A study on Zerovalent Iron Nanoparticles, Fe^<2+> and Fe^<3+> enhanced double chamber microbial fuel cells

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A study on Zerovalent Iron Nanoparticles, Fe^{2+} and Fe^{3+} enhanced double chamber microbial fuel cells

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Abstract: *Microbial fuel cells (MFCs) are a promising and rapidly evolving discipline, which is mainly applied to convert chemical energy of urban wastewater into electrical energy. Based on past research achievements, iron components (zerovalent iron nanoparticles, Fe^{2+} and Fe^{3+}) has positive enhancements on MFCs by increasing the activity of electroactive bacteria on anode chamber. In this study, the effect of zerovalent iron nanoparticles Fe^{2+}, Fe^{3+} and their combinations on a lab-scale double chamber MFCs was evaluated. Based on the results achieved, the best performance was 10 mg/L Fe^{2+} modified anolyte, which achieved accumulative voltage improvement of 216.8%. Furthermore, it was found that more than 20 mg/L of total iron components reduces the power generation of MFCs and this is deemed excessively high for this application. Lastly, a removal rate of 91.5% for Total Volatile Solids (TVS) was achieved after 30 days of operation.*

Keywords: Microbial Fuel Cells; Zerovalent Iron Nanoparticles; Wastewater Treatment; Renewable Energy

1. Introduction

As global energy consumption continues to rise significantly, studies on climate change unveil substantial and pressing concerns. As Fossil fuels show their limit and people are in need of an abundant, and renewable energy sourcesto support a more sustainable future . According to Japan Education Center of Environmental Sanitation, The average Japanese citizen produces 200 L wastewater containing 40 g organic matter (BOD) every day, which requires plenty of energy to purify in a traditional wastewater treatment plant. However, there is an abundance of energy hidden in organic matter which produces more than twice the energy required to run a wastewater treatment plant if put to good use. Microbial fuel cells (MFCs) are one of such solutions which generate electrical power and purify organic pollutants in wastewater by anaerobic respiration of electroactive bacteria. As a promising and rapidly evolving discipline, there are many solutions that help reduce the construction and maintenance cost of MFCs and improve their power production efficiency, which may be a comprehensive solution in solving our energy and environmental problems.

Iron is one of the most abundant metals in Earth's crust, which is considered an economically friendly material. Furthermore, , as iron is widely distributed in Earth's crust, there are many microorganisms that require this iron or can boost biological life, including electroactive microorganisms which play a vital role in microbial fuel cells by decomposing organic matter and releasing electrons and protons. It has been proven that Fe^{2+} and Fe^{3+} helps to enhance the microbial strategies for extracellular electron transfer (EET), such as cytochromes, electron shuttle or e-pili, of both gram-negative bacteria, gram-positive bacteria, and archaea, thus increase the power generation of microbial fuel cells [1]. Moreover,, nanoscale zero valent iron (novia) can also strongly optimize MFCs by providing an ideal conductor attachment for various electroactive microorganisms which enhances the direct interspecies electron transfer (DIET) [2] while releasing Fe^{2+} and Fe^{3+} at a constant rate thus increasing the energy generation of MFCs [3].

In this study, the effects of nZVI, Fe^{2+} and Fe^{3+} on MFCs is evaluated by analyzing the daily voltage, accumulative voltage, and power density per gram of total volatile solid (P_{gTVS}). Moreover, the performance mechanism of the iron component in MFCs is thoroughly discussed, leading to a comprehensive conclusion.

2. Materials and Methods

2.1 Reagents

Sodium borohydride (>99.0%, Junsei Chemical Co., Japan), ferric chloride hexahydrate (>99.0%, Junsei Chemical Co., Japan), ferrous chloride (98%, Sigma-aldrich Inc., USA), ferric chloride (97%, Sigma-aldrich Inc., USA). Chemical oxygen demand (COD), total iron and total ferrous are measured by HACH DR3900.

