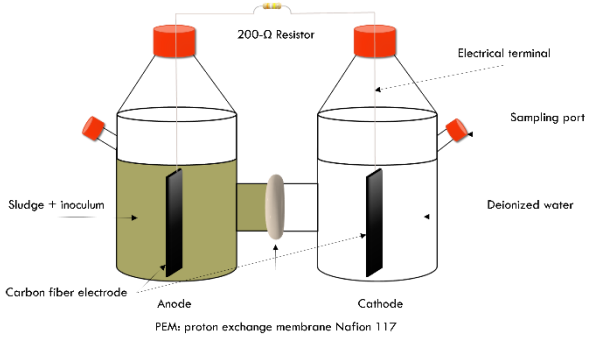

Fig. 2: Schematic design of the lab-scale double chamber microbial fuel cell (MFC).

Fig. 3: Real illustration of the lab-scale H-type MFCs used for our study.

All MFCs were operated in a batch mode and incubated at a constant temperature (40 °C) for 45 days of operation. Cathode chambers were filled with water. Fe(II), Fe(III), and Fe(II)/Fe(III) couple with a ratio of 50 % were introduced separately to the anode solution with a concentration of 10 mg/L to evaluate their effect on MFC's performances. The temperature value was selected based on the system conditions' significant impact on the initial biofilm formation process. Biofilms grown at higher temperatures (5 to 45 °C) tend to be more electrochemically active than in lower temperatures. A magnetic stirring was utilized to ensure a continuous mixing of the solution (500 rpm), which leads to a homogeneous medium and mass transfer resistance reduction. At the start of experiments, nitrogen gas N_2 was bubbled into the anodic chamber for 10 min to ensure anaerobic conditions.

2.3 Data measurements and analysis

The voltage output (V) was recorded every day using a multimeter. According to ohm's law, the current (I=V/R) and the power output (P=VI) were calculated using the external resistance R and the measure voltage V. The power density P_{an} (mW/m^2) was normalized to the anodic surface area following the equation below:

$$P_{an} = \frac{V^2}{A_{an} R} \quad (4)$$

Where: V is the measured voltage (mV), A_{an} is the anode surface area (cm^2), and R is the external resistance (Ω).
The coulombic efficiency was measured as the ratio between the experimentally measured coulombic values transferred from the microorganisms to the anode surface and the theoretically produced electrons from the degradation of the organic matter in the anode chamber as detailed in the equation below:

$$CE = \frac{M \int_0^t Idt}{F\ b\ Van\ \Delta COD} \quad (5)$$

Where: M is the oxygen molecular weight (32g/mol), F is the Faraday's constant (96485.34 C/mol), b is equal to 4 and it indicates the number of electrons exchanged per mole of oxygen, and Van is the anode chamber volume (500mL). The concentrations of ferrous, ferric, and total dissolved iron were recorded using a UV spectrophotometer. The bacterial growth was examined using the counting plate method.

2.4 Reagents

Sodium hydroxide (>93 %, Wako Co., Japan) and hydrochloric acid (35-37 %, Wako Co., Japan) were used for pH adjustment. Nitrogen gas was used for deoxygenating the deionized water through bubbling for 15 min before solutions could be prepared. Cl_3Fe and Cl_2Fe powder were directly added to the anode chambers of the studied MFCs.

2.5 Bacterial growth analysis

Our study focuses on using Mesophiles, which are middle-loving bacteria, growing at temperatures between 25 to 40oC. Also, most bacteria are best suited to neutral or slightly basic environments. We target the anaerobic bacteria cultured from real waste sludges, which are considered the powerhouse of microbial fuel cell technology. Bacterial growth rate was estimated by the plate count method using colony forming unit (CFU) technique. To do so, 1 mL from the original mixed culture inoculum was diluted 5 times in series to insure a countable plate. Then, 1 mL from each diluted sample was placed in the center of a Petri dish using a sterile pipette. The molten agar (15 mL) was poured into the Petri dish and mixed well. After solidification, the plate was saved for 24 hours overnight at 37 °C.
Figure 4 illustrates the different steps for bacterial growth counting and Figure 5 presents the melted agar and an example of a real Petri dish after 24 hours. Each growth colony was carefully counted and represented a CFU:

