Cobalt Ions Improve the Performance of Shewanella Oneidensis MR-1 Microbial Fuel Cells

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Cobalt Ions Improve the Performance of *Shewanella Oneidensis* MR-1 Microbial Fuel Cells

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Abstract: This study investigates the composition of metal ions using micronutrients to improve energy generated through MFC. Metal ions as micronutrients were investigated, utilizing various metal ions: pumblum, calcium, cobalt, cadmium, chromium, and zinc at various concentrations. Food waste was used to sustain mimicrooragnisms, which were converted into electrons. Metal ions promote bacterial metabolism through the transformation and distribution electrons. The study found that cobalt at 3 μg/L produced the highest voltage, current density, and power density of 4.5 V; 522 mA/m^2; and 1,085 mW/m^2, respectively. Our findings from studies reveal that cobalt succeeds in enhancing electricity. Keywords: cobalt, electricity; micronutrient; microbial fuel cell; electron transfer.

Keywords: cobalt, microbial fuel cell, *Shewanella oneidensis MR-1*

1. Introduction

Global economic developments have a positive impact on the international economy. However, from the perspective of waste, worldwide economic growth enhances the amount of waste, for example, food waste[1,2]. According to the United States Environmental Protection Agency (USEPA), the world wastes more than 34 million tons per year[3,4]. According to the Food and Agriculture Organization (FAO), more food goes to waste[5,6]. The management of food waste was inadequate, negatively affecting both health and environment[7,8]. This discovery is quite fascinating for researchers, especially the use of food waste to generate bio-electricity. Sugar reduction is a single stage in the synthesis of chemical and biofuels,such as glucose from starch[9,10]. Some studies suggest that glucose might be utilized as an energy source throughout the microbial fuel cell process. Furthermore, nowadays energy should be ecologically safe, along with the ability to reduce existing waste. MFC is one technology suitable for the criteria for eligibility. Some experts predict that by 2040, energy consumption will escalate to 37% over what it is at present. The electricity distribution ratio in Indonesia is only 95.35%, according to data from the Ministry of Energy and Mineral Resources in 2017[11,12]. Moreover, the Indonesian government and other countries were developing electric motorcycles, which have an influence on electricity usage. Almost all of the world's energy resources are derived from fossil and coal fuels, which are not environmentally friendly[13,14]. The presently developed strategy among researchers is for supplying energy demand while ensuring the stability of energy resources. According to data on worldwide renewable energy in 2020, renewable energy from biofuel will reach approximately six gigawatt Bioenergy production, often known as biomass, is a renewable and environmentally friendly source of energy with great potential[15,16].

In the MFC process, degraded food waste has been transformed into energy. Although Conventional Aerobic Activated Sludge (CAAS) can eliminate enormous amounts of organic waste, the process leads to more activated sludge, which does not happen with MFC. Food waste still contains several organic compounds, for example, glucose, protein, and fat. Bacteria continue to transform them as carbon sources into electrons via the Entner-Doudoroff pathway [17]. Acetobacter and Gluconobacter bacteria have the ability to convert organic components, including glucose into acetic acid [18,19]. The Sidoarjo mud contains both acetobacter and gluconobacter bacteria. Acetobacter and Gluconobacter bacteria create acetic acid, which is turned into electrons by electrogenesis bacteria such as Shewanella oneidensis MR-1. Many microorganisms might generate electricity, called electrogenic bacteria, such as *Geobacter sulfurreducens* , *Escherichia coli* , *Lactococcus lactis* , *Saccharomyces cerevisiae* , and *Shewanella oneidensis MR-1*[20,21].

Several factors may affect the performance of MFC, such as electrode designation, active area of electrode, configuration of MFC, electrode modification, and others. One of the most essential considerations when selecting an electrode is the electrical conductivity[22]. This study utilized carbon cloth, which has a high electrical conductivity. Another factor influencing MFC performance is the rate of electrons transport inside the MFC. Several possibilities for improving electricity are currently being studied[23,24]. Micronutrient addition is one of the factors that might enhance the generation of electricity through electron transport inside the MFC[25]. Micronutrients are chemicals that stimulate riboflavin production. Riboflavin accelerates metabolic activity in the *Shewanella oneidensis MR-1* bacteria. Riboflavin is the primary endogenous of EET (Extracellular Electron Transfer) of *Shewanella oneidensis MR-1,* which improves electron transport between bacteria and electrodes[26,27]. Increasing the electron movement between bacteria and electrodes generates electricity. Therefore, the addition of metal ion micronutrients may enhance electrical energy production. MFC has been studied in several kinds of substrates. However, food waste has never been studied previously. This study aims to evaluate the composition of metal ions as micronutrients to increase the generation of electricity.