2.2 nZVI synthesis

nZVI is synthesized by the following equation:

$$4Fe^{3+}+3BH_4^-+9H_2O \rightarrow 4Fe\downarrow +3H_2BO_3^-+12H^++6H_2\uparrow$$

Sodium borohydride was added to ferric chloride by peristaltic pump under 20 mL/min. The synthesis reaction was under anaerobic environment (achieved by continuous nitrogen supplement), 400 RPM vigorous mixing and 30 ℃ water bath. nZVI was then separated by vacuum filtration [4].

2.3 Sludge preparation

The sludge used as an anolyte was collected from the Mikasagawa Domestic Wastewater Purification Center (Fukuoka, Japan). Sludge was stored in an anaerobic environment at 4 ℃ prior to use. Measuring the Total volatile solids (TVS) was chosen as the method of analysis as it demonstrates more accurate sludge nutrient content consumed by bacteria compared to evaluating the chemical oxygen demand (COD). Additionally, This experiment used Fe^{2+} and Fe^{3+} to modify the anolyte [5]. Therefore, the initial iron concentrations of sludge were measured, and the result was low compared to 10 mg/L

Fe^{2+} or Fe^{3+} modification, therefore does not interfere with the final results. The parameters of sludge are shown in Table 1 and fig.1:

Table 1
Initial sludge parameters

Parameter	Value
COD (mg/L)	5270
pH	7.55
TS (mg/L)	3753
TVS (mg/L)	3417
TVS/TS	0.91

COD: chemical oxygen demand (mg/L), TS: total solid (mg/L), TVS: total volatile solid (mg/L).

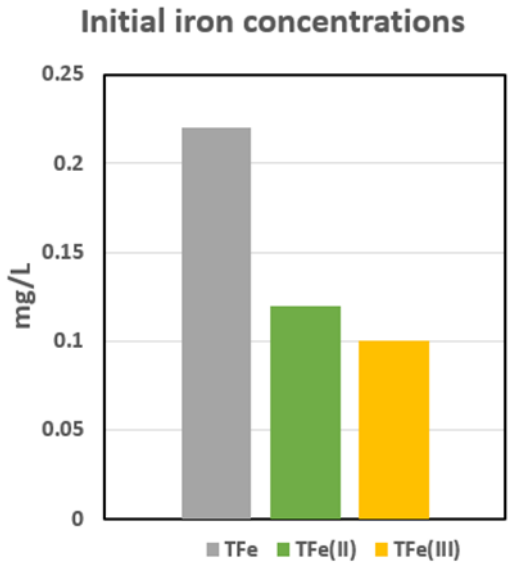


Fig.1 Initial iron concentrations. TFe: 0.22 mg/L, TFe^{2+}: 0.12 mg/L, TFe^{3+}: 0.10 mg/L.

2.4 MFCs preparation

The membrane of the double chamber MFCs was composed of a Nafion 117 proton exchange membrane. Both anode and cathode were carbon felt. MFCs were functioning in an anaerobic environment by introducing N_2 during operation. Magnetic stirrers were used to provide uniform temperature and maintain anolyte homogeneous. The parameters of MFCs are shown in Table 2:

Table 2
MFCs parameters:

Parameter	Value
Anode chamber volume (mL)	500
Cathode chamber volume (mL)	500
Electrode surface area (cm^2)	24
External resistance (Ω)	200
Operation temperature (°C)	40
Operation rotating speed (rpm)	1000

2.5 P_{gTVS} calculation

Different MFC experiments adopt other external resistance and scale (chamber size from several mL to hundreds of L). The voltage can show electric generation results clearly in one experiment. But to compare one experiment with another, power density per gram total volatile solid (P_{gTVS}), which based on both voltage, external resistance and anolyte nutrients content is necessary. P_{gTVS} can be calculated by the following equation:

