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Research article

Effect of electrode modification on the production of electrical energy and degradation of Cr (VI) waste using tubular microbial fuel cell

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Abstract: Carcinogenic hexavalent chromium is increasing worldwide due to the increased electroplating, welding and textile industry. On the other hand, molasses, the sugar factory's byproduct with high organic compounds (sugars), may pollute the environment if it is not processed. However, microbial fuel cell (MFC) seems to be a promising technology due to its ability to produce electrical energy from pollutant degradation using microbes while reducing hexavalent chromium to trivalent chromium with less toxicity. Carbon felt was used at both electrodes. This research aimed to determine the effect of modifying the anode with rice bran and cathode with Cu catalyst towards electricity generation and pollutant removal in molasses and reducing Cr (VI) into Cr (III) using tubular microbial fuel cells. Moreover, the effect of mixing Sidoarjo mud and *Shewanella oneidensis* MR-1 as electricigen bacteria toward electrical energy production and pollutant removal was determined. Experiments revealed that the S/CM/AM variable, which only used *Shewanella oneidensis* MR-1 as an electricigen bacteria with both modified electrodes, produced the highest total power density of 530.42 mW/m^2 and the highest percentage of Cr (VI) reduction of 98.87%. In contrast, the highest microbial population of 66.5×10^{10} cells/mL, 61.28% of Biological Oxygen Demand (BOD_5) removal and 59.49% of Chemical Oxygen Demand (COD) were achieved by SSi/CM/AM variable, mixing *Shewanella oneidensis* MR-1 and Sidoarjo mud as an electricigen bacteria with both modified electrodes. Therefore, this study indicates that double chamber tubular microbial fuel cells may be a sustainable solution for managing molasses and carcinogen hexavalent chromium.

Keywords: hexavalent chromium; electrode modification; Sidoarjo mud; *Shewanella oneidensis* MR-1; carbon felt; tubular microbial fuel cell

1. Introduction

Energy is the basis of sustainable development. Currently, most human activities are supported by fossil fuels [1]. Energy demand will continue to increase every year while the availability of fossil fuels is limited. In addition, the ongoing use of fossil fuels is not sustainable due to greenhouse gas emissions [2]. Replacing non-renewable fossil fuels with something more sustainable is a universal issue affecting communities and the environment. The extensive use of fossil fuels harms the environment due to carbon dioxide emissions, which result in air pollution, global warming, and health issues [3]. The depletion of energy reserves on earth requires humans to develop alternative energy. Several technologies have been proposed, such as solar photovoltaic cells, wind turbines, fuel cells, and geothermal energy to replace fossil fuels [4]. Microbial fuel cells (MFCs) seem to be new technology, one type of fuel cell currently being developed.

MFC has attracted significant research interest due to its potential for generating energy in an environmentally sustainable manner. Recently, researchers reported MFCs as a new technology for directly converting chemical energy stored in organic substances to electricity through catalytic reactions of exoelectrogenic bacteria [5]. The basic principle behind the MFC technology is that the chemical energy compounds are converted into electrical energy during the decomposition of organic and inorganic matters with the help of catalytic action of microorganisms and electron transfer efficiency [6]. MFC has several advantages over conventional processing technology. It is a high potential green energy technology because of its easy operation and excellent waste treatment capability with zero pollution emission since it is environmentally friendly [7,8]. However, MFC presents several challenges for their wide applications due to low power generation and costly fabrication materials of MFCs.

The performance of the MFC in generating electrical energy is influenced by several factors such as the type of microbe used [9], the type of contaminants degraded [10], types of the electrode [11], electrode modification [12], the type of configuration [3], type of separator used and the operating conditions of the MFC, including but not limited to pH and temperature [5]. In MFCs, the biodegradation of organic matter and electron transfer efficiency determine the generation of bioelectric energy [11]. Moreover, the internal resistance of the system and sustainability of electron transfer are also determining factors for a bio-electricity generation [13,14]. The exoelectrogenic bacteria produce free electrons to be collected by the electrode for power generation and are essential in MFC to remove pollutants while producing electrical energy [9,15]. Researchers revealed that the mixture of exoelectrogenic bacteria might boost electricity production [5]. The authors used *Shewanella oneidensis* MR-1, an exoelectrogenic bacteria with high productivity [16], and Sidoarjo mud-containing bacteria that might play a key role in MFC electron transfer. Darmawan et al. revealed that Sidoarjo mud could be utilised as an alternative source for bioelectricity production [17]. Sidoarjo mudflow is due to the mud volcano eruption in May 2016 in Sidoarjo, East Java, Indonesia. It consists of 70% solids and 30% water, with a 32%–40% salinity, pH 6.6–7, cation exchange capacity (CEC) 3.89–35.42 (meq/100 g), and total organic carbon 54.75%–55.47% [18].

The Author used hexavalent chromium waste as a catholyte and molasses as an anolyte in this research. Hexavalent chromium is a heavy metal mainly used in electroplating, welding, wood preservation and textile dyes. It is well-known for its natural and negative health effects due to its poisonous quality and mutagens [19]. The United States Environmental Protection Agency (US EPA) identified Cr (VI) as one of seventeen chemicals that endanger human health [20]. Costello et al. [21] revealed that Cr (VI) is around 100 times more poisonous compared to Cr (III). Intensive research was developed to reduce Cr (VI) to Cr (III). Different pre-treatment methods were used, including but not limited to membrane filtration, ion exchange and chemical precipitation. However, these methods aren't sustainable since they require much energy and mainly generate hazardous waste [19]. So, MFC seems to be a promising technology that may reduce Cr (VI) to Cr (III) environmentally sustainable. On the other hand, molasses, the sugar factory's most valuable by-product [22], was used as an anolyte since it contains high organic compounds (sugars) that might be used as a carbon source in MFC. Molasses could also be used in yeast fermentation, pharmaceutical, animal feed industries, feedstock for fuel ethanol production, paddy soil to absorb cadmium and arsenic, and feedstock for anaerobic digestion to produce biogas [23,24]. In this research, molasses was first sterilised by autoclave to kill any microorganisms that may live in it before its use.

