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Nitrite pre-treatment of dewatered sludge for microbial fuel cell application

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ABSTRACT

The effect of pre-treatment of dewatered sludge using different nitrite concentrations and pH for microbial fuel cell (MFC) application was investigated. The results show that the addition of nitrite was feasible to increase the solubilization rate of the sludge and may reduce mass transfer limitation at the anode. This helped the MFC to reach higher voltage and to generate more power. The higher free nitrous acid (FNA) concentration under the acidic condition helped to increase sludge solubilization. However, under an alkaline condition, during which the FNA concentration was relatively low, the solubilization of the sludge was higher. The highest voltage and power density produced was 390 mV and 153 mW/m^2, respectively, with the addition of nitrite at 100 mg-N/L and pH 9. Furthermore, it was found that elevated levels of FNA could inhibit electrogenic bacteria thus reducing power generation.

Introduction

The generation of waste biomass, especially from wastewater treatment plants (WTPs), has become a problem for the environment and for society. Engineers and administrators are concerned with the intense energy consumption involved in the treatment of biosolid waste/dewatered sludge from WTPs (Biradar et al., 2010); while at the same time, solid waste has the potential to be used as a source of renewable energy. The application of solid waste as a source of renewable energy could alleviate problems such as the scarcity of energy sources, environmental impacts from open dumping of solid waste, and greenhouse gas emissions. As a result, many researchers have studied ways to utilize solid waste and convert it to a renewable energy resource. One among the technologies that have been developed recently for this purpose is the microbial fuel cell (MFC) (Meng et al., 2017).

The MFC is a promising technology that could simultaneously address the issues of treating organic pollutants and producing electricity using electrogenic bacteria and organic matter as catalyst and substrate, respectively (Youn et al., 2015). MFC is unique compared to other types of fuel cells because it needs no metal catalyst at the anode. Instead of a metal catalyst, electrogenic bacteria covering the anode act as the biocatalyst for the fuel cell and oxidize the organic substrate. Electrogenic type bacteria have the capability of reducing iron by accepting electrons in the absence of oxygen and can produce electricity in the MFC. Any source of biodegradable organic matter has the potential to be used as the substrate for generating electricity in a MFC. This includes simple molecules of organic matter such as glucose and acetate, larger molecules such as proteins, and complex mixtures of organic matter such as those found in domestic wastewater, food waste, and waste sludge (Logan, 2009).

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Solid substrates such as dewatered sludge have higher organic matter concentrations and density compared to liquid substrates. These can be used as substrates for solid phase MFCs; however, there are problems concerning the utilization of high-solid sludge as a carbon source for MFCs. Although solid waste potentially contains higher energy density compared to a liquid substrate, the electricity production from MFCs using solid substrate has been quite low compared to MFCs using a liquid substrate (Lee and Nirmalakhandan, 2011). The limitation of a solid substrate such as dewatered sludge is that the solid organic particulate matter needs to undergo hydrolysis before it can be consumed by the electrogenic bacteria in MFC. As a consequence, the content of soluble organic matter in the solid waste is quite low. In anaerobic processes, hydrolysis is the rate-limiting process and increase in the rate of hydrolysis would increase the rate of anaerobic digestion, thus producing more readily available substrates for use by the electrogenic bacteria.

A variety of pre-treatment techniques including mechanical, thermal, and chemical treatments have been studied to accelerate the fermentation treatment of sludge (Wu et al., 2014). Pre-treatment of sludge can be done to cause cell lysis and disintegration of cell walls. This allows the intracellular material to solubilize to liquid phase and thus enhance the transformation of refractory material into biodegradable substances (Appels et al., 2008). Nitrite pre-treatment is one of the chemical treatments that can be used to hydrolyze the particulate material in sludge to more soluble organic matter. Nitrite pre-treatment of sludge could break down the sludge particles and disperse the extracellular polymeric substance (EPS) in its soluble phase (Huang et al., 2015a). Thus, nitrite has the potential to be used for chemical pre-treatment of dewatered sludge for MFC application. Further, nitrite is produced during the nitrification process as the intermediate product in biological nitrogen removal of wastewater. Normally nitrite will undergo the denitrification step to completely remove the nitrogenous compound from the wastewater. Therefore, utilizing nitrite for the pre-treatment of sludge not only could potentially increase the hydrolysis of the sludge but also could reduce the need for denitrification process (Wang et al., 2013). This offers considerable savings in the demand for carbon sources during the denitrification step in biological nitrogen removal from wastewater. Hence, in this study, dewatered sludge from a WTP was used as the organic substrate in the MFC. The sludge was pre-treated with nitrite and the performance of the MFC using pre-treated sludge was evaluated.