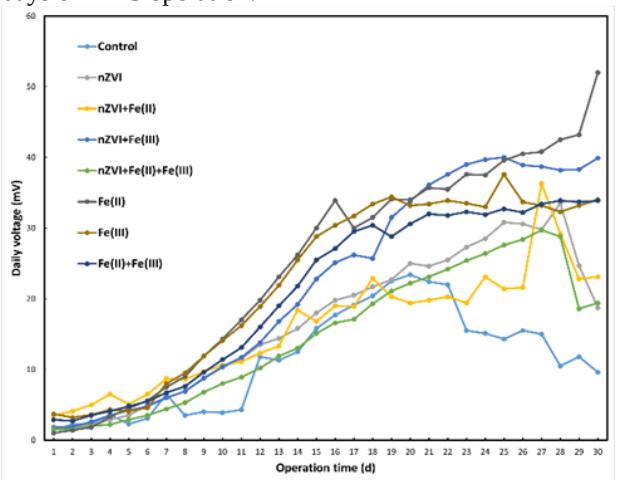
$$P_{gTVS} = \frac{U^2}{R \times V \times TVS}$$

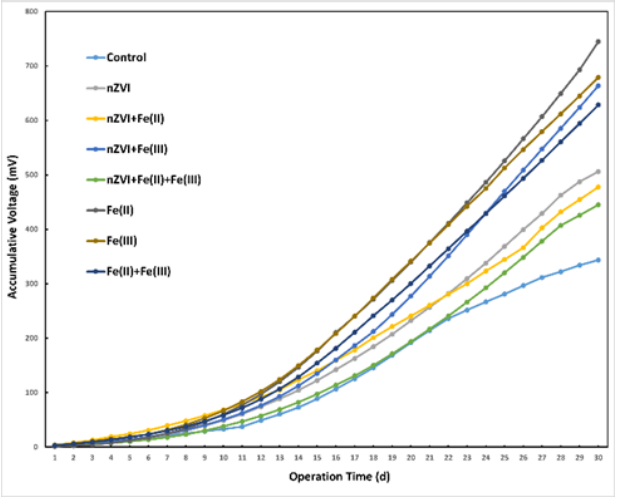
P_{gTVS}: power density per gram total volatile solid (W/g), U: daily voltage (V), R: external resistance (Ω), TVS: initial anolyte total volatile solid (g/L), V: anolyte volume (L).

3. Results and Discussion

3.1 Electric generation

Anolyte of MFCs was modified by 10 mg/L nZVI, 10 mg/L Fe^{2+}, 10 mg/L Fe^{3+} and their combination. The following data was recorded for daily voltage, accumulated voltage and P_{gTVS} cumulative during 30 days of MFC operation.





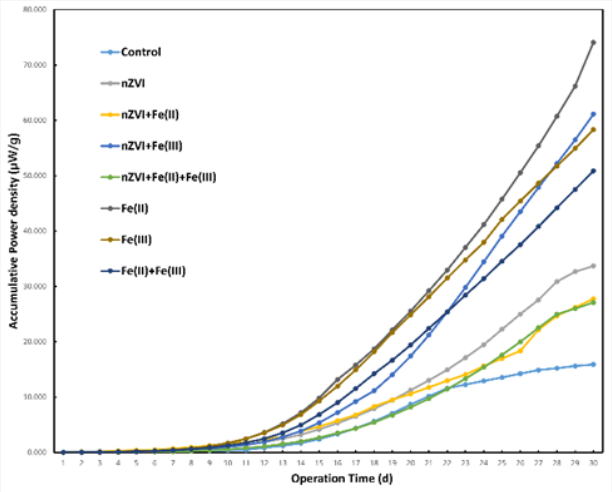


Fig.2 (A) daily voltage; (B) accumulative voltage; (C) accumulative power density.