Electrode materials play a significant role in MFC Performance [11]. Different materials may be used to make a perfect anode for MFCs, which require a large surface area to enhance the electron transfer rate. The large electrode surface area can capture more electrons and allow microorganisms to move effectively on the electrode's surface [25]. Anodic materials are also necessary because they help microbes oxidise organic substrate by increasing their metabolic rate [26]. This research selected carbon felt at both electrodes since it has several advantages. These include enormous porosity value, chemical and thermal stability, higher specific area and mechanical strength with high electrical conductivity [27]. The research conducted by Yaqoob et al. [28] showed that the electrode's modification to achieve a high surface area, good electron transferability, and bacterial adhesion is needed to enhance electricity production. In this study, the anode was modified by rice bran. The rice milling process resulted in rice bran as a byproduct. Annually, 29.3 million tons are supplied globally [29]. It contains about 11.3%–14.9% protein, 34.0%–62.0% carbohydrates and 15.0%–19.7% oil [30]. Rice bran nutrients with high organic content may increase bacterial growth, resulting in high electricity generation. Moreover, the rice bran contains a high silica concentration, increasing surface area [31] for bacterial attachment. High silica concentration can stick around the anode surface, modifying the anode to increase the surface area for bacterial attachment [31]. This study used rice bran to increase the contact area between microbes and the electrode. On the other hand, A highly active catalyst Cu modified cathode to improve the performance of the MFC. A catalyst at the cathode causes the cathodic reaction's activation energy to be lower and increases the reaction rate [12]. Cu was selected due to its perfect Oxygen Reduction Reaction. Pt metal with the highest ORR activity compared to other metals wasn't selected since it is costly and its scarcity limit its large scale application. Moreover, Pt has a poor stability in long-term use [32]. Therefore, Cu was used in this study for the cost and availability compared to Pt. Fan et al. [33] revealed that the MFC's power density was increased by 51.91% for a modified electrode with MnO_2, more than that of an unmodified electrode. The power density were 6.8 and 10.33 mW/m^2 before and after modification, respectively.

In this study, a double chamber Tubular MFC configuration was used. Researchers revealed that using Tubular MFC has advantages such as low cost of construction, high power output and waste degradation, and ease of construction [34]. A double chamber is an MFC configuration with two chambers, namely the anode chamber and the cathode chamber, and two chambers are connected through a membrane separator [35,36]. Li et al.[37] revealed that the absence of a separator increases oxygen and substrate diffusion, lowering the anode microorganism's electrocatalytic activity. However, one of the difficulties in separator applications is the slow transfer of protons from the anode to the cathode chamber, leading to pH splitting and decreasing MFC performance. In this study, Gore-Tex® was selected since it is the most efficient and effective separator with affordable cost, simple construction, and low internal resistance. Gore-Tex® was able to become an efficient and effective separator in MFC applications due to its characteristics of being nonselective to ion transport and microporous structure, as well as its thin thickness. It enhanced the proton transfer rate and oxygen diffusion compared to ion-exchange membranes [38,39]. Three layers of Gore-Tex® with a 1 cm distance between the separator and the cathode were used. Therefore, this research aimed to determine the effect of modifying the anode with rice bran and cathode with active catalyst Cu towards bioelectricity generation and pollutant removal in molasses and reduction of carcinogen Cr (VI) into Cr (III) using tubular microbial fuel cells. Moreover, the effect of mixing Sidoarjo mud and *Shewanella oneidensis* MR-1 as electricigen bacteria toward bioelectricity production was determined.

2. Materials and methods

2.1. Materials

2.1.1. Type of materials used

Materials used in this study are molasses, $K_2Cr_2O_7$, Sidoarjo mud, *Shewanella oneidensis* MR-1, Copper wire, Carbon powder, carbon felt 3F, Gore-Tex® separator, rice bran and Copper (II) sulfate. Sidoarjo mud was collected 30–45 cm below the mud's outer surface at coordinates 7°31′45.6″S and 112°42′43.6″E in Porong, Sidoarjo, East Java (Indonesia). Molasses were collected at P.T Energi Agro Nusantara, located in East Java (Indonesia) and $K_2Cr_2O_7$ from Ferak Berlin GmbH (German). The carbon powder (KB 600) was obtained from Gemmy Industrial Corp (Taiwan), whereas the carbon felt (GF-20, Nippon carbon) 3F with a size of 500 cm × 1000 cm and a density of 320 g/m^2 was purchased from Japan. Gore-Tex® separator and *Shewanella oneidensis* MR-1 were found in the Laboratory of Industrial Wastewater Treatment & Biomass, Institut Teknologi Sepuluh Nopember (ITS). On the other hand, all the other chemicals not listed used in this research are from Merck (Darmstadt, Germany).

2.1.2. MFCs reactors

A double chamber Tubular MFC reactor was used in this research. The anode and cathode chamber dimensions were 24 cm × 5.08 cm and 24 cm × 7.62 cm, respectively. Carbon felt was used at both electrodes with 12.5 cm × 20 cm for the anode and 20 cm × 24 cm for the cathode. The distance between the separator and cathode was 1 cm, as shown in Figure 3. Three layers of the Gore-Tex® (60 cm × 15.5 cm in size) separate both chambers. As shown in Figure 2, the external circuit was connected to the cathode and anode by Copper wire, with an external resistance of 1 kΩ soldered to the Printed Circuit Board (PCB). The digital multimeter monitored the Voltage output and electrical current.

Figure 1. Prototype tubular microbial fuel cell.

2.2. Methods

2.2.1. Media preparation and sterilisation

Liquid Nutrient Broth media was made with 0.6 grams of Nutrient Broth Powder and 0.225 grams of D-glucose mixed in 75 mL distilled water. The media was then autoclaved at 121 °C for 20 minutes at 15 psi to sterilise it.

2.2.2. Microorganism growth

A spore of *Shewanella oneidensis* MR-1 has grown aseptically on a nutrient broth fresh medium. The incubation was carried out at 30 °C. A hemocytometer was used to monitor the growth phase of bacterial growth [40].

2.2.3. Substrate preparation for anode chamber

Molasses and distilled water were sterilised by autoclave to kill any microorganisms that may live in them. Then, the pH of the acidic molasses was neutralised with a 5M KOH solution to pH 7, and a phosphate buffer solution of pH 7 was added. Neutral buffers were used to stabilise the electrode pH at an optimal level to get a good performance of MFC [5].

