Study of electrode modification and microbial concentration for microbial fuel cell effectivity from molasses waste and reduction of heavy metal Cr (VI) by continue dual chamber reactor

I F Nuryana¹, R Puspitasari¹, S. R Juliastuti¹
¹ Chemical Engineering Department, Institut Teknologi Sepuluh Nopember, Surabaya 60111, Indonesia
E-mail: julizainul@gmail.com

Abstract. The rapid growth of textile industry has resulted in increasing amounts of heavy metal chromium waste. Metal waste production reaches 80% of the total amount of water used in the batik process. Microbial Fuel Cell (MFC) is a promising alternative because of its ability to reduce metal waste Cr⁶⁺ while processing organic waste and producing bioelectric. In addition, molasses still contain 50-60% sugar which has the potential to be degraded. Shewanella oneidensis MR-1 was used in this research due to its high productivity. MFC used dual chamber continue to optimize the process. The objective of this research is to analyze the effect of carbon modification and bacterial concentration on MFC electricity production, reduction of Cr⁶⁺ and reduction of BOD. The higher concentration of bacteria and modification of electrodes could produce greater electricity and can reduce more hexavalent chromium metal Cr⁶⁺ also able to reduce the most optimum BOD. Variable of 10¹¹ cells/mL bacteria with electrode modification produced the best performance by producing electricity that was relatively more stable and the most optimum with total power density of 690.9mW/m², reduced heavy metals Cr⁶⁺ to Cr³⁺ up to 92.78% and BOD removal of 33.11%.

1. Introduction
Industry in Indonesia is rapidly growing, one of which is the textile and textile products (TTP) industry. Exported TTP increased by 3% in 2017 from the previous period. With the rapid growth of the industry, the amount of waste produced has also increased. The production process produces liquid metal waste which amounts to 80% of the total amount of water used in the batik process [1]. Chromium, one of metal waste, is one of the most dangerous waste for the environment because it cannot decompose. Generally, waste is disposed of in the water, so that it causes a negative impact on the ecosystem and food chain of living things in the water. Chromium metal will contaminate aquatic organisms (plants, fish, etc.) which will be consumed by humans. So, even though exposure to contamination in small amounts and indirectly, it can still endanger human health [2]. Moreover, the toxicity of this waste depends on the form of chromium, its oxidation number, and pH. Thus, acid pH and higher temperatures will increase the toxicity of Cr⁶⁺ to aquatic organisms [2].

There are several conventional methods for treating Cr⁶⁺ waste, such as reduction with ferro sulfate, binding of chromium compounds with resin (ion exchange) and thickening [3]. However, these three
methods require other chemical compounds which are less stable so that they require additional processing and costs. Microbial Fuel Cell (MFC) is a promising alternative because of the ability to reduce Cr\(^{6+}\) metal waste while at the same time processing organic waste and producing bioelectric (double track research). Microbial Fuel Cell (MFC) is a biology-based fuel cell that converts chemical energy into electrical energy by catalytic reactions of microorganisms [4]. MFC by utilizing industrial waste is able to become a promising alternative energy source. In addition, molasses as organic waste becomes particular concern because of its high production of 1.56 million tons in 2010 [5]. While more than 30% of molasses waste is still not optimally utilized. Molasses still contain 50-60% sugar which has the potential to be degraded and utilized further as a substrate agent of Microbial Fuel Cell (MFC) with higher productivity than other substrates [5].

The use of both wastes, heavy metal chromium and molasses waste in MFC system will be able to obtain 3 benefits at once, such as heavy metal processing, molasses waste treatment, and bioenergy production. So far, mostly developed-MFC [6] still use a batch system. The use of a batch system has several disadvantages on an industrial scale, which requires a large volume and space, other than that the bacteria used are counted. Thus, Microbial Fuel Cell (MFC) is needed to develop alternative energy from molasses waste and reduce Cr (VI) in heavy metal waste with dual batch systems that use continuous systems which require small volume and space.