MFCs generate electric power through the metabolic activity of special kinds of bacteria called electroactive bacteria. Some of these bacteria decompose organic matters and release protons and electrons on the anode chamber. Protons will pass through proton exchange membrane to the cathode chamber in MFCs reaction circle while electrons will pass through an external wire (and external resistance) to the cathode chamber and react with the protons and oxygen molecules on the cathode side. Electric power is generated by the movement of these electrons. It is evidentthat the greater the presence of active electroactive bacteria, along with a higher ratio, results in the increased release of protons and electrons, consequently leading to a higher electricity generation.. Usually, it takes time for electroactive bacteria to reproduced to considerable numbers after putted them into the MFC device, which is an ideal habitat for electroactive bacteria. In fig.2 (A), the daily voltage of MFCs was low in the first 7 days due to the low total group number of electroactive bacteria. The voltage then increased in the following 7 days due to the rapid breed of electroactive bacteria and remained relatively constant as the number of bacteria achieved the environmental capacity of the tiny ecosystem within the anode chamber. In the last few days of operation, the voltage became unstable and decreasing significantly due the depletion of nutrients at the anolyte was running out and no longer an ideal habitat for electroactive bacteria. As the supply of organic matters decreased, the anode chamber released less electrons and reduced the power generation of within the MFC.

Raising the quantity of organic matter in the anolyte sourced from urban wastewater poses a challenged, although, after decades of research, scientist have found various methods to enhance the activity of electroactive bacteria through other means. For example, researchers have found that electroactive bacteria release electrons through a special mechanism of energy-conserving respiration called extracellular electron transfer (EET).

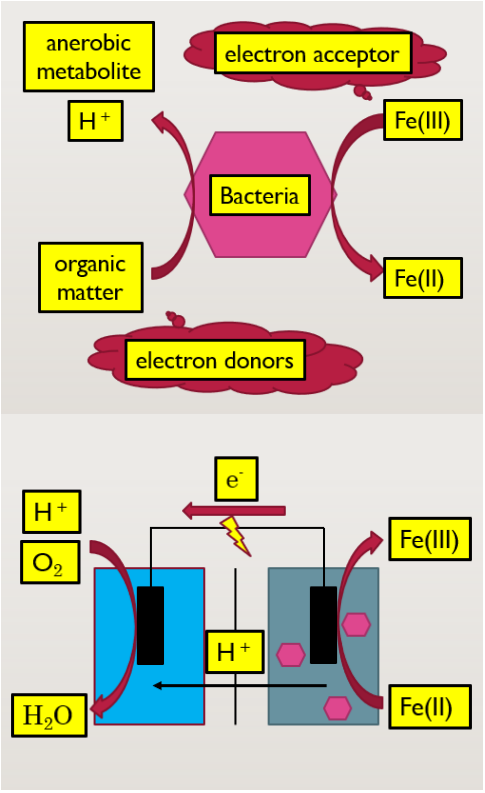


Fig.3 Mechanism of Fe^{2+}, Fe^{3+} enhancement on MFCs. (A) by extracellular electron transfer (EET) of bacteria (B) by electron transport mediator of MFCs anode chamber.

In fact, electroactive bacteria consume organic matters by anaerobic respiration and release anerobic metabolites, protons, and electrons, but this process happens inside their cell. The release of electrons is not achieved by anaerobic respiration only, but also by extracellular electron transfer (EET) which transfers electrons from inside the cell to the outside. Electroactive bacteria have various ways of acheiving EET: by using the cytochrome as a passage that allows the electrons to reduce ferric or ferric oxide outside the cell, by using ferric chelation to carry ferric inside the cell and absorb electrons, or by using electron shuttle which transports electrons outside and react with ferric. In most cases, iron ion or oxide plays a significant role as the extracellular electron acceptor in EET. This is because iron ions are easily converted between divalent and trivalent (ferrous and ferric), which means they receive and lose electrons, respectively.. Furthermore, electroactive bacteria are anaerobic creatures which live beneath the surface. Such layers contain an abundance of iron reserves that can be used for EET (in some situations, manganese can be a possible alternative for iron).