1. Materials and methods

1.1. *Dewatered sludge*

Dewatered waste sludge collected from the Juru Indah Water Konsortium (IWK) wastewater treatment plant was used as the substrate for the operation of an air-cathode MFC. The sludge was kept at 4 ± 1°C. The solid waste biomass had total solid content of 0.21 ± 0.01 g/g sludge and volatile solid content of 0.14 ± 0.01 g/g sludge, with pH 7.0 ± 0.5, and ammonium content of 77.6 ± 0.5 mg NH_4-N/L. Before being used in the MFC, the sludge was allowed to reach room temperature. The raw dewatered sludge had moisture content around 77% ± 2% and deionized water was added to the sludge to maintain the moisture content at 82% for all the MFCs.

1.2. *MFC construction and operation*

The single chambered air-cathode MFCs were fabricated using a glass material (diameter 8 cm; height 9.5 cm). Each MFC was operated without a membrane separating the cathode and anode (a schematic of the MFC is shown in Appendix A). The presence of a membrane in the MFC could lower the power density of the fuel cell because the membrane might limit the mass transfer of protons from the anode to the cathode (Tanikkul and Pisutpaisal, 2015). Furthermore, single-chamber membrane-less MFCs are cheaper and easier to construct and handle than are MFCs with membranes. Non-catalyzed graphite felt (5.0 × 5.0 cm; 0.3 cm thick) was used as electrodes. The electrodes were washed with 1 mol/L HCl followed by 1 mol/L NaOH, each for 24 hr, to remove biomass and metal contamination before use. Titanium wire, (diameter 0.81 mm, 99.7% trace metals basis, ALDRICH) was woven and connected to the anode and cathode to complete the circuit. The wire from the anode was sealed with epoxy and insulator tape to avoid short-circuits in the MFC. The top portion of the cathode was exposed to air to allow the oxygen reduction reaction (ORR) to occur. Atmospheric oxygen acted as the electron acceptor at the cathode in this MFC.

Each chamber of a MFC was filled with 200 g of dewatered sludge under a variety of pre-treatment conditions. Fifty grams of sludge was used at the anode, while the rest (150 g) of the sludge was placed between the electrodes to separate the cathode and anode. In the first study, pre-treatment of dewatered sludge was done with nitrite at concentrations of 0, 50, 100, 150, and 200 mg NO_2-N/L. Sodium nitrite ($NaNO_2$) was used as a source of nitrite for the sludge pre-treatment. The sludge was mixed thoroughly with the various concentrations of nitrite solution in a beaker before being used as the substrate in a MFC. The sludge used in this study was around pH 7. The MFCs were operated without any pH adjustment. The MFCs were allowed to run for 14 days. During the operation, sludge samples were carefully taken around the anode section. The samples were taken on days 0, 3, 7, and 14 of operation. The samples were then analyzed for soluble chemical oxygen demand (SCOD), protein, and carbohydrate. All MFCs were operated inside an incubator chamber at 35 ± 1°C to maintain a constant temperature. During MFC operation, a resistor (1000 Ω) was connected to each MFC as an external load. A digital multimeter (UNI-T UT33D) was used to check the voltage production every 6 hr during the entire MFC operation. The formula used to determine the concentration of free nitrous acid (FNA) is as below (Anthonisen et al., 1976):

$$\mathrm{FNA} = S_{\mathrm{NO2^-{-}N}}/\left(K_a \times 10^{\mathrm{pH}}\right), \text{where } K_a = e^{(-2300/(T+273))}, \text{for a temperature } T\ (^\circ\mathrm{C})$$

For the second study, to determine the effect of FNA, the pH of the dewatered sludge was varied between pH 5 and 9 with amounts of nitrite fixed at 100 mg-N/L. The pH was controlled using 1 mol/L HCl and NaOH. The other conditions of the MFCs used for the second study were similar to those in the first study.