2.2.4. Cathode solution preparation

Cr (VI) solution was prepared by dissolving 30 mg of potassium dichromate ($K_2Cr_2O_7$) in 300 mL distilled water. This reference is according to Indonesia Government Rule Number 22 of 2021 on class II river water quality standards [41]. Then, Buffer citrate was used to maintain the cathode acidity at pH 3.

2.2.5. Electrode modifications

2.2.5.1. Cathode modification

The impact of electrode modifications on MFC performance was analysed in this research. Cu modified cathode since a highly active catalyst causes the cathodic reaction's activation energy to lower and increase the reaction rate [12]. The $CuSO_4$ was dissolved into 87.8 wt% distilled water. Then, added 20 mL of ethylene glycol. Wang et al. revealed that 87.8 wt% Pt/C catalysts synthesised with a 20 mL Ethylene Glycol/23 mL H_2O ratio exhibit comparable electrocatalytic properties such as Oxygen Reduction Reaction (ORR) and stability. It was concluded that the controlled addition of water to the Ethylene Glycol solvent during the catalyst synthesis process promotes not only smaller particle sizes and more uniform size distribution but also favourable electrochemical properties of the catalyst in terms of mass activity and durability. Because of the synthesis process's simplicity and ease of filtering when water is added to Ethylene Glycol, these positive impacts have significant implications for applications in the large-scale production of Pt/C catalysts for PEM fuel cells [42]. These changes were applicable when the $CuSO_4$ catalyst was used in this research. On the other side, carbon felt was put into the mixture of 25 mL of ethylene glycol and 23 mL of distilled water. Then, $CuSO_4$ solution was added dropwise into the carbon felt container. The pH was adjusted to 7 using 1M NaOH solution. The mixture was refluxed at 72 °C within 3 hours. Then, the modified carbon felt was dried in an oven at 110 °C.

2.2.5.2. Anode modification

Rice bran, containing high silica [31] able to stick around the anode surface, modified the anode to increase the surface area for bacterial attachment. Anode modification was prepared by spreading the rice bran-carbon paste on the carbon felt surface. The modification paste was made by dissolving 8 grams rice bran with distilled water, and then 25 mL of rice bran solution was mixed with 0.125 grams carbon powder.

2.3. MFC experiment

This study used double tubular MFC reactors, as shown in Figure 1. The cathode and anode chambers were separated using a Gore-Tex® cloth separator, as shown in Figures 2 and 3. We used 3 layers of the Gore-Tex® in each experiment. 6 variables were analysed, as seen in Table 1. The cathode chamber was filled with Cr (VI) solution. The anode chamber was filled with sterilised molasses and 10^{11} cells of *Shewanella oneidensis* MR-1 at their log phases mixed with/without 15 mL of Sidoarjo mud as electricigen bacteria. Carbon felt was used at both electrodes. The carbon felt was cut into 12.5 cm × 20 cm for the anode and 20 cm × 24 cm for the cathode and sewn with copper wire. Then, the anode is varied with/without modification of rice bran, and the cathode is varied with/without modification of Cu catalyst. The distance between the separator and cathode is 1.0 cm, as shown in Figure 3.

The MFC chamber was connected to an external electrical circuit and observed for 12 days. A digital multimeter was used to analyse the electrical current and voltage produced daily. The power density was calculated as shown in Eq 1, where V is voltage (V), I is current (mA) and A is electrode area (m^2) [43]. The BOD_5 (biochemical oxygen demand) analysis was carried out every four days, referring to SNI 6989.72: 2009. SNI stands for Indonesian National Standard. COD analysis was carried out on the first and last days of observation, referring to SNI 6989.2: 2009 and Cr (VI) analysis every four days, referring to SNI 6989.71: 2009. The BOD_5, COD and Cr (VI) removal percentage can be calculated using Eq 2.

$$power\ density\ (mW/m^2) = \frac{I\ (mA)\ x\ V\ (volt)}{A\ (m^2)} \tag{1}$$

$$\%Removal = \frac{Initial - Outlet}{Initial}\ x\ 100\% \tag{2}$$

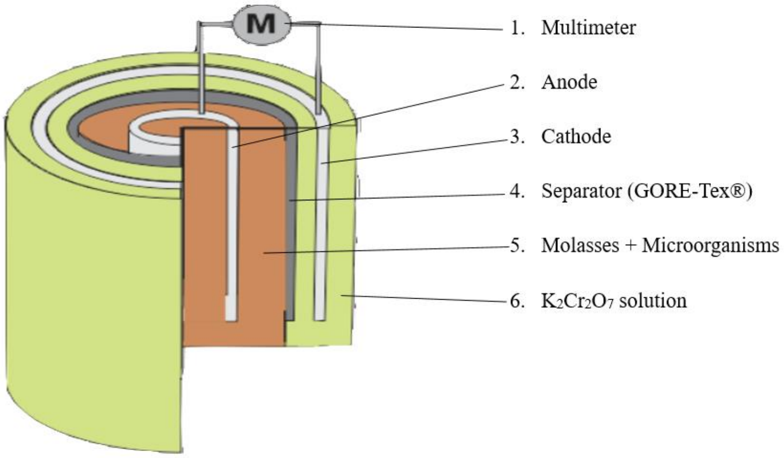

Figure 2. Tubular microbial fuel cell diagram.

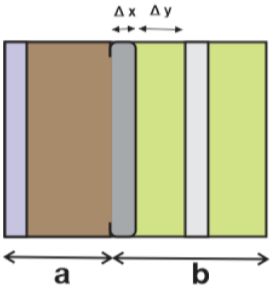

Figure 3. Illustration of tubular microbial fuel cell variables.

Note: a is the anode chamber diameter; b is the cathode chamber diameter; Δx is the number of layers of the Gore-Tex® separator (3); Δy Cathode-separator distance (1 cm); Size for anode = 12.5 cm × 20 cm; Size for cathode = 20 cm × 24 cm; Separator Size = 60 cm × 15.5 cm (3 layers).

Table 1. Sample code and different variations.