Extracellular is not the final destination of electron's movement in an MFC operation circle. As fig.2 (B) suggests, electrons need to reach anode, go through the external wire to the cathode, react with the oxygen and protons that came from the anode chamber and finally, generate water all within a single MFC operation circle. That means electrons should go from the extracellular space to the anode. Iron ions also play an important part as electron transport intermediate in this procedure. On EET stage, ferric ions plays a crucial role in facilitating the help transport of electrons from within the cell to the external environment while undergoing a reduction into

ferrous ions. In electron intermediate stage, as the electric potential is different between anode and cathode, ferrous ions will lose electrons at the surface of the anode and become oxidized to ferric ions once again. Following this step, the electrons then pass through the external wire and generate electric power while ferric ions will work for EET again.

In conclusion, iron ions can enhance the MFCs power generation by playing as electron acceptor in EET and electron transport intermediate. As fig.1 shows, the initial total iron of urban wastewater sludge is 0.22 mg/L. Fig.2 (B) illustrates the control scenarioin which urban wastewater sludge is directly used, resulting in the lower power generation when compared to other MFCs that have been modified with iron components. Therefore, 0.22 mg/L total iron can be considered a low dosage while further optimization might be necessary. The modification which achieved acumulative voltage improvement of 216.8% was by adding 10 mg/L Fe^{2+} to the anode chamber. 10 mg/L Fe^{3+} modified MFCs also showed good result next to Fe^{2+}. This is because 10 mg/L total iron may be the closest dose if we compare it to 10 mg/L Fe^{2+} and additionally,10 mg/L Fe^{3+} modified MFCs (with total iron of 20 mg/L) showed the lowest power generation. Both Fe^{2+} and Fe^{3+} can enhance the performance of MFCs. However, Fe^{2+} is more effective at releasing electrons at the anode from the outset, whereas Fe^{3+} relies on obtaining electrons from bacteria before releasing them at the anode. The effect between Fe^{2+} and Fe^{3+} is not significant. Therefore, if we consider the cost, 10 mg/L Fe^{3+} may be the better alternative. An iron dosage of 20 mg/L can be regarded as high resulting in reduced power generation and less favorable economic benefits compared to an iron dosage of 10 mg/L. Total iron of 30 mg/L (if nZVI can be regarded as total iron) showed low power generation but better than the control scenario.Thus, high dosage of Iron modifications should avoided.

Another modification method besides iron ions is the use of nZVI. nZVI is a highly active nanoparticles which decomposes and releases iron ions and electrons at a constant rate . Both iron ions and released electrons in the anode chamber can enhance the MFCs efficiency. Also, nZVI can provide an ideal conductive base for bacteria to generate biofilm due to its wide surface area, however,when it comes to power generation, nZVI and its combination with iron ions exhibited lower power generation than modifications with iron ions alone. One possible explanation could be an improper dose: The concentration of total iron ions is consistently below 10 mg/L when using 10 mg/L of nZVI alone and above 10 mg/L when using 10 mg/L of nZVI in combination with 10 mg/L of ferric or ferrous ions. For this reason, the optimized dose of iron ion modification should be very close to 10 mg/L. Another potential reason of low power generation of nZVI is the nZVI enhanced direct interspecies electron transfer (DIET) between electroactive bacteria and methanogen (fig.4). Methanogen consumes electrons thus causing a decrease in power generation of MFCs.

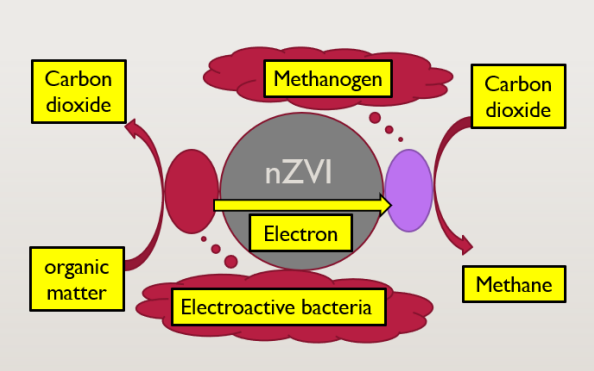


Fig.4 Mechanism of nZVI enhanced direct interspecies electron transfer (DIET)