CFU = CFU × dilution f actor × 1/aliquot

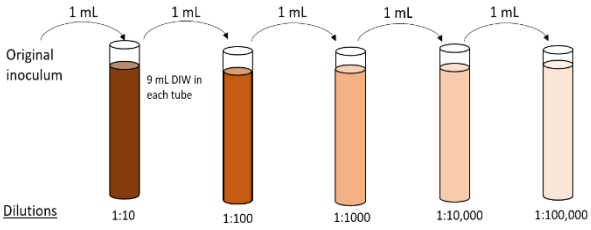

Fig. 4: Pour plate method for bacterial growth measurements.

Fig. 5: (A) Bacterial colonies in a Petri dish after 24 hours. (B) Prepared standard plate count agar (APHA) CM0463 (OXOID LTD., England).

3. RESULTS and DISCUSSION

3.1 Voltage generation

The MFC performance improved in time and the voltage output rapidly increased in MFCs supplemented with Fe(II), Fe(III), and Fe(II)/Fe(III) ions compared to the control MFC. Figure 6 illustrates the daily voltage evolution during 45 days of operation. Although the voltage was not stable all along with the experiments, it continuously increased in time. The voltage recorded was initially negligible (<0.1 mV, across an external resistance of 200 Ω). However, a steep increase of electricity production was observed after 7 days, which indicated successful colonization of the anode by electro-chemically active bacteria. A steep increase in electricity production was observed starting from day 1, and the voltage reached its maximum values at day 14 and day 18 for Fe(II) and control MFCs, respectively, suggesting that the addition of iron ions accelerated the start-up of the MFCs. This shortened start-up time could be attributed to the promotion of the bacterial adhesion to the anode material. The daily voltage increased, and maximum values were 31.6 mV (control), 57.8 mV (Fe2+), 67 mV (Fe3+), and 56.9 mV (Fe3+/Fe2+).

The accumulative cell voltages of all MFCs were recorded to be 38.01 %, 98.86 %, and 82.29 % for Fe2+, Fe3+, and Fe3+/Fe2+, respectively. The accumulative voltage increased continuously to reach 0.892 V (control), 1.231 V (Fe2+), 1.774 V (Fe3+), and 1.626 V (Fe3+/Fe2+). The maximal power output density increased by **77.42 %**, **70.00%** by using ferric and ferrous ions compared to control MFC, respectively. The mixed ferrous-ferric salts enhanced the maximal power output by **68.66 %** compared to control MFC.

3.2 Iron concnetration analysis

The iron concentrations in the anolyte are shown in Figure 7. In the beginning of the experiments, the initial Fe2+, Fe3+, and total iron concentrations were 21.68, 21.21, and 42.89 mg/L, respectively, at an initial pH equal to 7.17, 6.97, 7.00, and 6.46, respectively for control and iron ions supplemented MFCs. The ratio of Fe3+/Fe2+ was around 50 %, and it decreased in the end of the experiment, to be 90 % for control MFC, and 100 % for Fe3+-supplemented MFC. It was clear that most of the iron was in the form of Fe2+ after 45 days of operation in control and Fe3+-supplemented MFCs.