The MFCs were operated for 14 days and samples were taken at the same time interval as in the previous study for soluble organic content analysis.

1.3. Analytical measurements and process monitoring

Total solid (TS) was determined by drying the sludge at 105°C inside an oven for 24 hr. The concentration of volatile solids (VS) was determined by combustion of dry sludge in a furnace at 550°C for 30 min. Liquid samples were extracted from the sludge by diluting the sludge with distilled water (1:10, W/V) and mixing it to homogeneity in a vortex (Meng et al., 2017; Yu et al., 2015). The sample was centrifuged at 14,000 r/min and then filtered through a 0.22 μm membrane for further analysis. Samples from each MFC were taken to measure the nitrite, SCOD, carbohydrate, protein, and ammonium-nitrogen. All analysis was carried out according to standard methods (APHA, 2012).

Polarization curves were done to determine the maximum power density of the MFC by adjusting the external resistance from 270,000 to 2.2 Ω after the MFC reached stable voltage. A voltage reading was taken each time the external resistance was changed from high external resistance to low external resistance. The power of the MFC was determined based on Ohm's law: $R = \frac{V}{I}, P = IV$.The internal resistance (R_{int}) of the MFC was determined from the peak of power density curve as well as the slope generated from the polarization curve. The internal resistance of the cell was determined using the following equation for the slope of the polarization curve, $R_{int} = \Delta V_0/\Delta I$.

2. Results and discussion

2.1. Effect of nitrite addition on the concentration of SCOD

The changes in concentration of SCOD within the MFCs with different nitrite concentrations are shown in Fig. 1. The SCOD concentration represents the amount of soluble organic matter within the sludge. The trend of SCOD concentration was almost the same for all the MFCs within the test period of 14 days. It could be observed in Fig. 1 that the concentration of SCOD in the sludge with added nitrite, increased rapidly until day 3, and then started to decline until day 14. At the start of the experiment, the concentration of SCOD was around 150 mg/L. The greater the amount of nitrite added to the sludge, the higher concentration of SCOD was recorded at day 3. Within three days, the concentration of SCOD increased to 170, 270, 340, 360, and 410 mg/L with the addition of nitrite at 0, 50, 100, 150, and 200 mg-N/L, respectively. The addition of nitrite helped the solubilization of particulate matter, as evidenced by the increase of SCOD concentration on day 3 when more nitrite was added to the dewatered sludge. The recorded concentration of SCOD at day 14 was 170, 202, 216, and 260 mg/L with the addition of nitrite at 50, 100, 150, and 200 mg-N/L. The concentration of SCOD in the control sludge did not show much variation in the 14 days: the concentration of SCOD was around 140 ± 10 mg/L throughout the whole experiment.

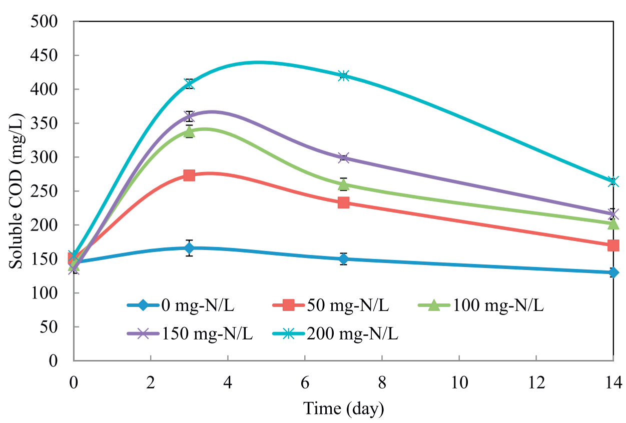

Fig. 1 – The concentration of soluble chemical oxygen demand (SCOD) with different concentration of nitrite pre-treatment during microbial fuel cell (MFC) operation.