2. Materials and Methods

2.1 Material

The materials used in this study such as 333 mg of food waste, *Aspergillus oryzae RIB40 (ATCC® 42149™), Aspergillus aculeatus 36411^{TM}, Candida rugosa NCPF 8452, Shewanella oneidensis MR-1 ATCC 700550*, Sidoarjo mud, $CaCl_2$, $CrCl_3$, $CdCl_2$, $ZnCl_2$, $CoCl_2$, $PbCl_2$, distilled water, carbon cloth, copper wire, cable, resistor 1 kΩ, and plastic bottles. Current and voltage production was observed using a digital multimeter and the biofilm surface was analyzed by Scanning Electron Microscope (SEM) (HITACHI FLEXSEM 100.

2.2 Preparing Microorganisms

The strain *Shewanella oneidensis MR-1 ATCC 700550* was obtained from the American Type Culture Collection. Before conducting in MFC process, *Shewanella oneidensis* was activated using a Nutrient Broth (NB) medium at 30^{o}C. The strain of three kinds of fungi, such as *Aspergillus oryzae RIB40 (ATCC® 42149™), Aspergillus aculeatus Iizuka 36411™,* and *Candida rugosa NCPF 8452* was adapted from American Type Culture Collection were activated using Yeast Extract-Peptone-Dextrose (YPD) Medium (Merck, German) at 30 ^{o}C. Three kinds of fungi and *Shewanella oneidensis MR-1* before being added to foodwaste make a growth curve. The growth rate curve makes to know the number of cells on the log phase condition, so added to the MFC process in the maximum number of cells. The growth rate curve of *Shewanella oneidensis MR-1* was observed every 2 hours for 24 hours of growth. While three kinds of fungi were observed every day for 20 days of growth.

2.3 Hydrolysis of Food – Pretreatment Process

The food waste use contains 60% of glucose, 30% of protein, and 10% fat. Hydrolysis of food waste was started by crushing food waste with a blender (Panasonic, Japan). Then 333 grams food waste was placed on a beaker glass (Merck, Germany) then dissolved with 167 mL aquadest. After that, *Aspergillus oryzae, Aspergillus aculeatus*, and *Candida rugosa* were added to the food waste at log phase condition, hydrolysis was started for 24 hours.

2.4 Preparing Metal Ions as Micronutrients

Metal ion as micronutrient was added in various concentrations at 3, 5, 7, and 10 (µg/L), using Pumblum II Chloride, Calcium II Chloride, Cobalt II Chloride, Zinc Chloride Anhydrate, Chromium III Chloride, and Cadmium II Chloride (Merck, German). Metal ions were variated as kinds of micronutrients and concentration of micronutrients was added. 3 µg/L metal ions were made with 3 mg dissolved in 1 L aquadest, then taken 1 mL and dissolved again in 1 L aquadest, so the concentration was 3µg/L.

2.5 Preparing electrode of MFC

Anode and Cathode were made from Carbon cloth Twill A-2205 (Shangai Passion Composites Co., Ltd, China). Anode and cathode were made by measuring carbon cloth 5cm in length, 2 cm in width, and 2 mm in thickness, then sewing with copper wire. Before the anode and cathode were placed in MFC, carbon cloth was activated by Chloride acid and Natrium Hydroxide. Carbon cloth was submersed in 1 M of Chloride acid for 24 hours, then in 1 M Natrium Hydroxide for 24 hours. Carbon cloth was washed using aquadest, then used as an anode and cathode.

2.6 Preparing Sidoarjo mud

Sidoarjo mud was added to MFC because it contains an electrogenic bacteria culture source. Some bacteria in Sidoarjo mud, such as Geobacter sulfurredusence, Bacillus subtilis, Clostridium, Streptomyces, Coliform, Salmonella, and Staphylococcus aureus. Sidoarjo mud was extracted from 30–45 cm below the mud near Lapindo at coordinates 7^{o}31'45.6" S and 112^{o}42'43.6" E in Renokenongo Village, Porong District, Sidoarjo, East Java.

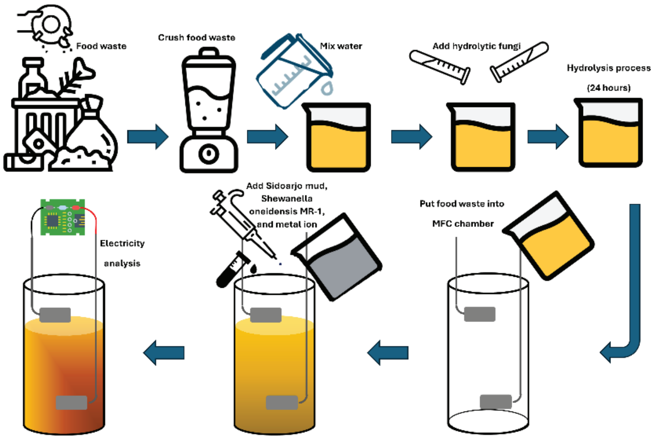

Fig. 1: Schematic MFC Process

2.7 Construction and operation of MFC

A single-chamber MFC was constructed with a working volume of 1000 ml in each chamber (Aqua, Indonesia). Each chamber was filled with 500 ml (a mixture of food waste and water) and 500 ml of Sidoarjo mud[28]. The anode was placed 3 cm from the bottom of the chamber and the cathode was placed on top of the MFC solution. As an external circuit, the electrodes are connected with a copper wire having an external resistance of 1000 Ω. The specific surface area of the electrode for each application is calculated using the cylindrical equivalent projected area (Meshack Simeon, 2019) :