Code	Substrate	Types of electricigen Bacteria used	cathode	Anode
S/CWM/AWM	Molasses + hexavalent chromium	*Shewanella oneidensis* MR-1	Cathode without modification	Anode without modification
S/CM/AWM	Molasses + hexavalent chromium	*Shewanella oneidensis* MR-1	Cathode modified with Cu Catalyst	Anode without modification
S/CM/AM	Molasses + hexavalent chromium	*Shewanella oneidensis* MR-1	Cathode modified with Cu Catalyst	Anode modified with rice bran
SSi/CWM/AWM	Molasses + hexavalent chromium	*Shewanella oneidensis* MR-1 + Sidoarjo mud	Cathode without modification	Anode without modification
SSi/CM/AWM	Molasses + hexavalent chromium	*Shewanella oneidensis* MR-1 + Sidoarjo mud	Cathode modified with Cu Catalyst	Anode without modification
SSi/CM/AM	Molasses + hexavalent chromium	*Shewanella oneidensis* MR-1 + Sidoarjo mud	Cathode modified with Cu Catalyst	Anode modified with rice bran

Note: S: *Shewanella oneidensis* MR-1; Si: Sidoarjo mud; CM: cathode modified with Cu Catalyst; CWM: cathode without modification; AWM: anode without modification; AM: anode modified with rice bran.

3. Results and discussion

3.1. Growth curve of *Shewanella oneidensis MR-1*

A Nutrient broth Agar (NBA) fresh medium was used aseptically to grow a spore of *Shewanella oneidensis* MR-1. The bacteria were used in their log phase. The log phase is when the number of cells increases exponentially with time [44], and cells in this phase are the healthiest [45]. Therefore, before the bacteria *Shewanella oneidensis* MR-1 was added to the reactor for electricity production, it was necessary to know the log phase of this bacteria and the optimum time. The optimum time for the log phase is when the highest bacterial population growth is achieved, after which the bacteria undergo a death phase marked by a significant decrease in bacterial populations [46]. The growth curve for *Shewanella oneidensis* MR-1 was observed every hour during 24 hours of incubation to determine the optimum time. Regularly counting the bacterial population in the culture media was performed using a hemocytometer.

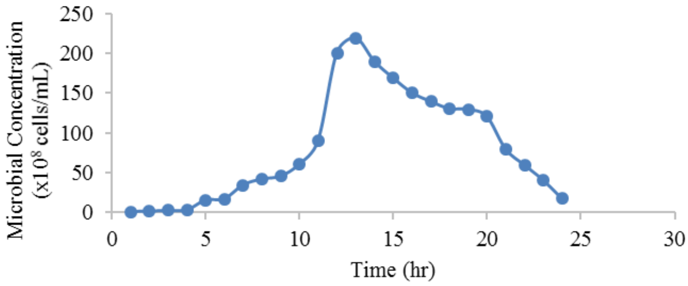

Figure 4. Growth curve of *Shewanella Oneidensis* MR-1.

As shown in Figure 4, it was noticed that there was a considerable increase in the number of cells at the log phase [47]. The log phase for *Shewanella oneidensis* MR-1 is between the 11–13th hour. After the 13th hour, the bacteria experienced a death phase. Therefore, the bacterial culture was transferred to the MFC chamber at the 13th hour.

3.2. pH control during MFCs operation

MFC utilises exoelectrogenic bacteria to reduce pollutants and directly produce electrons and protons then converted into electricity [48]. The rate of microbial growth is influenced by pH [49]. Since microorganisms play an important role in an MFC reactor, the pH of an electrolyte is among the parameters that may affect the efficiency and performance of MFC. The transport of cationic ions (Na^+, K^+, etc.) in the cathodic chamber could raise the pH and conductivity. Because protons are consumed in the cathode reaction, transport of cation ions (other than H^+) results in an increased pH in the cathode chamber, which reduces MFC performance [5]. Hence, the pH of both electrodes should be kept at an optimal level to get a good performance of MFC. Neutral buffers are techniques that may be used to stabilise the electrode pH.

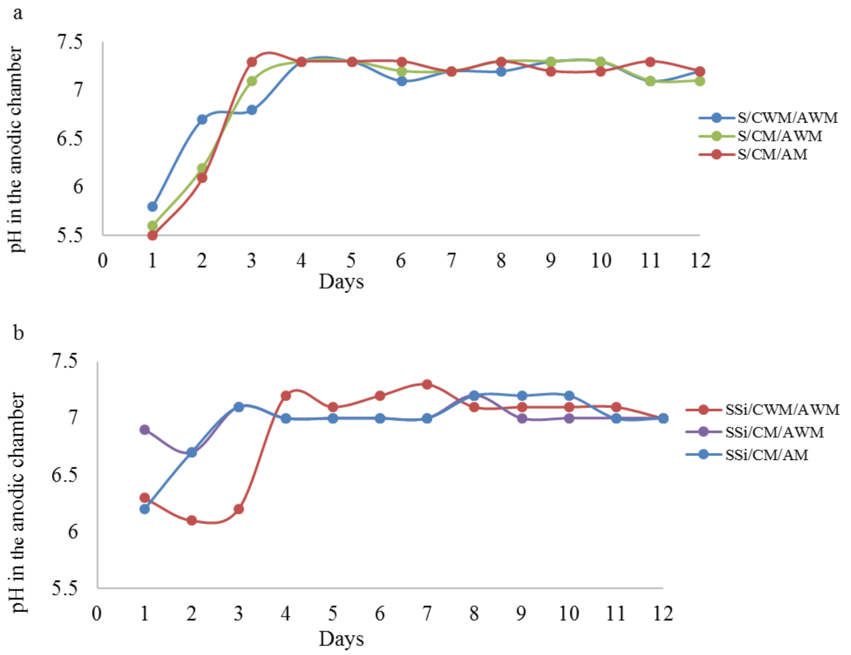

Figure 5. pH in the anodic chamber for all variables within 12 days of MFC operation. a: pH in the anodic chamber using only *Shewanella oneidensis* MR-1 as an electricigen bacteria; b: pH in the anodic chamber using *Shewanella oneidensis* MR-1 mixed with bacteria in Sidoarjo mud as electricigen bacteria.