nZVI provids an ideal habitat for electroactive bacteria which works in favour ofMFCs, but not only for electroactive bacteria. There are anaerobic bacteria called methanogen, which absorb electrons and converts CO_2 to methane. This process consumes electrons from the anode chamber and decreases the efficiency of the MFC. With the existence of nZVI, the electrons released by electroactive bacteria are transported by this conductive nanoparticle to methanogen in this very efficient way of DIET [6]. Furthermore, nZVI itself can release electrons which is ideal for methanogenesis and increases their number of species. In fact, nZVI is a proven material to enhance anaerobic digestion, a wastewater treatment technology which convert organic matters to methane by means of methanogens and other bacteria. Although the electric energy generation of nZVI modified MFC was lower than iron ions, the chemical energy generation (methane) should be higher. It is feasible to harvest the gas produced in the anode chamber as a byproduct when the methane concentration reaches a sufficient level. Concequently, nZVI-modified MFCs remains a promising area for further research.

3.2 TVS removal

After 30 days of operation, TVS removal of MFCs was concluded as follow:

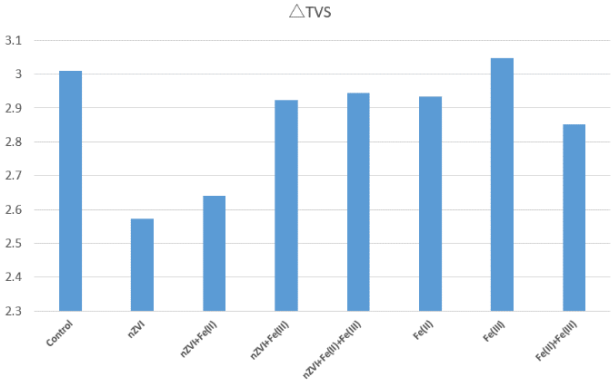


Fig.5 TVS removal of MFCs

MFCs is a wastewater treatment method after all. We cannot discard wastewater with a high total volatile solid (TVS) concentration, otherwise bacteria and algae will overpopulate and create a separate environment concern. Any wastewater treatment method that includes MFCs should provide satisfactory TVS removal efficiency.

In this study, 10 mg/L Fe^{3+} modified MFCs achieved the highest TVS removal of 3.047 g/L, which is 91.5% of the initial TVS. That indicates that the bacteria were highly

active, resulting in the production of the cleanest purified wastewater. These results are also reflected in electric generation. Fe^{3+} modified MFCs achieved near best power generation. In theory, Fe^{2+} modified MFCs attain higher power generation by following a mechanism that is not primarily reliant on bacteria. However, it is worth noting that they exhibit lower initial EET efficiency.

10 mg/L nZVI modified MFC achieved lowest TVS removal. One potential reason is that the existence of nZVI changed the bacteria species number within the MFC, which may produce different TVS removal result. Another possible explanation is the initial iron ions of nZVI modified MFCs was low and do not have enough enhancement effect towards the electroactive bacteria. The combination of nZVI and iron ions showes better result than nZVI on its own.

4. Future Works

4.1 iron ions modification

In this study, the results show that 10 mg/L Fe^{2+} or Fe^{3+} modified MFCs represent the closest and most optimized dosage. 20 mg/L of total iron should be considered too high. Further research should be undertaken to determine the optimal dose between the range of 0 mg/L and 20 mg/L. Also, Fe^{2+} or Fe^{3+} modified MFCs had a negligible difference in performance. If we take cost and TVS removal into consideration, we can say Fe^{3+} modified MFCs is the best alternative. However, there are ideas for new combinations or materials that we have not explored yet. For example, initial ferric ions can enhance EET which increases the activity of electroactive bacteria, while initial ferrous ions can release electrons and enhance the electron transport from the EET stage to the anode. The combination of ferric and ferrous may be a better candidate then using these materials separately. The results for the combination in this study showed unsatisfactory result which may be due to the high dosage and not the singular use of ferric or ferrous. An alternative approach is to consider using ferric oxide or ferric hydroxide instead of ferric chloride hexahydrate to reduce expenses. Although ferric chloride is cheaper than ferrous chloride and the optimal dose should not be incredibly significant, it is still possible to increase the economic benefit. In fact, there is no ferric chloride in the environment of electroactive bacteria because it is not stable and is easily converted to ferric oxide and ferric hydroxide (even in the anode chamber during the experimental process). Some EET mechanisms can use such oxide or hydroxide directly. Ferric hydroxide comes at a lower cost than ferric chloride, and ferric oxide is even more economical. It is worthwhile to experiment with these options before concluding that ferric chloride is the most optimal choice.