$$A_e = 2lw + 2lt + 2wt \quad (1)$$

Where A_e, l, w, and t represent a projected area, the electrode length, width, and thickness, respectively.

At the start of the process, hydrolyzate of food waste as a substrate of the MFC. 20 mL *Shewanella oneidensis* MR-1 in 10 hours inoculation were added and also 500 mL Sidoarjo mud. 1 mL of metal ions as micronutrients were added into each chamber by variated in kinds (Pb^{2+}, Ca^{2+}, Co^{2+}, Cd^{2+}, Cr^{2+}, Zn^{2+}, and Control variable) and also variated in concentration (3 µg/L, 5 µg/L, 7 µg/L, and 10 µg/L) then the process was carried out on a batch scale.

Voltages, currents, and power densities were measured at varying micronutrient concentrations from 3 µg/L, 5 µg/L, 7 µg/L, and 10 µg/L when the maximum available output voltage of the MFC was reached and plotted. The voltage and current are measured every day by using a digital multimeter (Fluke, Canada). Then the power density is calculated by using Eq.(2) (Meshack Simeon, 2019).

$$P = VI / A \quad (2)$$

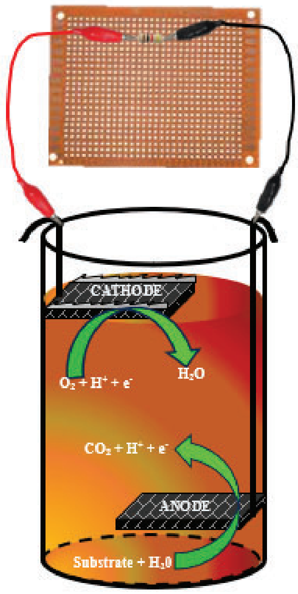

Fig. 2: MFC Construction

3. Results and Discussion

3.1 Voltage generation

In the present study, we developed a single-chamber MFC with food waste as fuel to generate electrical energy[17]. Food waste utilized for MFC fuel has the highest amount of glucose, up to 60%. As a result, huge quantities of glucose are transformed into electrical energy. Glucose is transformed into electrons via the Entner-Doudoroff pathway. The Entner-Doudoroff pathway transforms one molecule of glucose into two molecules of pyruvate and acetate[29,30]. It also generates an ATP molecule from an excess of two nicotinamide adenine dinucleotide phosphates (NAD(P)H)). The acetate continues to transform into pyruvate molecules, which also produce ATP. Nicotinamide adenine dinucleotide (NAD^+) and nicotinamide adenine dinucleotide phosphate ($NADP^+$) were utilized to regenerate acetate[17]. After the long process in the Entner-Duodroff pathway, it generates energy for bacteria metabolic processes along with electrons. Several different micronutrients have been added to the MFC for improve the electricity generation. The voltage increases significantly through the cycle, which consists of three phases; ascending, stationary, and declining phases[28]. According to the reference, Figure 1 shows the initial voltage of MFC (V vs t). The graph in Fig 3 reveals that V in 3 µg/L achieves the maximum for the Cobalt ion. After 5 days of operation, all the micronutrients increased to the highest voltage of 5 mV. Figs.4, 5, and 6 demonstrate micronutrient concentration of 3 µg/L, 5 µg/L, 7 µg/L, and 10 µg/L, leading to maximum voltages 4.5 mV, 3.2 mV, 3 mV, and 2.5 mV, respectively.

Cobalt at 3 µg/L has a greater rate of reaction than other micronutrients, leading to improved MFC performance[31]. It is caused by cobalt-producing cobalamin, which forms Vitamin B12 [32]. Cobalamin, or Vitamin B12, is required for bacteria development, including *Acetobacterium, Clostridium, Streptococcus, Streptomyces, Shewanella, Lactobacillus sakei*[33], which are found in Sidoarjo mud. Thus, cobalt may develop a faster reaction rate than other metal ions. After the 10th day of operation, every kindof micronutrients decreased voltage generation. After 20th days of operation, the voltage had decreased under the limit voltage of approximately 0.5 mV. The particular model of MFC leads to decreased voltage. We used a batch MFC system, which indicated that the electricity would decrease when the substrate were exhausted. This design requires sufficient support to transform continuous substrate into electrons[34]. Moreover, decreased substrate was provided in the system, and the voltage continued to drop, revealing that the voltage did not have the ability to stabilize in this batch system[35]. The cascade of voltage is steady in several micronutrients (3 μg/L, 5 μg/L, 7 μg/L, and 10 μg/L), whereas non-micronutrients generate low voltage. The graph with different kinds and concentrations of metal ions showed the same trend, gradually rising at first, reaching a peak, and then gradually decreasing. Therefore, the four metal ion concentrations are being investigated to find out which generates the most electricity over 20 days of operation[36].