Both *Shewanella oneidensis* MR-1 and electricigen bacteria in Sidoarjo Mud have the optimum pH growth of 7 [50]**.** The slow transfer of protons from anode to cathode and the accumulation of H^+ protons in the anode chamber decreased the pH value. Therefore, a strong base, 5M KOH, kept the pH neutral. Here, the neutral buffer wouldn't be sufficient since the rate of H^+ transferred was slower than the rate of H^+ formed. Figure 5 shows that the pH control was done every 3 hours from 0 to the 3rd day of MFC operation for the variables with no cathode modification. From 0 to the 2nd day of MFC operation for the variables with the modification of cathodes with actively Cu catalysts. Therefore, the electroneutrality for electrodes modified by Cu catalysts is faster than for electrodes without modification in a tubular MFC reactor. Kaul et al.[51] revealed that modified cathode using Cu metal increases the reaction rate. From the 3rd to the rest of the days, the anode chamber's pH change was insignificant. Therefore, pH control wasn't necessary. There was a balance between the rate of transfer of electrons and the rate of proton transfer. This condition is called electroneutrality[12].

3.3. *Effect of electrode modification on the production of power density*

MFC removes pollutants while simultaneously producing electricity [52]. MFC works by oxidising the organic substrate into smaller molecules using the microorganism and producing electrons and proton H^+. Then, the produced electrons are transported to the cathode through an external electrical circuit and converted into electrical energy while the protons migrate into the cathode surface. The potential difference between both electrodes causes the flow of electrons. The cathode's protons and electrons are consumed by reducing soluble electron acceptors such as oxygen. In brief, the reactions that occur in MFC are [53]:

$$anode: organic\ substrate \rightarrow H^+ + e^- + oxidized\ substrate \tag{3}$$

$$cathode: 4H^+ + 4e^- + O_2 \rightarrow 2H_2O \tag{4}$$

This study used a double tubular MFC with hexavalent chromium waste as a catholyte and molasses as an anolyte. *Shewanella oneidensis* MR-1 mixed with bacteria in Sidoarjo mud was utilised as an electricigen bacteria that oxidised the molasses and transferred the produced electrons to the anode. The flow of produced electrons streamed through an external electrical circuit and copper wire while the protons migrated into the Gore-Tex® separator from the anode to the cathode chamber. The cathode's protons and electrons are consumed by reducing hexavalent dichromate ions ($Cr_2O_7^{2-}$) into Cr^{3+} ions. The following reactions took place[54]:

$$Cr_2O_7{}^{2-} + 14H^+ + 6^{e-} \rightarrow 2Cr^{3+} + 7H_2O \tag{5}$$

The power density generated in the first days was low due to the adaptation of electricigen bacteria to the new environment. In this study, we had a continuous reactor, so by adding the substrate, the power density increased and decreased at a certain time. As seen in Figure 6a, we only used *Shewanella oneidensis* MR-1 as an electricigen bacteria. The power density was high on the 5th to 6th day of MFC operation, then declined until the 12th day of MFC operation. In contrast, by mixing *Shewanella oneidensis* MR-1 and Sidoarjo mud from days 10–12, we continue to experience the power density increase as seen in Figure 6b. The authors suspect that the electricigen bacteria competed with non-electricigen bacteria until the 10th day, even though no microbial community analysis was performed. Therefore, from the 0–10th day, the produced bacteria were mostly non-electricigen bacteria. S/CM/AM, which only used *Shewanella oneidensis* MR-1 as an electricigen bacteria with both modified electrodes, achieved the highest power density of 3.13 mW/m^2 on the 6th day of MFC operation.

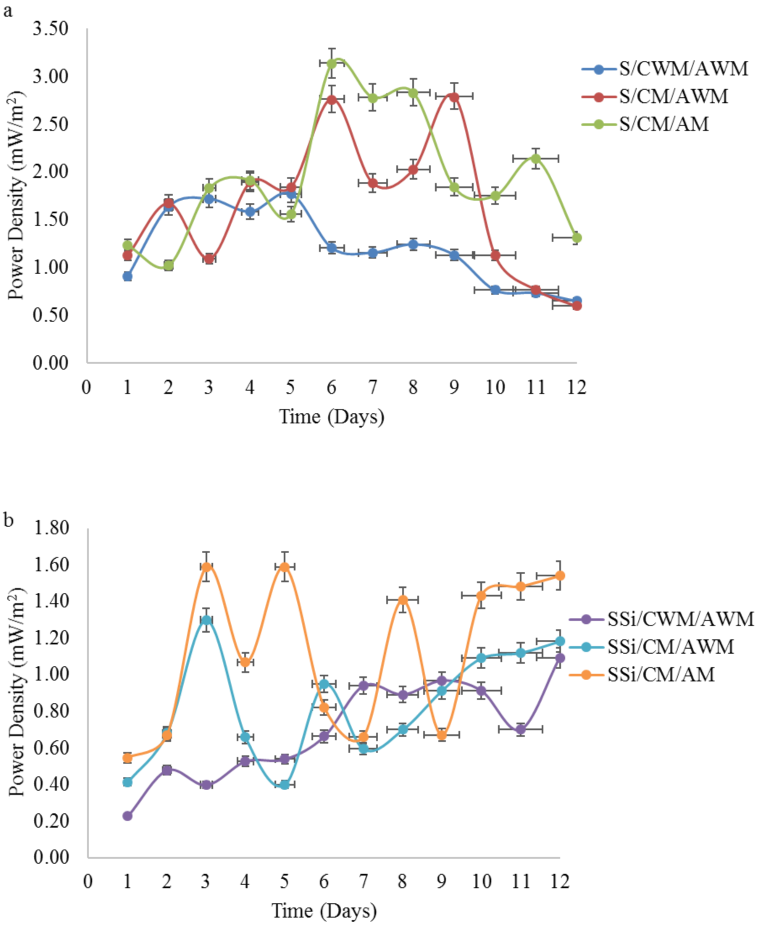

Figure 6. Power density produced using a double chamber Tubular MFC within 12 days. a: power density produced using only *Shewanella oneidensis* MR-1 as an electricigen bacteria; b: power density produced using *Shewanella oneidensis* MR-1 mixed with bacteria in Sidoarjo mud as electricigen bacteria.

