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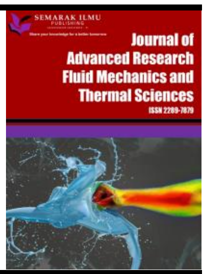

MFC Performance with Additional Micronutrients in Food Waste Substrate

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ABSTRACT

Microbial Fuel Cell (MFC) are the one of utilization of waste for renewable energy continues to be developed. According to the FAO, 32% of all food for human consumption is discarded about 1.3 billion tonnes per year. In this study, Microbial Fuel Cells used an organic source in the form of food waste that had been hydrolyzed by Aspergillus oryzae, Aspergillus aculeatus, and Candida rugosa. The results of the hydrolysis are entered into the MFC system. In the MFC system it is mixed with Sidoarjo mud and Shewanella oneidensis MR-1, then put into a Single Chamber microbial fuel cell (SC-MFC) to generate electricity. In this research, also added micronutrients (Mg^{2+}, Ni^{2+}, Cu^{2+}, Ca^{2+}, Pb^{2+}, Co^{2+}, Cd^{2+}, Cr^{2+}, and Zn^{2+}) to increase the metabolic of *Shewanella oneidensis MR-1* bacteria, so can elevate electric currents. electrons and protons are produced by microorganisms by changing organic compounds in the substrate. The results showed that the best power density was 6.652 W/m2 with BOD 89.362% and COD removal 77.273% achieved with a ratio of food waste to water of 2:1 M. Food hydrolysis is capable of hydrolyzing 40% food waste into glucose within 24 hours. The greatest percentage of glucose decreased was achieved by Cobalt micronutrient addition with 77% of glucose decreased. Therefore, MFC can be greatly enhance food waste degradation to become a carbon source in a microbial fuel cell for electricity production.

1. Introduction

Nowadays, most researcher in the world focus on environmental problem [1]. Among all environmental problems, need more efforts to treat wastewater. Not only wastewater, but also foodwaste, that contain many organic components can cause pollution too. Food waste contains many organic components, as well as dye-firm waste which contains chemicals which are also harmful to the environment [2]. Most of the researchers in the world are trying to develop renewable energy, mainly sourced from waste [3]. It's related with the increase in world population [4]. Several conventional technologies have been developed to reduce waste in the world. Several waste treatment technologies have been developed, including WWTP, anaerobic digestion, and

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conventional aerobic activated sludge (CAAS) [5]. Now has a lot of waste treatment that aims to produce energy. One technology that is able to produce energy from waste is Microbial Fuel Cell (MFC). MFC is a bio-electrochemical technology that converts organic substrates into electricity with the help of microbes as catalysts [6]. This technology is in accordance with the needs in recent years, which an terrace energy demand in the world [7]. Inside the MFC, organic and inorganic substrates are always used. The organic substrate used in MFC contains microbes that are capable to produce electrical energy [8]. Substrate is one of the important components in MFC that affects the production of electrical energy because functions as a source of energy microbial growth [9]. In addition, MFC is also able to degrade organic and inorganic components in the substrate. Substrates that have been used in MFC, such as waste from tofu, molasses, chromium [10], septic tank, fishery [11], domestic, and brewing [9]. Besides that, substrate of MFC also can use marine sediments and water from starch processing. In addition substrate from pure component, like glucose, ethanol and cysteine are also used as MFC substrates [9].

MFC also able to reduce Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) along with the electrical energy production in the MFC system [6]. Organic matters in waste would be decomposed into electrical energy. This makes the outlet waste from MFC safe and environment friendly. From literature, low organic in substrate produces low electrical energy, because it not enough to supply food for the bactery growth [7]. In this study, was using food waste which contains a lot of organic compounds, so the requirement bacteria growth fulfilled [5].

In this study, enzymatic pretreatment was used through the use hydrolytic fungi. From the reference, hydrolysis through enzymes is the best method compared to hydrothermal, ultrasonic, alkaline, microwave, and mechanical methods [12]. After the food waste is hydrolyzed using enzymes from fungi, the electrogenic bacteria, *Shewanella oneidensis MR-1* added to substrate, which convert organic compounds into electron and transfer it to anode [13]. This research also uses electrogenesis bacteria inside Sidoarjo mud, so the optimal conversion can be achieve.