*Shewanella oneidensis MR-1* is a good bacterial agent to degrade molasses substrate and capable to produce high energy densities of 2 W/m\(^2\) [7]. This bacterium will be used in this research. In addition, electrodes are also a very influential component in the process of reduction and oxidation. The anode surface area affects bacterial activity and electron transfer. In this study, the anode in the form of a carbon rod will be modified with the addition of an area. Rice bran contains high silica [8], so it can be used to increase the anode contact area. Silica will stick to the carbon rod so that additional particles form. This study analyse the effect of bacterial concentration and the effect of electrode modification on the effectiveness of MFC dual batch using a continuous recirculation system, where it is expected that MFC can degrade molasses and reduce chromium (reduce toxicity) while producing the optimum bioelectric. The objectives of this research are to analyze the effect of carbon modification and bacterial concentration on MFC electricity production, reduction of Cr\(^{6+}\), and reduction of BOD.

2. Method

The system was conducted in at atmospheric condition with temperature of 30°C and pressure 1 atm. Molasse waste was used as feed in anode chamber and chromium solution as representative of contaminated water was used as feed in cathode chamber. The anode and cathode reactor was separated with salt bridge. The bacteria used in this research were *Shewanella oneidensis* MR-1 with 2 variable concentrations of \(10^6\) cell/mL and \(10^{11}\) cell/mL.

The experiment was conducted using a dual-chamber reactor with recirculated continue system, as illustrated in figure 1. Due to continue process, the fresh feed would be injected with flowrate 0.4 L/hour for both anode and cathode chamber. The experiment was running during 96 hours, so fresh feed should be prepared every 24 hours to maintain continue system.

The molasse fresh feed was poured into chamber number 11 and chromium solution into chamber number 12. In the first 25 hours, the fresh feed was used to fulfill the reactor anode and cathode first; then after 25 hours, valve number 10 (to chamber) was opened to fill chambers number 7 and 8. After 25 hours, the bottom valve number 10 (bottom product) was opened and at the same time, the pump was turned on to return the bottom chamber feed into reactor again. Recycle ratio was 0.85 or in another sentence, the pump was operated with flowrate 0.34 L/hour. The system was maintained until 96 hours. The analysis was conducted every 12 hours such as BOD (Biological Oxygen Demand), Chromium Hexavalent concentration, bacterial concentration by counting chamber and acidity. However, power or electricity production was analyzed every 6 hours.
2.1. **Substrate preparation for anode chamber**

500 mL of molasse and distilled water were sterilized using an autoclave to ensure there is no other microorganisms will be involved in the process. To prepare starter of *Shewanella oneidensis MR-1*, 1.5 L nutrient broth was prepared and sterilized. Then, inoculated bacteria were displaced into nutrient broth and incubated during 13 hours in temperature 30°C. After 13 hours, the bacteria would be counted if it was qualified for the concentration variable, starter was ready to use. Starter, molasse and buffer phosphate were mixed together then distilled water was added up to 10 L of total volume. In the continuo MFC system, substrate for anode chamber should be prepared every 25 hours.

2.2. **Cathode solution preparation**

Chromium hexavalent 8 ppm solution was prepared by diluting 0.226-gram K_2Cr_2O_7 in distilled water up to 10 L. Buffer citrate pH 3 was added into solution in order to maintain the acidity in pH 3.

2.3. **Electrode modification**

Modification was the variable of this research in order to analyze the impact of anode modification. Chemical-base modification was used to activate the anode by soaking the electrode in HCl 1 M for 24 hours then rinse the anode by distilled water. After that, the anode was soaked again in KOH 1 M for 24 hours then rinsed by distilled water [9]. In order to increase the surface area, modification using rice bran was conducted. Rice bran contained high amount of silica which semiconductor and able to stick around the electrode’s surface [8]. 320 grams of rice bran with homogenous size of 50 mesh was added into 2000 mL of distilled water then stirred around an hour. Then, rice bran was precipitated gravitationally. 1500 mL of solution was displaced and mixed with 100 grams of activated carbon powder.

2.4. **Salt bridge preparation**

Salt bridge was used to transfer the proton produced by the system from anode to cathode chamber. In this research, KCl-agar salt bridge was used. 30 grams of solid agar was diluted in 1000mL hot distilled water. Then, 40 grams of potassium chloride was added into agar solution. The salt agar solution was poured into containment and cooled until it turned into solid.