It should be noted that both hydrolysis and consumption of organic matter occurred simultaneously in all of the MFCs. During the hydrolysis process, the insoluble and complex particulate matter within the sludge was converted into simpler and more soluble molecules. The hydrolysis of the sludge was proven by the increase in the SCOD concentration compared to the initial SCOD concentration. On the other hand, the presence of voltage or current during the operation of MFCs from the start to the end of the experiment confirmed that consumption of organic matter occurred within the sludge. This is because, to generate electricity, the electrogenic bacteria need to consume organic substrate as their carbon source. In the biofilm around the anode, the soluble organic substrate was oxidized by the electrogenic bacteria to mobilize electrons and protons. The electrons from the bacterial cells were then transferred to the anode, which acts as the electron acceptor under anaerobic conditions. The electrons move through the external circuit from the anode to the cathode and this generates current in the MFC. Simultaneously, the protons cross the sludge from the anode to the cathode. The electrons and protons then react with the oxygen at the cathode to form water.

During the initial phase, the rate of hydrolysis was higher than the rate of consumption for the sludge that was pre-treated with nitrite. The higher rate of hydrolysis caused the accumulation of soluble organic matter until day 3. However, after day 3, the concentration of SCOD started to decline. The consumption of organic substrate by the electrogenic bacteria and other processes (such as acidogenesis and acetogenesis) probably increased the consumption rate, compared to the hydrolysis rate alone, within the MFCs after day 3.

The particulate matter from the dewatered sludge was mainly composed of bacterial cells and EPS, which is mainly of polysaccharides and proteins. The amount of EPS released contributed to the higher SCOD content for the pre-treated sludge. This is because soluble EPS makes up a portion of the total SCOD. Fig. 2 shows the concentration of soluble EPS within the dewatered sludge at day 3. The concentration of soluble EPS shows the same trend as the concentration of SCOD under different nitrite concentrations. The amount of soluble EPS released was greater as more nitrite was added to the dewatered sludge. On day 3, the EPS concentration in the soluble phase was 145, 153, 178, 237, and 252 mg/L with the addition of 0, 50, 100, 150, and 150 mg-N/L of nitrite, respectively.

The higher the concentration of nitrite that was added, the longer it took for the nitrite to deplete completely. Nitrite was depleted from the MFCs after 1, 1.3, 2, and 2.4 days given initial

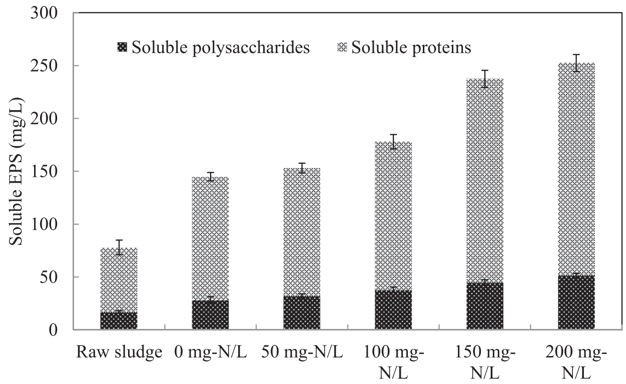

Fig. 2 – The concentration of soluble polysaccharides, proteins and, soluble extracellular polymeric substance (EPS) into the liquid phase with different nitrite dosage after 72 hr.

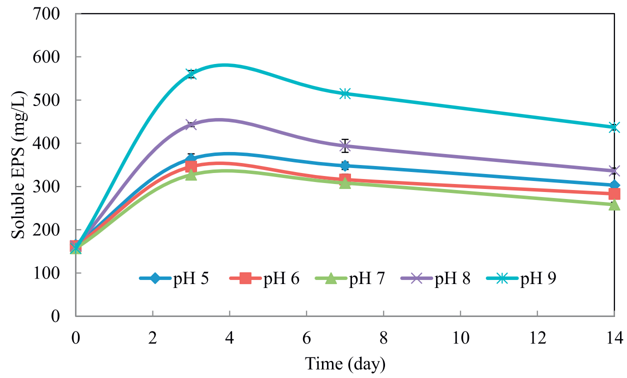

Fig. 3 – The concentration of soluble chemical oxygen demand (SCOD) with nitrite pre-treatment fixed at 100 mg-N/L and different pH during MFCs operation.