Micronutrients (Mg^{2+}, Ni^{2+}, Cu^{2+}, Ca^{2+}, Pb^{2+}, Co^{2+}, Cd^{2+}, Cr^{2+}, and Zn^{2+}) added to increase the metabolic reactions of *Shewanella oneidensis MR-1* bacteria and other electrogeetic bacteria, so that they can trigger electron transfer and generate electric currents. It also can achieve optimal BOD and COD degredation [14]. The electrodes used in here was carbon cloth which has characteristic biocompatible, chemically and heat stable [15]. From this experiment, will be shown the effect of adding various types of micronutrients to the electrical energy produced, decreasing the content of BOD and COD, and another factors that affect the MFC.

2. Methodology
2.1 Material

The main materials of this research are food waste, that contain glucose, protein, and fat. Material also using three kinds of fungi (*Aspergillus aculeatus, Aspergillus oryzae* and *Candida rugosa)* was used to hydrolysed component glucose, protein, and fat. In here used electrogenic bacteria *Shewanella oneidensis MR-1* and also used microbes inside Sidoarjo mud. In here used micronutrient Mg^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Ca^{2+}, Co^{2+}, Cd^{2+}, Cr^{2+}, Zn^{2+} to influences increasing of *Shewanella oneidensis MR-1* bacteria. Electrode was used Carbon cloth twill A 220 that tied with copper wire. We purchase fungi from Airlannga University. We buy all chemical from Merck (Darmstadt, Germany). Collected Sidoarjo mud from Porong, Sidoarjo, East Java.

2.2 The MFC's Reactor

Single Chamber MFC with Batch system are used in this research. Chamber of MFC was used from 1.5-liter mineral water bottle waste. Carbon cloth used as cathode and anode with cut into 5 cm in length, 2 cm in width, and 2 mm thickness, with total surface area 22.8 cm^2. For cathode placed on the surface of MFC and anode take at 3 cm from base of chamber. Cathode and anode connected with copper wire, whereas connected with 1 kΩ resistance at Printed Circuit Board. Voltage and electrical current as the result can monitored by digital multimeter.

2.3 Methods

Bacteria and Fungi preliminaries and sterilisation: The media used a Potato Dextrose Agar (PDA) 39 g dissolved with 1 L water, then stir homogeneous and boiled. Then, sterilized by autoclaved at 121°C for 15 minutes.

Micronutrient preparation: 1 μg/L micronutrient Mg^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Ca^{2+}, Co^{2+}, Cd^{2+}, Cr^{2+}, Zn^{2+}) was prepared by dissolving 1 mg of micronutrients in 1 L of water. Then, take 1 ml of the solution and dissolving again in 1 Liter of water.

Hydrolysis of Food waste: Food waste was crushed immediately, then diluted 333-gram food waste in 167 ml aquadest. Then food waste hydrolysed by three kinds of fungi (*Aspergillus oryzae, Aspergillus aculeatus*, and *Candida rugosa*) for 24 hours.

Process in MFC Experiment: In this research, using Single Chamber of MFC from bottle mineral waste, then enter 500 grams of mix of food waste and water, and also 500 grams of Sidoarjo mud. Then add 1 μg/L micronutrient to each different chamber. Cutting carbon cloth with 5 cm in length, 2 cm in width, and 2 mm thickness, then solved with 1 M NaOH and 1 M HCl solution to activated. After carbon cloth activated, then tied with copper wire. Take one of electrode at the 3 sm from the base of chamber, it called anode. And the other carbon cloth was placed on surface of chamber, it called cathode. Electricity can be measured if the system connected with external resistor, so the cathode and anode was connected with external resistor using cable. This research was observed every day in 20 days using a a digital multimeter.