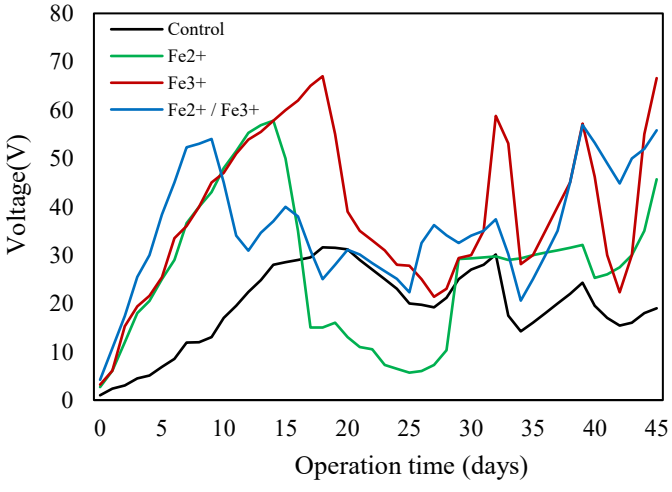

Fig. 6: Daily voltage generation in MFCs using Fe2+, Fe3+, and Fe3+/Fe2+ iron salts with 10 mg/L at the anode throughout the operation period.

Iron analysis showed that their Fe2+ concentrations were 12.44 and 17.81 mg/L, at pH values equal to 7.12 and 6.49 respectively. Therefore, the biological oxidation-reduction rate was good enough to sustain the produced current in the Fe3+-supplemented MFC. The Fe3+/Fe2+ ratio was always 50 % for the mixed Fe3+/Fe2+ ions supplemented MFCs, and the correspond iron ions concentrations at day 45 were 19.06, 18.15, and 37.21 mg/L for Fe2+, Fe3+, and total iron at pH values equal to 6.29. Besides, iron analysis showed that the Fe2+, Fe3+, and Fe3+/Fe2+ concentrations were 15.93, 8.58, and 24.51 mg/L, respectively at a pH value equal to 6.80 for Fe2+ supplemented MFC.

Iron analysis showed that iron ions concentration did not stay constant but decreased with time. The lower ratio Fe3+/Fe2+ during the end of the experiments for Fe3+-supplemented MFC may be caused by "the higher current density (3.697 A/cm^2) for which a higher reduction rate of ferric ions is needed. The iron oxidizers' limited activity resulted from higher Fe2+ concentrations, which can have an inhibitory effect on growth and oxidizing capacity. A prior study, where the iron reduction process was investigated in the cathode chamber of a scaled-up MFC, showed that iron ions precipitated at the end of the experiments. This observation was a reasonable explanation for the decrease in the iron ions. The authors inspected the obtained precipitates under microscope analysis, and they proved that these precipitates consisted of yellow-orange rod-shaped microorganisms combined with iron salts structures. The study suggested that the yellow precipitates were a combination of the iron oxidizers with iron salts. The study highlighted the risk of iron precipitation, which should be further discussed. Besides, the diffusion of iron ions through the membrane from the anode chamber to the cathode chamber could limit the availability of iron ions in the anolyte, which would further lower the iron ions concentrations.

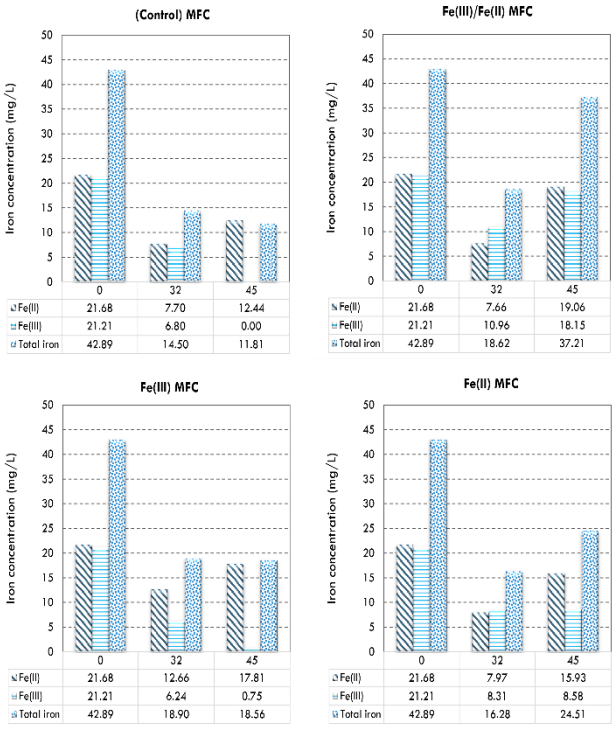

Fig. 7: Iron concentration in the anolyte for control and iron ions-supplemented MFCs throughout the days of operation.