nitrite concentration of 50, 100, 150, and 200 mg-N/L, respectively (shown in Appendix A). The depletion of nitrite could be related to the hydrolysis rate within the sludge, which was increased by the presence of nitrite. Beyond the depletion of nitrite, the amount of SCOD continually decreased. This result further proved that the presence of nitrite did play an important role in solubilizing the sludge within the MFC. Commonly, there are two mechanisms to remove nitrite: nitrification and denitrification. Nitrite oxidizing bacteria (NOB) are the primary organisms responsible for the conversion of nitrite to nitrate in the nitrification process. In nitrification, oxygen is needed by the NOB to oxidize nitrite to nitrate. In denitrification, nitrite becomes the electron acceptor and the carbon source present in the sludge will become the electron donor for the denitrifying bacteria. In the MFC, the anode part is devoid of oxygen and this factor rules out the nitrifying process as the cause of nitrite depletion in the MFC. The depletion of nitrite that was detected in this study showed that denitrification may occur in the anaerobic zone of the MFCs, to reduce nitrite and release nitrogen gas.

Although the results indicate that nitrite addition is one cause for the solubilization of sludge, many researchers have found that it is not nitrite alone. In fact, a derivative of nitrite (free nitrous acid: FNA) is also responsible for the solubilization of the sludge. The concentration of FNA is mainly dependent on the concentration of nitrite, the pH, and the temperature (Anthonisen et al., 1976). In this study, the sludge was around pH 7; thus, the concentration of FNA was at 0.0087, 0.0175, 0.0263, and 0.0350 mg-N/L with addition of 50, 100, 150, and 200 mg-N/L of nitrite, respectively. The results show that FNA probably plays a role in the solubilization of particulate organic matter within the sludge. To investigate this further, sludge pre-treatment was conducted under constant nitrite concentration, but at different pH, to determine if FNA is the main factor in the solubilization of dewatered sludge.

2.2. Effect of different pH combined with nitrite addition on the concentration of SCOD

Fig. 3 shows the concentrations of SCOD within the dewatered sludge in the MFCs with fixed 100 mg-N/L of nitrite and varying pH. On day 3, the concentration of SCOD across all MFCs increased to 360–560 mg/L from the initial SCOD concentration of 160 mg/L. The peak concentration of SCOD under acidic condition (pH 5 and 6) was 350 and 360 mg/L, while under alkaline condition (pH 8 and 9), the concentration of SCOD was around 440 and 560 mg/L. The peak concentration of SCOD was the lowest (330 mg/L) for the sludge at neutral pH 7. It was expected that the concentration of SCOD would be higher under acidic condition than under alkaline condition. This is because the concentration of FNA will increase significantly at lower pH and probably would increase the solubilization of the sludge. However, based on the results, the best nitrite pre-treatment condition to increase the solubilization of particulate matter was alkaline, followed by acidic and neutral conditions. In this study, it was shown that the concentration of SCOD was greater under alkaline condition although the concentration of FNA should be lower at higher pH.

Fig. 4 shows the concentration of soluble EPS for the dewatered sludge pre-treated with 100 mg-N/L of nitrite at varying pH after 3 days. As expected, the trend of EPS followed the trend of SCOD concentration. The amount of EPS released was 211%, 159%, 123%, 224%, and 238% higher (compared

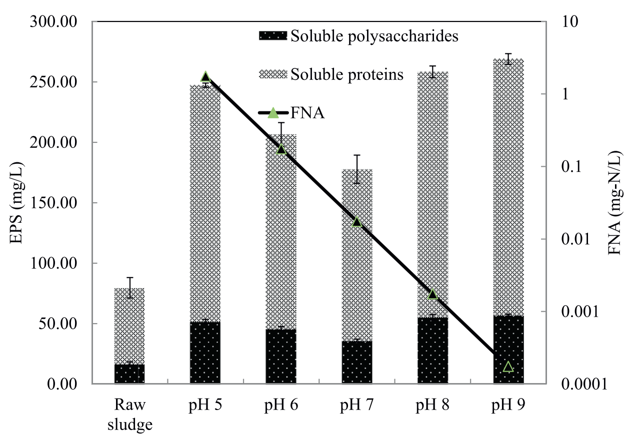

Fig. 4 – The concentration of soluble polysaccharides, proteins, soluble EPS and free nitrous acid (FNA) with fixed concentration of nitrite at 100 mg-N/L and different pH in MFCs after 72 hr.

to raw sludge) after pre-treatment at pH 5, 6, 7, 8, and 9, respectively.