More substrate in MFC provides more nutrients for bacteria. Furthermore, bacteria consumption enhances the activity of bacteria, which leads to developing electron transport and , as a result, fluctuating electrical outputs [37].

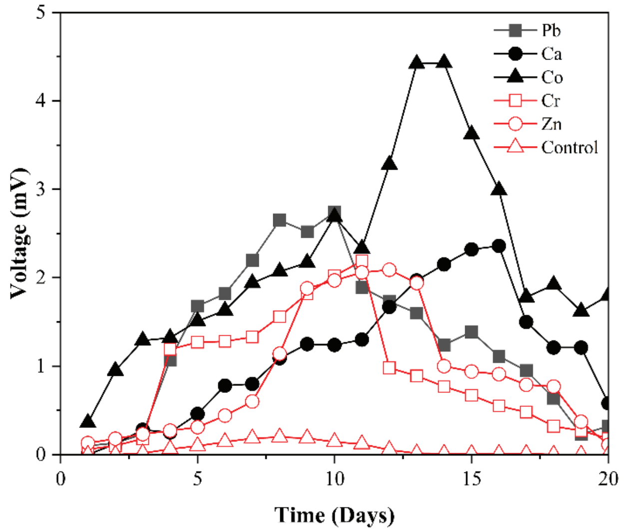

Fig. 3: Voltage performance of MFC by use 3 μg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc

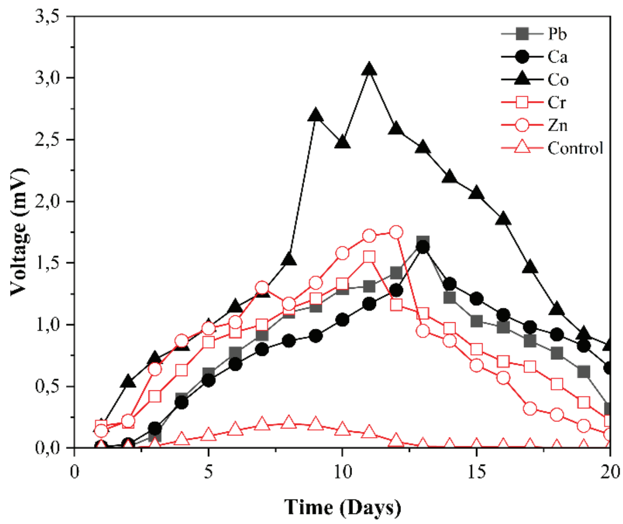

Fig. 4: Voltage performance of MFC by use 5 μg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc

3.2 Current density generation

For this study, we constructed a single-chamber MFC and added micronutrients of various types and concentrations to investigate how these subtances effected the performance of the MFC with respect to electricity generation. Generally, various micronutrients have different effects on generating electricity. The addition of micronutrients might encourage microbial growth in MFC and create ideal conditions for microbial growth and growth, thus boosting riboflavin formation and conducting electron transfer role in electricity generation[38]. During the operation process, the difference in voltage between the anode and cathode of the MFC conducts electricity current. Furthermore, to determine the performance of the current between the anode and cathode, the current density may be calculated. This equation carried into consideration the current density.

$$\text{Current density} = \text{Current}/(\text{Area of active electrode}) \quad (3)$$

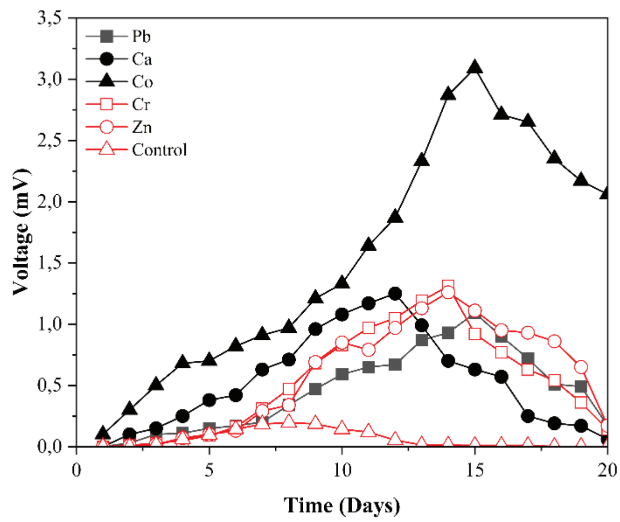

Fig. 5: Voltage performance of MFC by use 7 μg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc