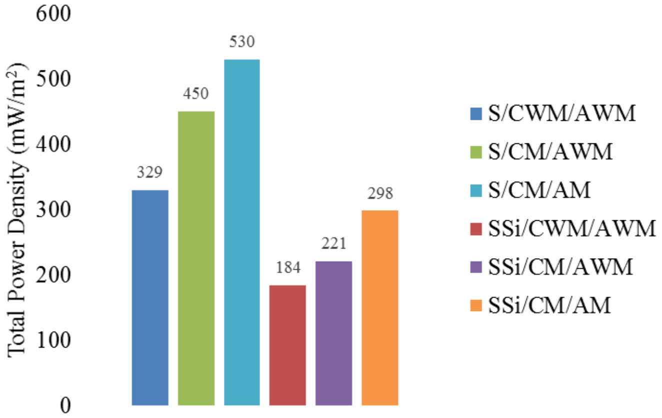

Figure 7. Total power density on each variable produced within 12 days of MFC operation.

The Trapezoidal Rule was used to calculate the area under the curve and obtain the total power density within 12 days of MFC operation. As shown in Figure 7, all the variables with electrode modifications produce an amount of power density than the unmodified electrode. Modifying the anode with rice bran increased the contact area [31] between electricigens bacteria and the anode resulting in a higher power density production. Moreover, rice bran contains a high organic compound, which increases bacteria growth. On the other hand, the cathode modification with Cu causes the cathodic reaction's activation energy to lower and increase the reaction rate [12]. The total power density produced by S/CM/AM, which only used *Shewanella oneidensis* MR-1 as an electricigen bacteria with both modified electrodes, was 1.6 times higher than S/CWM/AWM only used *Shewanella oneidensis* MR-1 as an electricigen bacteria with both unmodified electrodes.

Gul et al. [5] revealed that the mixture of exoelectrogenic bacteria might boost electricity production. This research used *Shewanella oneidensis* MR-1 and Sidoarjo mud as electricigens bacteria. As seen in Figure 6, the total power density produced by mixing Sidoarjo mud with *Shewanella oneidensis* MR-1 was low compared to the power density produced using only *Shewanella oneidensis* MR-1. The total power density produced by SSi/CM/AM, mixing *Shewanella oneidensis* MR-1 and Sidoarjo mud as an electricigen bacteria with both modified electrodes, was 221 mW/m^2, while the total power density produced by S/CM/AM, which only used *Shewanella oneidensis* MR-1 as an electricigen bacteria with both modified electrodes, was 530 mW/m^2. The result wasn't in accordance with Gul et al. [5] that a mixed culture of electricigen bacteria in MFC might enhance the power density produced. It was concluded that the bacteria in *Sidoarjo mud* inhibit the growth of *Shewanella oneidensis* MR-1, resulting in lower power density production.

3.4. The growth of microbial population in MFC

Researchers revealed that microbial growth depends on different factors, including but not limited to substrate concentration, pH and temperature [15]. As seen in Figure 8, the microbial population was low in the first days due to their adaptation to the new environment. The microbial population increases at a certain time and declines for the rest of the days since they have entered the death phase. It was attributed to the low availability of organic compounds over time. The highest microbial population (66.5 × 10^{10} cells/mL), as seen in Figure 8b, was achieved by SSi/CM/AM, mixing *Shewanella oneidensis* MR-1 and Sidoarjo mud as an electricigen bacteria with both modified electrodes variable, at the 6th day of MFC operation. In this study, the highest microbial population growth was seen where is a mixed culture of electricigen bacteria. It was due to the large size of inoculation in the mixed culture. In addition, an organic compound [18] in Sidoarjo mud may favour microbial growth. However, in Sidoarjo mud, there are the electricigen and non-electricigen bacteria. As seen in Figure 7, the highest total power density was achieved by the S/CM/AM variable even though the highest number of microbial growth was achieved by the SSi/CM/AM variable since the bacteria in Sidoarjo mud inhibit the growth of *Shewanella oneidensis* MR-1. On the other hand, the microbial population for variables with modified electrodes is higher than for variables without electrodes' modification. It shows that rice bran's organic content is essential to microbial growth.

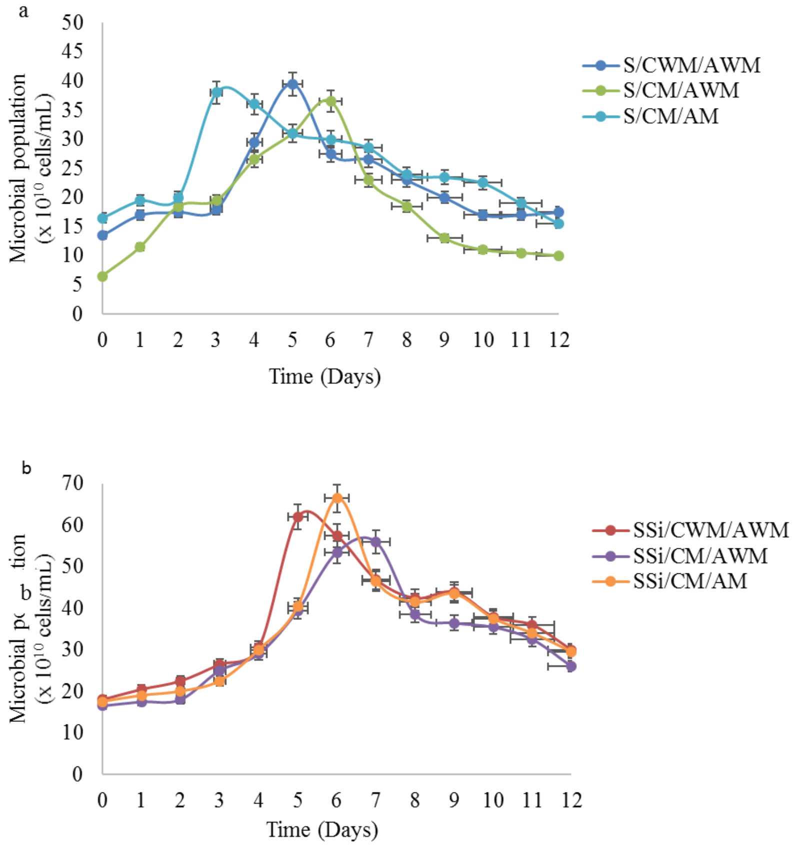

Figure 8. Microbial population growth in MFC within 12 days. a: Microbial population growth in variables using only *Shewanella oneidensis* MR-1 as an electricigen bacteria; b: Microbial population growth in variables using *Shewanella oneidensis* MR-1 mixed with bacteria in Sidoarjo mud as electricigen bacteria.