This research also oobserve number of cell by using a microscope with counting chamber method, one gram of sample was diluted with aquadest and the diluted 5 times. The BOD_5 (biochemical oxygen demand) analysis and COD analysis was carried out every three days observation. The power density was calculated as shown in Eq. (1) [3], where V is voltage (V), I is current (mA), and A is electrode area (m^2).

$$\text{Power density } (mW/m^2) = (I\ (mA) \times V\ (volt))/(A\ (m^2)) \quad (1)$$

3. Results

3.1 Power Density

Microbial Fuel Cell (MFC) utilize metabolism of bacteria to produce electricity and together to treats wastewater [16]. Therefore, if desired higher electricity produced, it can through enlarge and accelerate the metabolic processes of the bacteria used in the MFC. Metabolic process inside bacteria body can enlarge and faster when riboflavin production was higher. More riboflavin production able reach when micronutrient added. In this research use metal ions in microgram per liter as micronutrient [17]. Microbial Fuel Cells have been much research and developed with various substrates, bacteria, electrodes, electrode modifications, chamber variations, etc. However, so far the MFC still generates quite a small amount of electricity when compared to other power plants. In this study, it was tested by adding metal ions as micronutrients. The result of the addition without micronutrients produces a smaller voltage, current, and power density when compared to the addition of micronutrients. The micronutrients used in this study were 9 types of metal ions, including Mg^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Ca^{2+}, Co^{2+}, Cd^{2+}, Cr^{2+}, Zn^{2+}. The nine types of metal ions, Cobalt ions produced the highest energy density. Cobalt is capable of producing a maximum power density of 6,175,438.6 mW/m2, resulting from a maximum voltage of 275 mV and a current of 51.2 mA.

In this study, carried out in a batch system. From the batch system the total power density was calculated using the Simpson's Rule 1/3, the results are shown in Figure 5. The highest total power density was also achieved by the supplementary of Cobalt ions as a micronutrient. The total power density generated by Cobalt ions is 29,099,688.96 mW / m2 in 20 days. The highest total power density is 900 times higher than the total power density produced by the control variable (without the addition of metal ions), which is only able to produce a total power density of (32,431.46 mW/m2). This shows that the supplementary of cobalt ions as a micronutrient can improve the performance of MFC with its role as a trace element for bacteria [18].

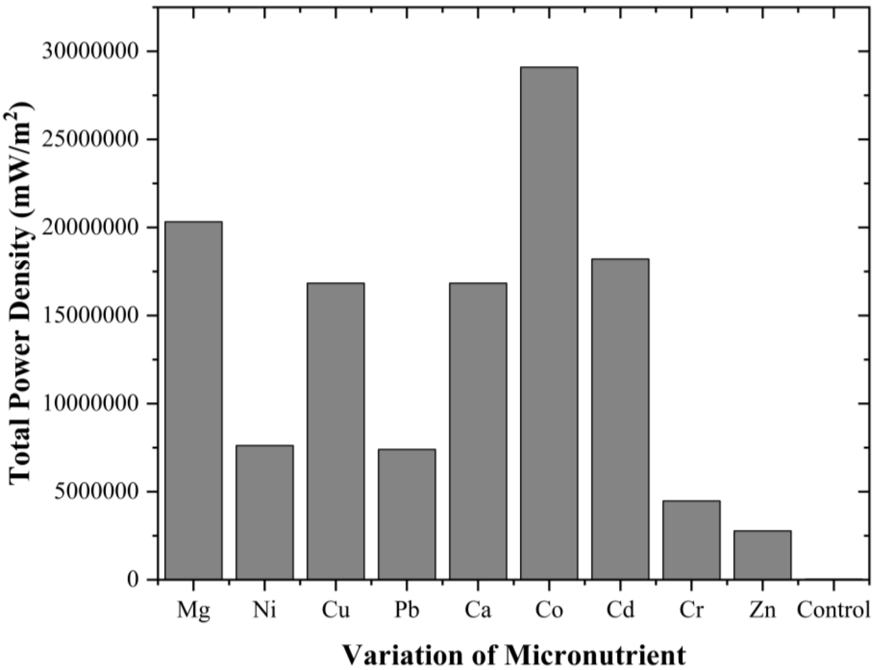

Fig. 1. Impact of Miconutrient Addition on Total Power Density Production

The total power density resulting from the MFC system in this study with the addition of Cobalt metal ions as a micronutrient was able to reach 29,099,689 mW/m2 or 29,099.69 kW/m2. Indonesia's electricity power target to be achieved is 2,949.58 MW. While the total power density generated from MFC for 20 days is 29,099,689 mW/m2 or 29,099.69 kW/m2. It can be said that MFC is likely to be able to meet Indonesia's energy needs by using a scale-up system with a substrate of around 51 tonnes of food waste and with the addition of the micronutrient cobalt of around 0.05 gram/L (raw calculation). Thus, electricity needs in Indonesia can be fulfilled through the MFC.