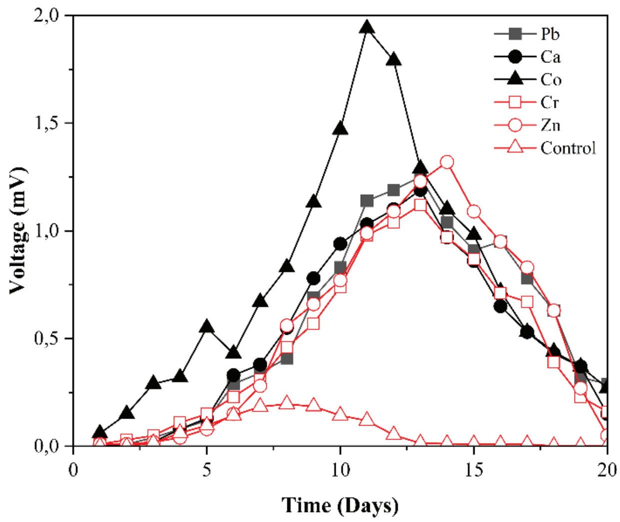

Fig. 6: Voltage performance of MFC by use 10 μg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc.

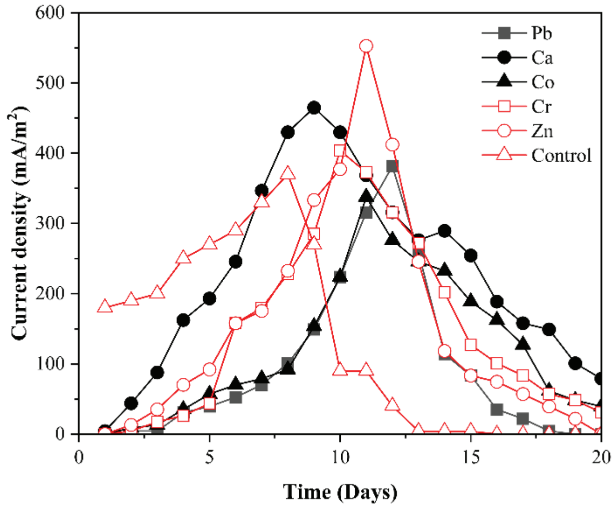

Fig. 7: Current density performance of MFC by use 3 μg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc.

As shown in Fig. 7, 8, 9, and 10, as the current density grew, the output power of the MFC increased steadily, with a maximum current density of 465; 386; 522; and 447 mA/m^2 for zinc 3 μg/L, Cobalt 5 μg/L, Cobalt 7 μg/L, and Cobalt 10 μg/L, respectively. The highest current density was achieved by Cobalt in 7 μg/L concentration, producing 522 mA/m^2. As a result of current density, micronutrients lead to electricity generation. However, when MFC operate for a longer period of time, the substrate reduces and bacteria consumption declines. The reductionof nutrients inside the MFC system inhibits bacterial development, resulting in the death of many microorganism. The decline of bacteria results in fewer electron moves and a decrease in generated energy. The fewernumbers of bacteria inhibited electron transport, decreasing the current density.

Several kinds of micronutrients provide high voltage and current density, whereas the control (non-micronutrient) variable generates high current density during 20 days of operation. After reaching the peak voltage and current density, it was gradually decreased. The decline in cell voltage generations was driven by promptly activation loss. Furthermore, it can be triggered by the exhaustion of the fuel in the substrate[39,40].

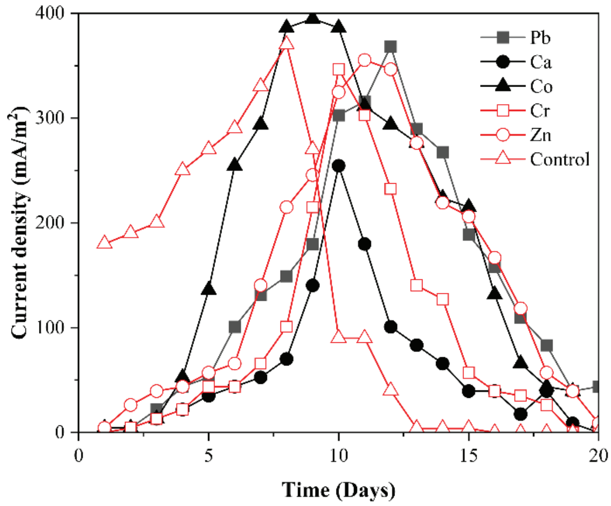

Fig. 8: Current density performance of MFC by use 5 μg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc.