3.5. The effect of electrode modification into BOD5 and COD removal

The highest BOD_5 and COD indicate pollution in water [55]. Gul et al. [5] revealed that the decrease of pollutant removal in MFC indicates that active bacteria oxidise the large molecules into smaller molecules while producing electrical energy. In this study, *Shewanella oneidensis* MR-1 and bacteria in Sidoarjo mud influenced the pollutant removal in organic molasses waste.

Figures 9 and 10 show that SSi/CM/AM, mixing *Shewanella oneidensis* MR-1 and Sidoarjo mud as an electricigen bacteria with both modified electrodes, achieved 61.28% and 59.49% of BOD_5 and COD removal, respectively, on the 12th day of MFC operation. The bacteria population mainly influences pollutants removal [50]. As shown in Figures 9 and 10, the most effective removal of BOD_5 and COD occurs between the 4th to 8th day of MFC operation; this was directly proportional to the highest microbial population present in the reactor, as seen in Figure 8. Moreover, the variables with modified electrodes could produce pollutant removal higher than for variables without electrodes' modification. It was due to the increased microbial population in modified electrodes, as shown in Figure 8. On the other hand, variables with a mixed culture of electricigen bacteria produce high pollutant removal than variables that used only *Shewanella oneidensis* MR-1, as shown in Figure 9 but produce lower total power density, as shown in Figure 7. It was because non-electricigen bacteria were dominant in mixed culture, resulting in lower total power density.

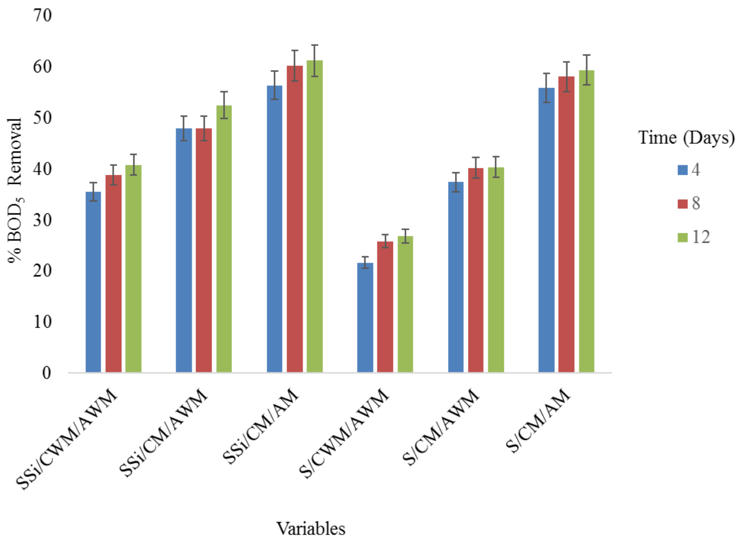

Figure 9. BOD5 removal of molasses substrate in a double chamber Tubular MFC reactor within 12 days of MFC operation.

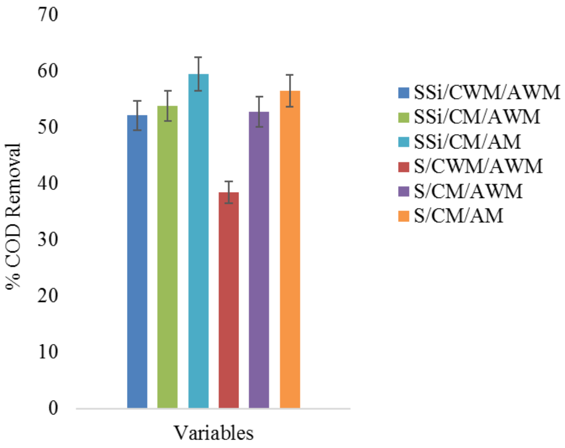

Figure 10. COD removal of molasses substrate in a double chamber Tubular MFC reactor within 12 days of MFC operation.

3.6. The effect of microbial population and electrode's modification for Cr^{6+} *removal*

In MFC, the potential difference between anode and cathode causes the flow of electrons and protons from reducing organic substrate by electricigen bacteria towards the cathode chamber. The protons and electrons at the cathode chamber reacted with hexavalent dichromate ions into trivalent chromium [56]. Reducing hexavalent chromium to trivalent chromium with less toxicity [19] is highly preferred for a sustainable future.

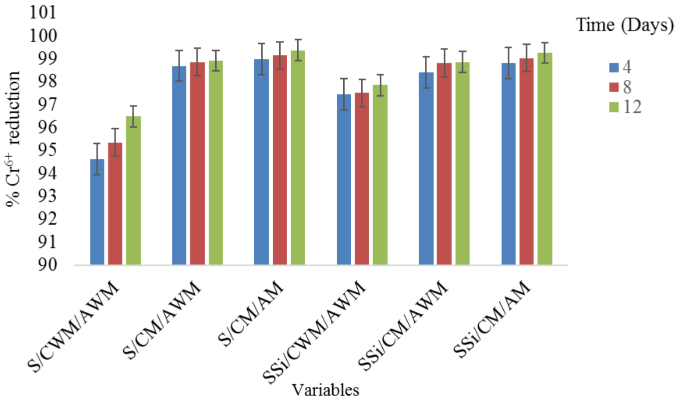

Figure 11. Cr^{6+} removal in a double chamber Tubular MFC reactor within 12 days of MFC operation.