4.2 nZVI modification

nZVI showed lower power generation than iron ions in this study but consequently, optimizes the MFCs.. nZVI can enhance DIET between electroactive bacteria and methanogens thus decrease power generation. There are two potential solutions for this problem: to eliminate methanogens or accept and symbiosis. Try to make an anolyte environment that is challenging for methanogens to survive is a feasible solution. Methanogens are good at adapting to extreme environment (like high pressure environments or boiling hot springs) and bad at competing with other bacteria in relatively good environment (especially aerobic environment). According to the results from the follow-up study, although electroactive bacteria is also an anaerobic bacteria, expose to air for an extended period of time before its use may increase the power generation of MFCs because methanogens are more sensitive to oxygen than almost any other anaerobic bacteria. Another perspective involves converting methane, produced by methanogens, back to carbon dioxide and electrons using a chemical method. Conversely, to accept the symbiotic relationship between electroactive bacteria and methanogens might be a more favourable approach. If electroactive bacteria release 100 units of electrons and methanogens accept 50 units, it indicates that both anaerobic digestion and MFCs are not ideal wastewater treatment technologies. Anaerobic digestion will waste 50 units of electrons released by electroactive bacteria while MFCs will waste 50 units of electrons adsorbed by methanogen (and generate strong greenhouse exhaust gas if not collected). Combination of these two technologies, collect both methane and produce electrical benefits while nZVI can be the bridge between these two technologies.

The optimal dose of nZVI is near 10 mg/L according to the previous research, but total iron concentrations can be regarded as a highly sensitive interference in the anode chamber of the MFCs.. Furthermore, adding initial iron ions can benefit nZVI modified MFC.. Considering that the optimal dose of iron ions is around 10 mg/L and 10 mg/L of nZVI consistently releases iron ions, this study suggests that the optimal dose of initial iron ions should be closer to 5 mg/L, rather than 10 mg/L

nZVI is a proven nanomaterial for the removal of various containments such as nitrite, phosphorus, chromium, or antibiotics due to its high activity of adsorption and oxidation. Existing wastewater treatment technologies can remove ordinary containments like TVS, nitrite and phosphorus, but meets certain challenges when dealing with contaminants of emerging concern that have only been recognized as harmful in recent years like chromium and various antibiotics. This is a promiment and important subject in the field of environmental engineering, and should be a focus point in any research related to wastewater treatment, with particular emphasis on nZVI research [7-18].

5. Conclusion

This study comprehensively explored the Performance of nZVI, Fe^{2+} and Fe^{3+} enhanced MFCs. MFCs modified with10 mg/L Fe^{2+} exhibited the most significant increase in the accumulative voltage improvement, amounting to an improvement of 216.8%. However, when taking into account both cost and TVS removal, 10 mg/L Fe^{3+} modified MFCs could serve as a viable and cost-effective alternative.. Iron ions can enhance MFCs by improving EET of electroactive bacteria and play as an intermediate electron transporter in the anode chamber. The dose of iron ions is highly sensitive, the optimal dose should be near 10 mg/L but less than 20 mg/L. nZVI modified MFCs achieved the lowerest power generation due to improper dose and enhancement of DIET between electroactive bacteria and methanogens. This study

effectively addresses future prospects. The practicality of MFCs remains a considerable challenge due to their low power generation and high construction costs. This research, however, aims to contribute as a modest yet meaningful step forward in the ever-evolving field.

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