3.3 Power density generation

Power density graphs were gathered during power generation to assess electron discharges. MFC often attain their peak power density during the ascending phase. As a consequence, Fig. 11, Fig. 12, Fig. 13, and Fig.14 represent the output power density curves of MFC with different types and concentrations of micronutrients. Cobalt produces the greatest power density at concentrations of 3 μg/L concentration (in Fig.11) , 5 μg/L (in Fig.12), 7 μg/L (in Fig.13), and 10 μg/L (in Fig.14). Cobalt ion at 3 μg/L has an increased reaction rate than other variables, thus providing the optimum concentration for micronutrient in MFC. Cobalt produced the highest power density, with maximums of 1,085; 1,061; 1,082; and 868 mW/m^2, respectively. Cobalt ion at 3 μg/L has a greater reaction rate than others variables, resulting the optimal concentration for micronutrient performance in MFC. After 20 days of operation, the MFC produced a maximum power density of 1,085 mW/m^2 (Fig. 11). The highest power density was attained with 3 µg/l Cobalt. During the 14th day MFC operation, most variables power densities climbed. After the 14th day of operation, the power density progressively decreased dropped until the final day of operation. Figure 11 shows that the output of the MFC progressively rose with increasing cobalt concentration up to 3 µg/L, with a maximum output of less than 5 µg/L, 7 µg/L, and 10 µg/L for various micronutrient concentration.

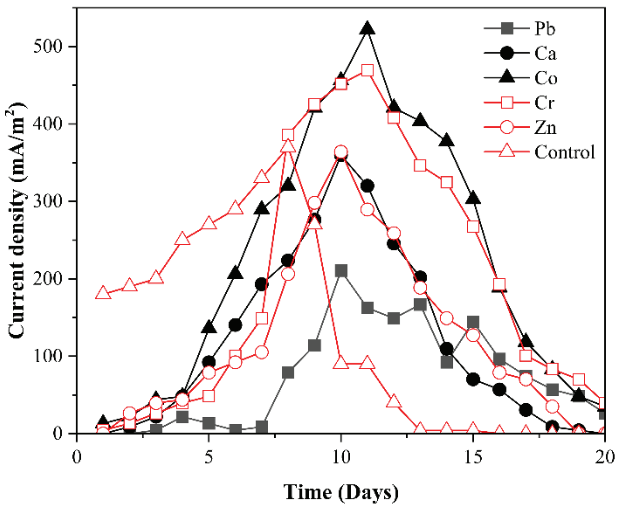

Fig. 9: Current density performance of MFC by use 7 µg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc.

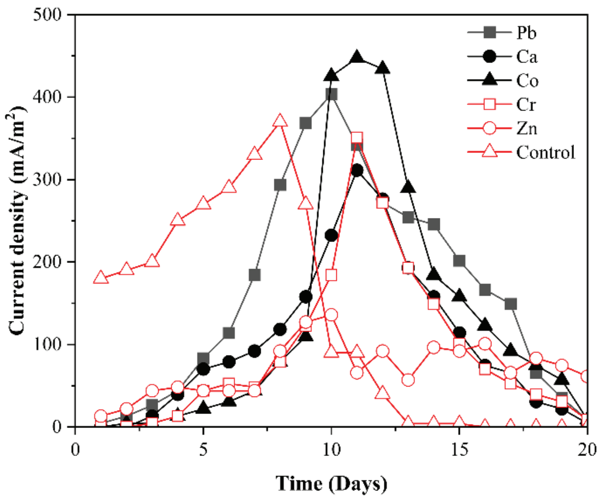

Fig. 10: Current density performance of MFC by use 10 µg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc.

Cobalt is poisonous at extreme levels (above 14 µg/L); nevertheless, in this study, it was utilized as a micronutrient at concentration below 14 µg/L. Thus, in this investigation, cobalt was not hazardous to microorganisms in the MFC. This might be attributed to cobalt's role as an effector in the synthesis of riboflavin[41,42]. Riboflavin stimulates the movement of *Shewanella oneidensis MR-1* extracellular pili. The extracellular pili of *Shewanella oneidensis MR-1* serve as the last electron acceptor, completing the electron transport chain of quinol. This is accomplished by the use of cytochromes and outer membrane proteins, as well as flavin. Riboflavin is formed using kobamida and koenzim, and it also stimulates metabolism in the *Shewanella oneidensis MR-1* bacteria, which also accelerating the electrons transport.

This study found that all metal ions, including cobalt, cadmium, chromium, and zinc, can boost power and MFC performance. The reasoning is as follows: (1) increasing *Shewanella oneidensis MR-1* to transfer electron metabolisms; (2) metal ions may improve the cathode performance. *Shewanella oneidensis MR-1*'s metabolic activity improves the transport of electrons and results in an advantage of cathodic potential[26]. Decreasing micronutrient concentration would limit the amount of electrons moved to the catholyte, resulting in fewer electron transfers, and therefore the reaction at the cathode would be significantly reduced, creating severe cathodic polarization[43,44]. The strong cathodic polarization would result in more inhibition within MFC performance[45].