Fig. 4 also shows the concentration of FNA at the start of the experiment for the sludge treated at differing pH. The acidic nature, combined with the effect of nitrite, helped to increase solubilization of the sludge. The acidic condition alone could help the solubilization of sludge. This is because the complex polymers that are the basic constituents of EPS easily break down into simpler monomers and oligomers under acidic conditions (Devlin et al., 2011). This breakdown would contribute to increase of the SCOD concentration within the dewatered sludge. Besides, the concentration of FNA was highest at pH 5 in this study. FNA has the capability to disrupt the structure of the cell and thus cause cell lysis, resulting in greater release of intracellular substances from the cells to the soluble phase (Jiang et al., 2011). Once FNA is formed, reactive derivatives such as NO, NO_2, and N_2O_3 can be generated. These small molecules have the ability to cross cell membranes and react with reduced thiols of the protein molecule to form nitrosothiols which will kill the cells (Jiang et al., 2011). FNA also has the ability to change the chemical structure of the EPS within the dewatered sludge. Under FNA exposure, mutagenesis of EPS occurred. This could cause the deaminative de-polymerization of proteins and also induce mutagenic changes in the nucleic acid due to deamination of amino bases. FNA is also capable of destroying the strong ring structure in the EPS, which would increase the biodegradability of complex EPS into simpler molecules (Zhang et al., 2015). The higher FNA content combined with the acidic nature of pre-treatment can be used to increase the release of soluble organic matter into the liquid phase.

On the other hand, the alkaline condition (at pH 9) was the best for sludge solubilization, as shown in Fig. 3. The SCOD concentration at pH 9 was 71% and 54% higher than the SCOD concentration at pH 7 and 5, respectively. Under alkaline condition, the solubilization of sludge was enhanced due to the effect of ionization by the hydroxide ion originating from NaOH, on the carboxyl groups forming the EPS. This ionization caused repulsion between the molecules of the EPS resulting in the breakdown of the EPS structure. This breakdown of EPS allowed solubilization of the complex protein and carbohydrate molecule chains into the liquid phase (Lee et al., 2014).

Previously, it had been concluded that high FNA concentrations can increase the solubilization of sludge. However, it is interesting to notice that for the pre-treatment of dewatered sludge at high pH, the concentration of FNA should be lower. Although the concentration of FNA was relatively low, the solubilization of the sludge was better under alkaline condition in this study. This was probably because the presence of hydroxide ion had more impact on sludge solubilization than did FNA at higher pH. Apart from FNA, other derivatives of nitrite such as nitric oxide could cause the solubilization of dewatered sludge. Nitric oxide can be formed during denitrification and has the capability to induce dispersal of bacterial biofilm. After exposure to nitric oxide, certain biofilm-forming bacteria, such as *Pseudomonas* sp., will respond to the exposure by detaching their cells from the biofilm. This allows other antimicrobial agents such as FNA to attack individual cells more easily (Barraud et al., 2006). Although at high pH, the concentration of FNA is relatively lower, the presence of another nitrite derivative such as NO can also enhance the solubilization of sludge.

2.3. Effect of nitrite addition on electricity generation

It took up 1, 2, 4, 6, or 10 days for MFCs to reach stable voltage production with 0, 50, 100, 150, and 200 mg-N/L of nitrite addition, respectively (shown in Appendix A). The higher the concentration of nitrite added, the more time was taken for the MFCs to reach their stable voltage. The reason for the difference in time taken for MFCs to reach stable voltage could be that the electrogenic bacteria needed time to adapt to the various concentrations of nitrite present within the sludge. The electrogenic bacteria within the sludge probably competed with the denitrifying bacteria for carbon, thus affecting the voltage production within the MFC. Under any condition, the electron acceptor with the highest redox potential will be reduced first. Species of bacteria that can use electron acceptors with higher redox potential will have an advantage in competing with other types of bacteria that use electron acceptors with lower redox potential (Achtnich et al., 1995). The presence of nitrite allowed the denitrifying bacteria to use the nitrite as their electron acceptor in the denitrifying process and thus converted the nitrite into nitrogen gas for their metabolic process (Huang et al., 2015b). After the nitrite in the MFC was depleted, the voltage generation started to stabilize and reached peak voltage shortly after that. This highlights that the elimination of nitrite from the MFC is essential to ensure high and stable generation of voltage in MFCs.