3.3 Analysis of internal resistance

The measured cell voltage is lower than the theoretical MFC potential. A useful way to express the energy loss in the MFC system is to control the anode chamber's resistivity during the experiments. Figure 8 illustrates the anode resistance throughout the operation period. Results proved that the total resistance decreased from 1000 Ω.cm^2 to 699, 666, 815, and 782 Ω.cm for control, Fe2+ ,Fe3+, and Fe3+/Fe2+-supplemented MFCs, respectively. Measurements showed that the total resistance decreased by 30.1 %, 33.4 %, 18.5 %, and 21.8 % for control, Fe2+ , Fe3+ , and Fe3+/Fe2+-supplemented MFCs, respectively, resulting in improving the MFCs performance with time.

However, these results explained that the high internal resistance limits the amount of power generated in the studied MFCs. The growth of the electroactive biofilm could explain the high resistance. It contributed to the improved current generation but simultaneously increased the anolyte's resistivity. Results showed that iron ions stimulated the electrochemically active bacteria present in the mixed culture microbial community. The maximum values of colonies were 1.42 E+11 colonies/g.VS for both Fe2+ and c-supplemented MFCs, whereas the Fe3+/Fe2+-supplemented MFC showed an increase up to 1.83E+11 colony/g.VS. The bacterial growth increased by 92.18 % using Fe2+ and Fe3+ions compared to control MFC. The Fe3+/Fe2+ enhanced the bacterial growth by 177 % compared to control MFC.

These results demonstrated a superior influence of iron ions on the behavior and activity of bacterial communities. A recent study studied the effect of the presence of Fe3+ ions on Shewanella oneidensis MR-1 which are known as iron-reducing bacteria. Electrochemical analysis proved that the biofilm is vital for the protection against local corrosion might occur during the experiments. Also, the multiplication of microorganisms that can completely oxidize the organic matter are the primary contributors to power production.

3.5 Wastewater treatment and organic matter removal

The performance of the constructed MFCs was reflected in terms of COD removal efficiencies and total TVS degradation.

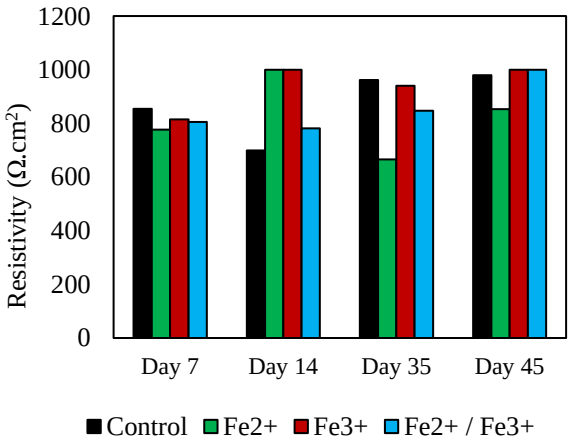

Fig. 8: Resistance of the anode chamber of MFCs using Fe2+, Fe3+, and Fe3+/Fe2+ iron salts with 10 mg/L throughout the operation period.

As shown in Figure 9, the control MFC presented a removal efficiency of 46.62 %, whereas Fe3+ supplemented MFC, Fe2+supplemented MFC, and the Fe3+/Fe2+ supplemented MFCs, the COD removal efficiencies were 78.49 %, 51.55 %, and 65.00 %, respectively at day 15. A decrease in the removal efficiency was observed at day 32, and then values increased at the end of the experiments. These variations in COD removal efficiencies could be attributed to the different phases of bacterial growth. An increase of bacterial colonies in the log phase would accelerate the organic matter degradation. However, a reduced number in the death phase will limit the digestion process, and thus COD values would remain high.