Cobalt is a trace metal ion, classified as a micronutrient in this study, that might alter the conversion of glucose via the Entner-Doudoroff pathway[17].

3.4 Biofilm formation

A biofilm is a layer of bacterial colonies on the surface of an electrode[46,47]. The anodic biofilm is more significant than the cathodic biofilm in producing and repairing micronutrient ions from the matrix. The thicker and wealthier the biofilm, the higher and more steady the power density produced by MFC[48]. Furthermore, biofilm on the electrode promotes bacterial immobilization and activity[42]. SEM was utilized to determine the morphology of biofilms in anodes. Figure 15, depicted the findings of SEM investigation, revealing the presence of bacteria in the anode. The SEM revealed that the biofilm develops compact microorganisms on the anode surface. Biofilm thickness reflects a balance between the population of electrogenic bacteria and the nutrients available in the MFC system. The dense, thick biofilm is composed of active bacteria on the surface of the carbon fiber electrode, as well as secreted extracellular materials and nutrient fluxes. As a result, the presence of the biofilm layer accelerates electron transmission, increasing the voltage and current produced by the MFC electrode[49]. Enhancement of attachment bacteria in anodic and the electron shuttle concentration would increase electricity; additionally, another factor related to improving electricity, such as cell metabolism and electron transport chain activity (e.g., c-type cytochromes), may be influenced by and play a role in improving energy performance[41]. This demonstrated that the biofilm was important for electricity generation[50]. However the biofilm shape remained unstable during the operation. It can be produced by the freshness of oxygen and the duration of incubation[51,52].

Fluctuating biofilm development in the MFC process is a method of bacterial protection when exposed to a poisononous substance[53].

Figure 15 illustrates the number of bacterial cells present throughout MFC operations. It demonstrates that at the beginning of the process, there were still around 6,000 million bacterial cells per milliliter. However, on the seventh working day, the total number of bacterial cells increased to 36,000 million cells/mL. This number of bacterial cells correlates to the results of SEM biofilm in Fig. 16, indicating that the bacterial cells are attached to the anode layer.

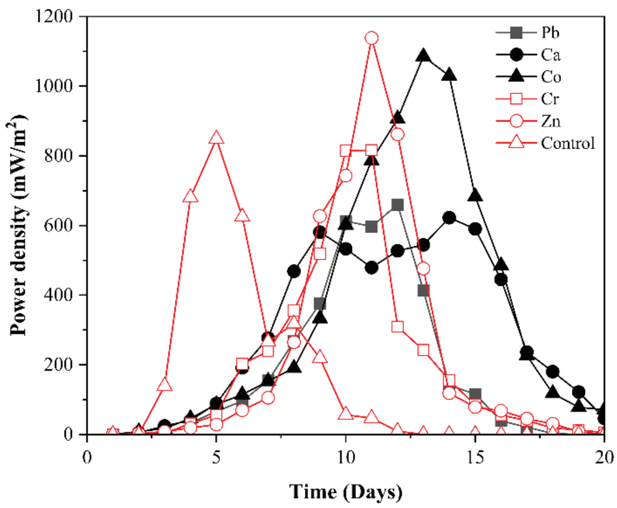

Fig. 11: Power density performance of MFC by use 3 μg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc.

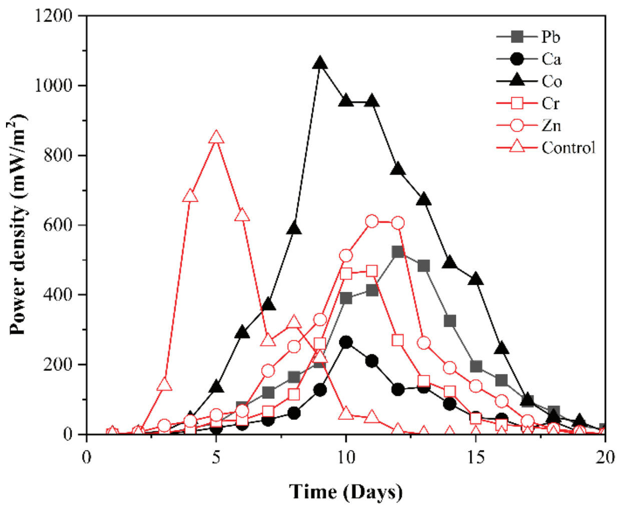

Fig. 12: Power density performance of MFC by use 5 μg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc.

Decreased bacterial numbers and bacterial capabilities, as demonstrated by biofilm, resulted in lower electricity generation[54]. This may result in bacterial cells being easily isolated from the outer layer of biofilm formed at the substrate's liquid-phase anode[55].