The FNA derivative of nitrite was present in the sludge until all the nitrite was depleted. The presence of FNA could possibly inhibit the electrogenic bacteria and thus cause the reduction of voltage generation for the MFCs containing nitrite. The presence of FNA within the MFCs caused various types of bacteria to lyse and this included electrogenic bacteria. However, it is also noted that certain species of electrogenic bacteria (such as *Shewanella* sp.) can withstand the effect of nitrite and FNA. As a strategy to overcome the toxicity of FNA, *Shewanella* sp. can secrete multiple oxidases or enzymes (such as bd-type oxidase) which have the ability to metabolize nitrite into NO and then N_2O (Giuffrè et al., 2014). During denitrification, carbon sources such as acetate are usually consumed by the bacteria as an energy source (Yoon et al., 2013). It has been reported that although *Shewanella* sp. is capable of surviving under exposure to FNA, the effect of FNA can still inhibit its growth (Zhang et al., 2013). This could explain why all the MFCs could generate electricity in the presence of nitrite, although at lower voltages, because only the growth of electrogenic bacteria was inhibited by the FNA. These results also clearly show that the depletion of nitrite affects the time taken for the MFCs to reach maximum voltage. Maximum voltage was achieved only after nitrite had been completely depleted from the MFCs.

Table 1 shows the maximum voltage and power density of MFCs with different nitrite concentrations. The highest max voltage was produced by the MFC (354 mV) using 100 mg-N/L of nitrite pre-treated dewatered sludge as the substrate. The concentrations of nitrite higher than 100 mg-N/L did not contribute to higher maximum voltage within the 14 days of MFC operation. Although the best nitrite concentration of nitrite for electricity generation using the sludge was at 100 mg-N/L, the initial voltage generation of the MFC with 100 mg-N/L of nitrite was lower compared to MFC using non-pretreated sludge.

The power density of each MFC was 83.81, 94.66, 101.07, 55.57 and 42.70 mW/m^2 when the amount of nitrite added to

Table 1 – Comparison of maximum voltage, power density and internal resistance with different nitrite concentration.

Nitrite (mg-N/L)	Max voltage (mV)	Power density (mW/m^2)	R_{int} (ohm)
0	325	83.81	797.69
50	349	94.66	640.74
100	354	101.07	249.75
150	310	55.57	369.79
200	281	42.70	478.38

Table 2 – Comparison of maximum voltage, power density and internal resistance with different pH and FNA concentration.

pH	FNA (mg-N/L)	Max voltage (mV)	Power density (mW/m^2)	R_{int} (ohm)
5	1.75	63	8.07	2080
6	1.75×10^{-1}	174	34.95	1240
7	1.75×10^{-2}	341	101.16	280
8	1.75×10^{-3}	363	118.15	240
9	1.75×10^{-4}	390	153.62	200

the dewatered sludge was 0, 50, 100, 150, and 200 mg-N/L, respectively, as shown in Table 1. The performance of a MFC can be expressed using the power density obtained from the polarization and power density curve (shown in Appendix A). The maximum power density of a MFC is obtained when its internal resistance is lowest. The internal resistance of a MFC can be affected by various factors, including pH, substrate concentration, and temperature.

The higher power density and lower internal resistance in the MFC using dewatered sludge with 100 mg-N/L of nitrite added, compared to the blank, was due to the higher concentration of substrate available in the pre-treated MFC. The higher concentration of substrate represented by the SCOD concentration caused a high substrate concentration gradient between the surface of the anode and the bulk substrate. The higher substrate concentration reduced the mass transfer limitation and thus contributed to higher power density for the MFC. The best nitrite concentration for highest power production in the MFC was 100 mg-N/L in this study. However, pre-treatment of nitrite exceeding 100 mg-N/L had a detrimental effect on the power density, although higher nitrite concentration increased the concentration of soluble organic matter for the electrogenic bacteria to consume. As described earlier, FNA has the capability to inhibit microorganisms. The negative response by the voltage generation and power density in the MFCs may be related to the toxic effect of the FNA on the electrogenic bacteria in the MFC after exceeding 100 mg-N/L of nitrite. This study demonstrated that nitrite addition at 100 mg-N/L can help to increase the power density of MFC in which dewatered sludge is used as the organic substrate.