3.2 Microbial Population Growth

From Figure 5 it known that the highest number of microbial populations are 16,800 x 106 cells/ml was achieved by addition of Magnesium in substrate. Even though, the highest power density is produced by the addition of cobalt ions, although magnesium has the highest number of microbial cells. It is due to the Cobalt can reach riboflavin production more than Magnesium, so bacteria with cobalt addition metabolism occur significantly. Magnesium can increase the number of microbial cells, but does not speed up their metabolism. It also may cause by the ineffective production of riboflavin in the bacterial cells of the addition of magnesium, the metabolism is also disturbed. So even though the number of cells is more, it is not able to produce high electricity. In Figure 4, it may be known that there was a steady increase in both Microbial growth and power density until the 8th to 14th day of operation. Increasing of number of cell electrogens bacteria could be directly kind of micronutrient added [20]. Most of the microbial growth in the MFC experienced the greatest increase on the 8th to 14th day of the MFC operation process. However, after the 14th to the 20th day of MFC operation, the microbial population decreased. This can happen because the MFC operates in a batch system, which causes the substrate to run out as microbial food. Microbes enter a period of death as the substrate in the MFC decreases.

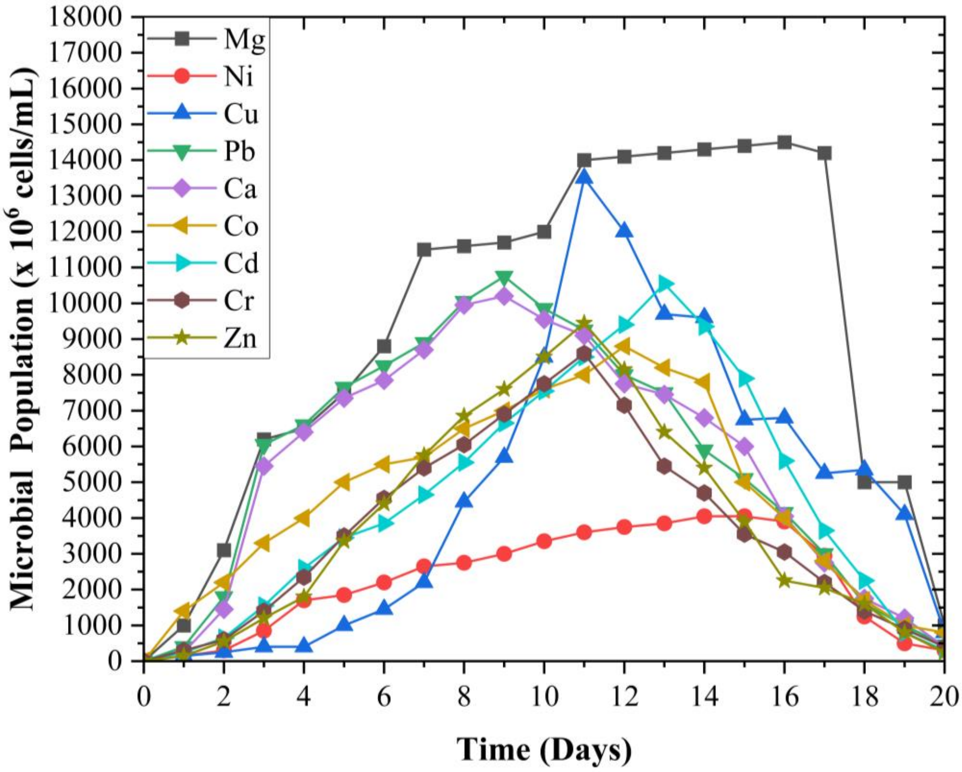

Fig. 2. Impact of micronutrient addition on microbial population growth in MFC

3.3 BOD and COD Removal

One way to detect water pollution is to do a BOD5 test [21]. The initial BOD_5 of Solid food waste in this study was 6750 mg/L. From Figure 6 and showed that Cobalt addition had the highest, 89.33 % BOD5 removal on the 20th day of MFC operation. The decrease in the BOD5 value in the MFC can be produced by degradation by the microbes in it. The combined use of the bacterium Shewanella oneidensis MR-1 and active microorganisms in Sidoarjo mud is the right combination to degrade waste in the MFC. Besides being able to degrade the food waste used, it is also able to decompose this waste as a source of MFC fuel. The highest degradation corresponded to the highest number of microbial populations, which was achieved by the addition of Magnesium ions.