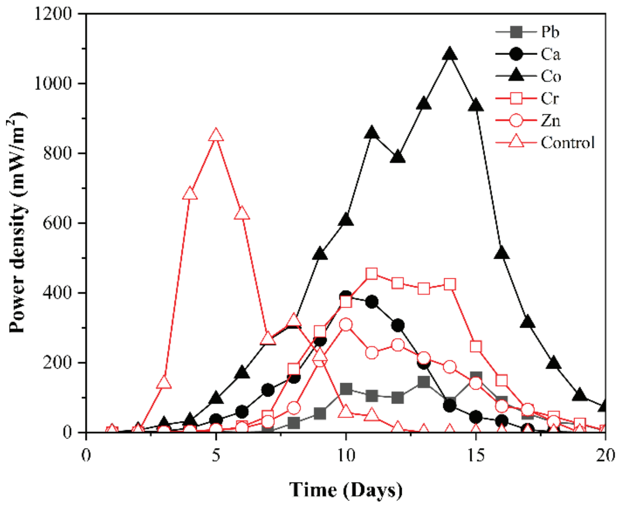

Fig. 13: Power density performance of MFC by use 7 μg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc.

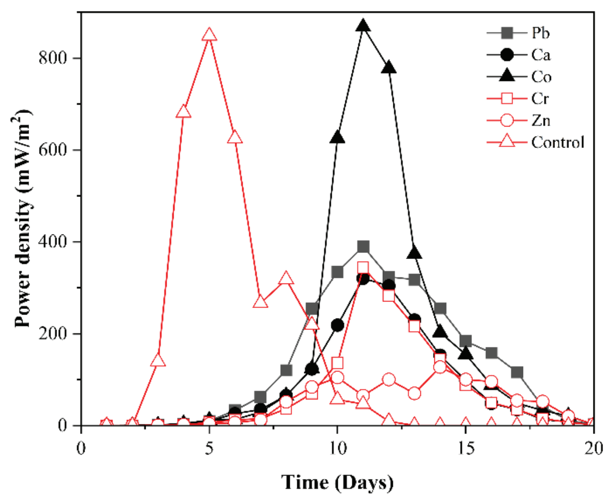

Fig. 14: Power density performance of MFC by use 10 μg/L concentration of micronutrient : Pumblum, Calcium, Cobalt, Cadmium, Chrom, and Zinc.

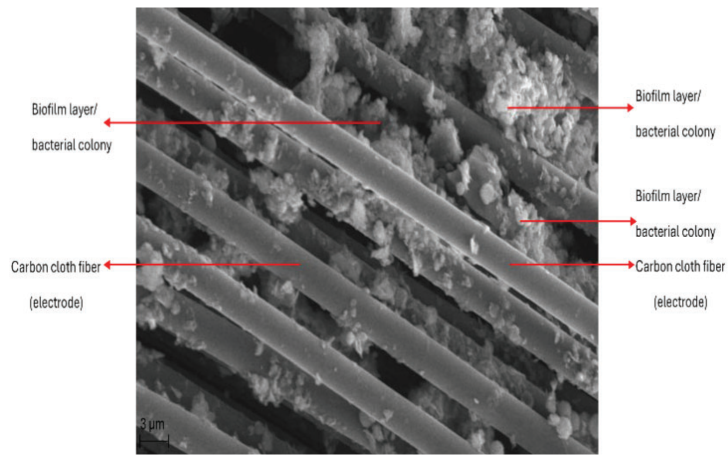

Fig. 15: SEM of carbon cloth electrode after an experiment for *Shewanella oneidensis MR-1* at magnification 1500 X.

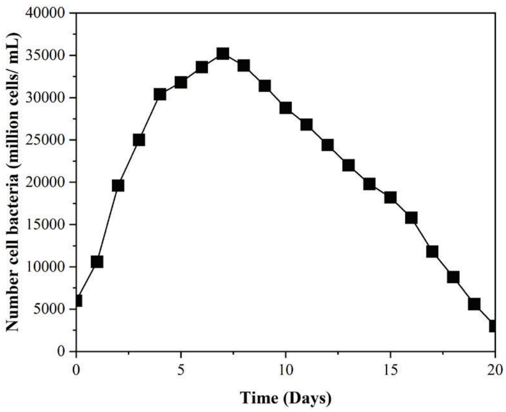

Fig. 16: Number cell of bacteria in MFC operation

4. Conclusion

Metal ion have been successfully generated as a micronutrient for *Shewanella oneidensis MR-1* in food waste MFC to improve its performance. In this study, the electricity generation was assessed using voltage output, current density output, and power density measurements. Using 3 µg/L of Cobalt micronutrient resulted in the highest voltage, current density, and power density of 4.5 mV; 522 mA/m^2; and 1,085 mW/m^2, respectively.

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