2.4. Effect of different pH combined with nitrite addition on electricity generation

The highest voltages produced by the MFCs were 63, 174, 341, 363, and 390 mV with dewatered sludge at pH 5, 6, 7, 8, and 9, respectively (shown in Appendix A). The voltage generation for all of these MFCs gave almost the same trend: the maximum voltages were achieved within 4–5 days and stabilized after that. At the end of the 14-day period, the voltages of all MFCs were slightly lower that their voltages at 4–5 days. The reduction of SCOD could contribute to the lower voltage of the MFCs after three days of operation.

Table 2 shows the amount of FNA at the start of the experiment, maximum voltage, and also the power density of each MFC. The acidic condition had a detrimental effect on the output voltage within the MFC as demonstrated in this study. The acidic condition could improve the oxygen reduction rate (ORR) at the cathode due to the high concentration of H^+. However, in this case, the acidic condition seemed not to increase the overall closed-circuit potential of the MFC. The acidic condition within the MFC, combined with the presence of nitrite, might disrupt the activity of electrogenic bacteria at the anode and thus reduce the overall performance of the fuel cell; although the ORR was improved at the cathode under the acidic condition. This may be due to a concentration of FNA that was too acidic. At pH 5, 1.75 mg-N/L of FNA was the level at which it had irreversible negative impact on the population of bacteria within the MFC. It had been reported previously that 0.5–2.0 mg-N/L of FNA could permanently damage up to 80% of the viable cells in sludge and that the activity of the cell did not recover at all after such exposure to FNA (Pijuan et al., 2012). The extracellular electron transfer to the anode is more effective under alkaline condition and this could contribute to a higher rate of biofilm formation at the anode (Behera and Ghangrekar, 2009). In a MFC, biofilms of electrogenic bacteria are needed to transfer the electrons from the bacterial cells to the surface of the anode effectively through direct electron transfer or through nanowires (Kishimoto et al., 2013). These factors combined probably contributed to the higher power generation under alkaline condition than under acidic condition in the MFC.

Polarization analyses were done on the MFCs operated with 100 mg-N/L of nitrite addition and different pH (shown in Appendix A). The internal resistance of MFC for pH 5, 6, 7, 8, and 9 was around 2080, 1240, 280, 240, and 200 Ω, respectively as shown in Table 2. The internal resistance of the MFC was lowest at pH 9 (alkaline condition). The high internal resistance under acidic condition could be due to the inhibition effect of the FNA and unfavorable pH effect on the population of electrogenic bacteria at the anode. Under alkaline condition, the higher substrate availability compared to that under neutral condition, and lower FNA inhibition, contributed to the lower internal resistance within the MFC and thus resulted in higher power density for the MFC. This study showed that the alkaline condition combined with nitrite pre-treatment of sludge is a viable way to increase the power generation of a MFC.

2.5. Microbial population at anode

Samples of isolated bacteria taken from the anode of the MFC were sent to Macrogen, South Korea, for microbial identification. The phylogenetic tree of most dominant bacteria populated at the anode is shown in Appendix A. The most common bacteria that dominated the anode region of MFC were from the genus *Pseudomonas* and *Bacillus* sp. Both of these bacteria shown to have potential to be a good exoelectrogenic bacterium for MFC. *Pseudomonas* have the ability to transfer electrons as they secrete phenazine-based metabolites at the surface of the anode, thus improving the generation of electricity. Similarly, it has been found that *Bacillus subtilis* acts as a good biocatalyst for MFC (Hassan et al., 2016; Jothinathan and Wilson, 2017).

3. Conclusions

The nitrite pre-treatment of dewatered sludge was a feasible way to increase the concentration of SCOD and thus enhance the performance of a MFC to produce electricity. The increase of SCOD helped to overcome the mass transfer limitation at the anode and thus increased the power density of the MFC. The pre-treatment of dewatered sludge at alkaline condition (pH 9) combined with the addition of nitrite (100 mg-N/L) produced the highest power by MFC at around 153 mW/m^2. It was also shown that nitrite concentrations higher than 100 mg-N/L, and pH lower than 7, were not beneficial in increasing the electricity generation in the MFCs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jes.2018.06.023.

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