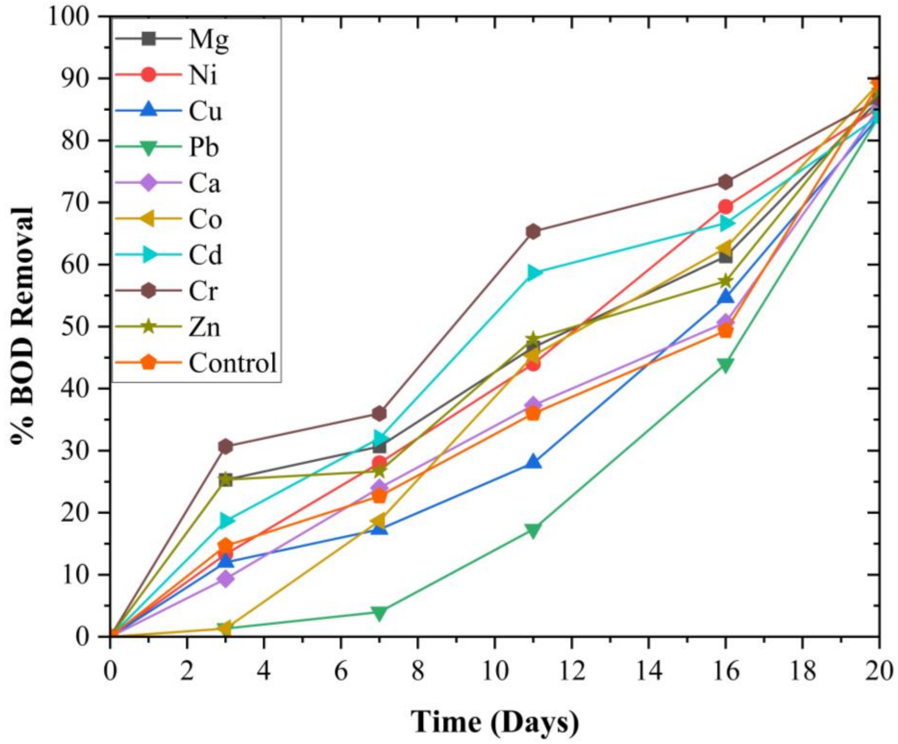

Fig. 3. Impact of micronutrient addition on BOD removal in MFC

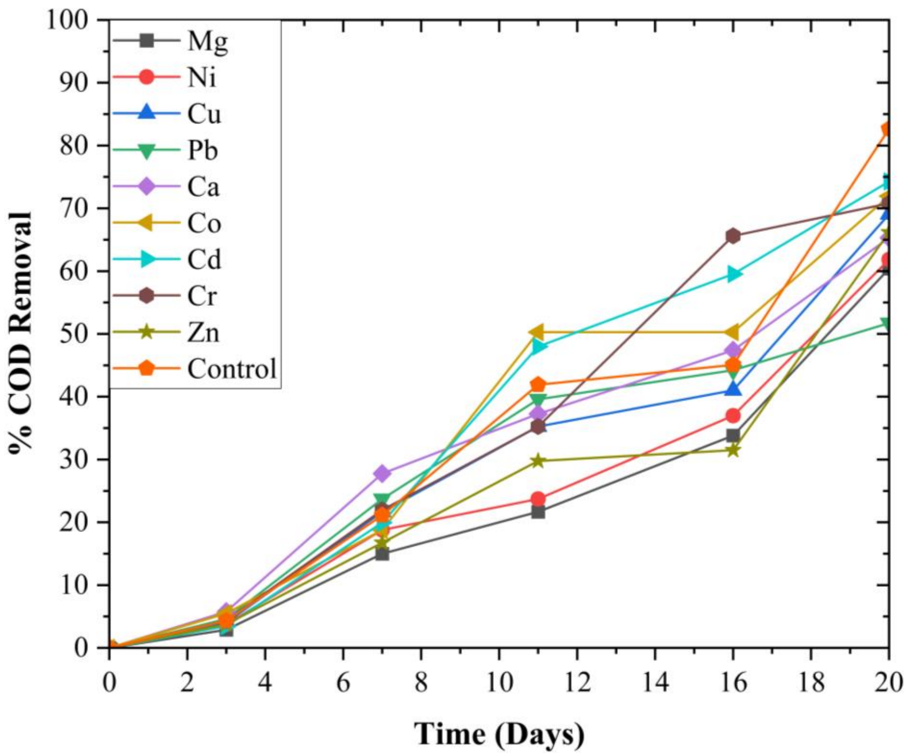

Fig. 4. Impact of micronutrient addition on COD removal in MFC

In addition to measuring BOD, to detect pollution of a water or waste can also be used to measure COD. The amount of organic matter that cannot be decomposed by microorganisms is referred to as Chemical Oxygen demand (COD) [22], and the highest COD is an indication of water pollution [21]. In this study, the initial COD level of food waste was 3,460 mg/L. The highest COD degradation reached 82.66%, which was achieved by the control variable during 20 days of operation. While the variable addition of metal ions, the highest COD degradation was 74.28% which was achieved by the addition of cadmium ions.

Microbial activity in MFC affects the removal or degradation of BOD and COD. Meanwhile, microbial activity in MFC is highly dependent on substrate availability, substrate concentration, microbial concentration, pH, and temperature [10]. This can be shown in. It can be said that there is a relationship between the amount of substrate, the number of microbes, and the degradation of BOD and COD. Figures 2 and 3 can be seen that the highest number of microbial populations and BOD degradation was achieved by the addition of Magnesium ions. With the number of microbial populations Based on the concentration of bacteria, 16,800 x 106 cells/mL was able to produce BOD degradation of 82, where the substrate added with micronutrients had the highest BOD and COD removal. Based on the bacterial concentration, 16,800 x 106 cells/mL of added Magnesium was able to degrade BOD by 89.3%.

3.4 Decreased of Glucose in MFC Process