The high performance of Fe3+ supplemented MFC might be attributed to the effects of redox reactions inside the anode chamber. Also, Microbes would be more activated when exposed to iron ions, which enhanced the degradation of the organic matter. Table 4.4 shows the total volatile solids variations during the experiments period. TVS concentrations were reduced by 48.05 %, 52.78 %, and 36.67 % by Fe2+, Fe3+, and Fe3+/Fe2+ supplemented MFCs, respectively compared to 30.83 % in control MFC. These results highlight the promising effect of Fe3+ for promoting organic matter removal, enhancing bacterial growth, and increasing power generation in MFCs.

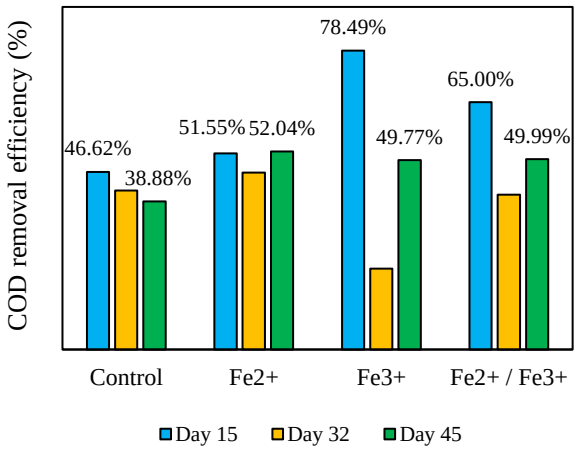

Fig. 9: COD removal using Fe^{2+} , Fe^{3+} , and Fe^{3+} / Fe^{2+} iron salts with 10 mg/L in the anode chamber throughout the operation period.

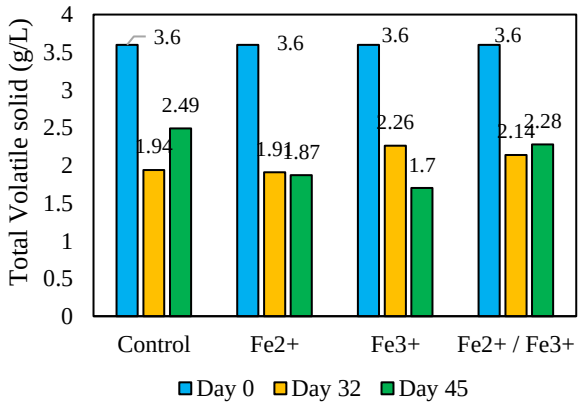

Fig. 10: TVS concentrations using Fe^{2+}, Fe^{3+}, and Fe^{3+} / Fe^{2+} iron salts with 10 mg/L in the anode chamber throughout the operation period.

4 CONCLUSION

In this study, Fe ions' addition exhibited a higher power output and a shorter start-up time. The addition of iron ions was favorable for the enrichment of bacteria that could reduce Fe(III) by oxidizing organic matters through anaerobic respiratory metabolism. The results correlated with power generation enhancement as a combination of bacterial growth increase, resistivity decrease, and organic matter degradation improvement. Fe(III) ions would favor the multiplication of bacterial colonies and the biofilm formation in the anode chamber, which resulted in high protection for bacteria against any corrosion effect. Besides, it will further promote organic matter degradation due to the strengthened activity and metabolic rate of electroactive bacteria, and power output would increase. However, a low removal efficiency could be obtained due to limitations in the electron donor's mass transfer to the microbes and the anodes. The amount of power generated in these MFCs was limited by the system's high internal resistance and the biological Fe(III) iron reduction rate that was high enough to limit the produced current. Therefore, suggested studies would be elaborated to 1. control the oxidation-reduction rate of iron ions in the anolyte, 2.improve the electron transfer rate, and 3. reduce the resistivity of both anodic and cathodic chambers.

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