Figure 11 shows that the highest percentage of hexavalent chromium reduction of 99.38% was achieved by S/CM/AM, which only used *Shewanella oneidensis* MR-1 as an electricigen bacteria with both modified electrodes. It was due to the highest electrons and protons produced, which reacted to chromium hexavalent in the cathodic chamber. The highest percentage of hexavalent chromium removal and total power density, as seen in Figure 7, were higher when we used only *Shewanella oneidensis* MR-1 as an electricigen bacteria than in mixed culture since the bacteria in Sidoarjo mud inhibit the growth of *Shewanella oneidensis* MR-1. On the other side, the highest percentage of hexavalent chromium was higher with modified electrodes than without modification. Cu metal facilitates the movement of electrons from the anode to the cathode through the electrical circuit. Therefore, the rate of chromium reduction reaction is faster. In addition, Cu metal causes the cathodic reaction's activation energy to lower and increase the reaction rate [12], resulting in higher power density and hexavalent chromium removal.

Table 2. Different variables on MFC performance and pollutant removal.

Sample code	BOD$_5$ removal (%)	COD removal (%)	Cr^{6+} removal (%)	Total power density (mW/m^2)
S/CWM/AWM	26.92	38.46	96.49	329
S/CM/AWM	40.36	52.84	98.92	450
S/CM/AM	59.41	56.53	99.38	530
SSi/CWM/AWM	40.82	52.21	97.85	184
SSi/CM/AWM	52.47	53.88	98.87	221
SSi/CM/AM	61.28	59.49	99.26	298

3.7. Uncertainly analysis

The results of the analysis of the experiments obtained are strongly influenced by several factors, such as: Sidoarjo Mud, molasses, and electrode modification with rice bran. With all of these factors, the Sidoarjo mud is one of the most significant uncertainties. This is due to the condition of the Sidoarjo mud which since 2006 had been gushing onto the earth until now and it is still releasing hot mud from the earth. The Lapindo mud is a hot mudflow event located in Sidoarjo, East Java—Indonesia on May 29, 2006 [57]. So that, the microbial community used in the study, from year to year also experiences differences. This is shown by research related to the analysis of microbial communities that has been carried out in 2018 (Figure 12) and 2022 (Figure 13), as shown in the following figures.

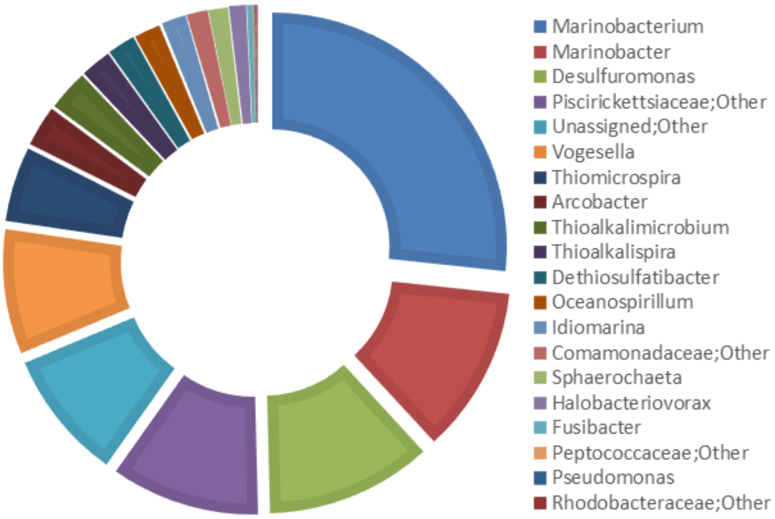

Figure 12. Microbial community analysis from Sidoarjo mud collected in 2018.

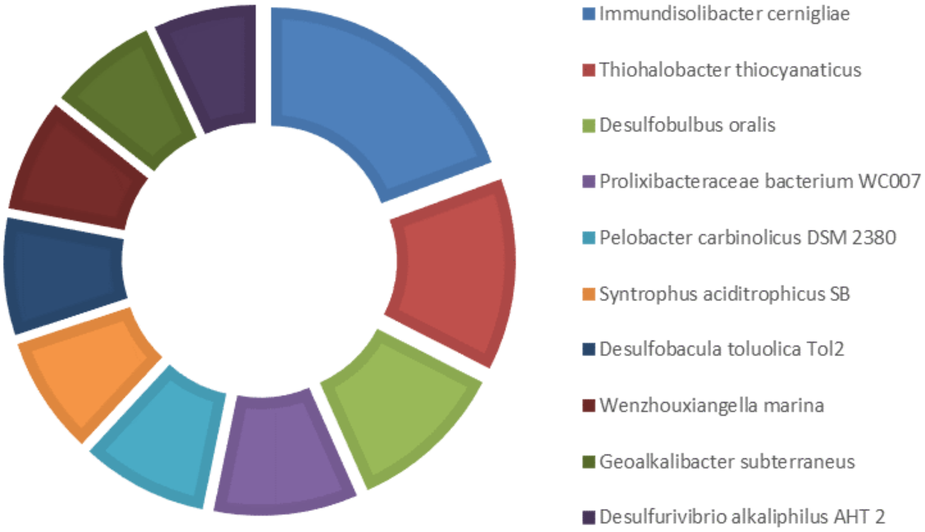

Figure 13. Microbial community analysis from Sidoarjo mud collected in 2022.

4. Conclusions

This research proposed an innovative and environmentally friendly method for waste management. The electric energy was produced from the organic compound in molasses while removing pollutants, and carcinogen hexavalent chromium was reduced to trivalent chromium using a double chamber tubular microbial fuel cell. *Shewanella oneidensis* MR-1 and Sidoarjo mud mixture as electricigen bacteria achieved the highest BOD_5 and COD removal of 61.28% and 59.49%, respectively. But, only *Shewanella oneidensis* MR-1 achieved higher power density production and hexavalent chromium removal of 530.42 mW/m^2 and 98.87%, respectively. It was due to the higher microbial population in mixed culture due to the large size of inoculation and organic compound in Sidoarjo mud that may favour microbial growth. But, non-electricigen bacteria were dominant in mixed culture, resulting in lower total power density and hexavalent chromium reduction. On the other hand, modified anode with rice bran and cathode with Cu metal resulted in higher power density production, pollutant removal and hexavalent reduction than unmodified electrodes. Therefore, the current finding indicates that double chamber tubular microbial fuel cells with modified electrodes may be a better solution for managing molasses and carcinogen hexavalent chromium.

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Conflict of interest

The authors declare no conflict of interest.

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