Commonly organic and anorganic substrates used as substrate in MFC process, in the liquid or solid form. Organic substrate could be ranging from a simple compound, such as glucose. Food waste which used in MFC contains glucose, that would be used microorganism as carbon source to production of electricity. Carbon source in MFC used to achieve enrichment of the anodic electrode with electrochemically active bacteria [23]. Food waste used in this research was analysed using DNS method, that containing 0.92 g/L glucose. The glucose in the food waste has not been completely used Therefore, a pretreatment is needed that is able to break down the food waste content into glucose monomers. Hydrolysis is a process of breaking polysaccharides in lignocellulosic biomass into sugar monomers. The complete hydrolysis of cellulose produces sugar. The hydrolysis process can be carried out acidic and enzymatically. Process enzymatic hydrolysis requires enzymes [24].

In this research used enzymatic hydrolysis involving three types of fungi, namely *Aspergillus oryzae, Aspergillus aculeatus*, and *Candida rugosa.* Where *Aspergillus oryzae* functions to break down into glucose monomers, *Aspergillus aculeatus* functions to break down into amino acid monomers, and *Candida rugosa* breaks down fats. Hydrolysis process in here started with crushed of food waste, then analysed glucose inside of food waste using DNS method by Spectrophotography. After that enters Aspergillus *oryzae, Aspergillus aculeatus*, and *Candida rugosa* into food waste. Three kinds of fungi was added to food waste when their in log phase growth and waited for 24 hours hydrolysis process. After 24 hours hydrolysis process, then glucose was analysed again, that containing 1.29 g/L glucose or there is an increase in the glucose content in food waste by 40%.

Hydrolyzed substrate entered to MFC Chamber and also added *Shewanella oneidensis MR-1*, Sidoarjo mud, and also micronutrient, it process for 20 days. After 20 days, we take substrate (food waste) to analyzed glucose concentration using DNS method. Glucose concentration was decreased in all of variables, it showed that glucose inside of food waste was used by microorganism as carbon source to electricity production. Decreased of glucose concentration for every kinds of micronutrient added showed in Figure 5, we know that Co^{2+} was the most greter of decreased glucose concentration in percentage 77% decereased of glucose. This is according with the power density produced by the Co^{2+} variable, where Co^{2+} was the most larger power density produced so as carbon source large consumed too. The low levels of glucose in the substrate caused the variable addition of Co2+ ions to microbial growth on the 14th day to decrease. The transfer of electrons and protons between the bacterial cell and the electrodes is weakened when glucose levels are low. However, adding a substrate containing a lot of glucose does not result in an increase in the mass of the bacterial cells, but instead causes the glucose level in the anode chamber to run out quickly [25].

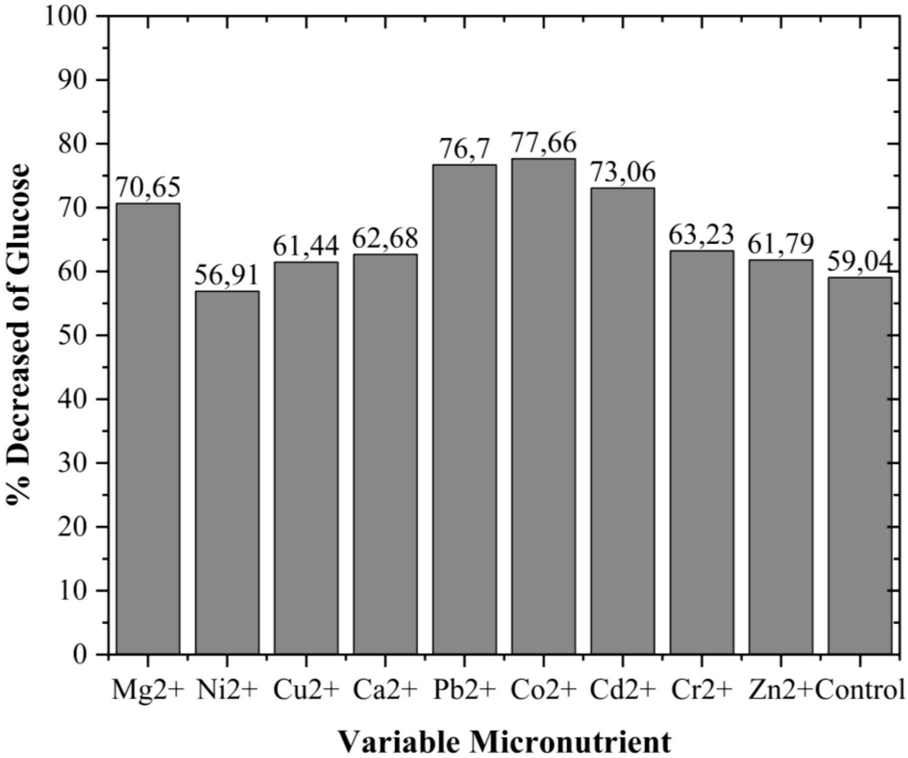

Fig. 5. Impact of micronutrient addition on decreased of glucose MFC

4. Conclusions

This study aims to determine the effect of adding metal ions as micronutrients on the growth of the bacterium Shewanella oneidensis MR-1, resulting in greater bioelectricity. Food waste that has been previously hydrolyzed using hydrolytic fungi (Aspergillus oryzae, Aspergillus aculeatus, and Candida rugosa) then enters the MFC system along with Shewanella bacteria, Sidoarjo mud, and micronutrients. Micronutrients were added with a variation of 9 types metal ion with 1 µg/L concentration. Observation of power density, microbial count, BOD removal, COD removal, and glucose reduction. The highest power density production of 6,175,438,596 mW/m2 was generated by adding Cobalt. The highest BOD5 and COD removal of 89.33% and 74.277% respectively were generated by the addition of Cobalt and Cadmium. All types of addition of micronutrients, microbial growth increased on day 1-14 and decreased on day 15 until the rest of the day. The greatest percentage of glucose reduction was achieved by adding the micronutrient Cobalt with 77% glucose reduction. In addition to being able to degrade food waste, MFC is also expected to be able to meet Indonesia's electricity needs with a scale-up system using 51 tons of food waste and 0.05 micrograms/liter. So, the development of MFC with the addition of micronutrients is expected to be applied commercially in the future.

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