2.5. **Experiment using dual chamber reactor**

The experiment was conducted using dual-chamber reactor with recirculated continuo system. Due to continuo process, the fresh feed would be injected with flowrate 0.4 L/hour for both anode and cathode chamber. The experiment was running for 96 hours, so fresh feed should be prepared every 24 hours to maintain continuo system.

![Figure 1. Dual chamber recirculated continuo system](image-url)
3. Experimental Result and Analysis

3.1. Microbial fuel cell – dual chamber continue

In this study, Microbial Fuel Cell (MFC) used a dual-chamber, which was distinguished between the anode chamber and the cathode chamber. The anode compartment contained organic sugar mill waste (molasses) with a concentration of 5% as a substrate. However, the cathode compartment contained chromium hexavalent solution as the representative of wastewater with Cr (VI) concentration of 8 ppm. *Shewanella oneidensis MR-1* carried out metabolism that involves molasses solution as a substrate which caused degradation of organic compounds in the anode compartment. This metabolism took place anaerobically which produces electrons (e⁻) and protons (H⁺) with reactions as follows:

\[ \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} \rightarrow 6\text{CO}_2 + 24\text{H}^+ + 24\text{e}^- \quad E^0 = 0.01 \text{ V} \] (1)

Electrons (e⁻) and protons (H⁺) that have been produced would be transferred to the cathode compartment by following the working principle of the voltaic cell. The electrons formed from the reaction accumulate on the electrode (graphite) in the anode compartment. The potential difference between the anode chamber and the cathode caused the flow of electrons to produce power density. The flow of electrons streamed through the electrode circuit and copper wire, while the protons (H⁺) pass the Proton Exchange Membrane (PEM) from the anode compartment to the cathode compartment. Then, electrons (e⁻) and protons (H⁺) reacted with dichromate ions (Cr₂O₇²⁻) in the cathode compartment, these ions were formed by the following reaction:

\[ \text{K}_2\text{Cr}_2\text{O}_7 \rightarrow 2\text{K}^+ + \text{Cr}_2\text{O}_7^{2-} \] (2)

Electrons (e⁻) and protons (H⁺) reacted with dichromate ions (Cr₂O₇²⁻) to form Cr³⁺ ions or commonly known as reduction reactions as follows:

\[ \text{Cr}_2\text{O}_7^{2-} + 14\text{H}^+ + 6\text{e}^- \rightarrow 2\text{Cr}^{3+} + 7\text{H}_2\text{O} \quad E^0 = 1.33 \text{ V} \] (3)

As a result of the transfer of electrons from the anode to the cathode, the charge on each chamber became unbalanced. Therefore, the load balancing process was occurred by utilizing a Proton Exchange Membrane (PEM) containing K⁺ and Cl⁻ ions. In order to stabilize anode compartment due to electron loss, the Cl⁻ ion of the PEM would move towards the anode compartment. Whereas in the cathode compartment that receive d electrons has a negative charge, so it requires K⁺ ions from PEM to stabilize the charge. K⁺ enters the cathode compartment to balance dichromate ion or reduced permanganate levels. Then the H⁺ ion from the anode compartment moves to the cathode compartment to replace the K⁺ ion.

3.2. The profile and effect of acidity to Shewanella oneidensis MR-1 growth

Bacteria played an important role in the process of Microbial Fuel Cells (MFC), which was to oxidize organic substrates. In this study, the bacteria used was *Shewanella oneidensis MR-1*. Bacteria were injected into molasses solution in the anode compartment when in the log phase with the amount corresponding to the concentration variable. In the log phase, bacteria experienced a rapid growth. In addition, the need for energy for bacteria in the log phase was higher compared to other growth phases. So, it was expected that many protons and electrons were produced from the metabolic processes of these bacteria [10]. In order to analyze the growth profile of *Shewanella oneidensis MR-1*, the experiment was conducted. The result was compared with the research of Jeong, 2006.
Based on Figure 2 (a), this *Shewanella oneidensis MR-1* was able to live at extreme temperatures close to 0°C even though the optimum temperature growth ranges from 30-35°C [11]. In lower temperatures, *Shewanella oneidensis MR-1* experienced a lower growth rate and vice versa. The profile of the microbial growth of the experiment in Figure 2 (b) had a tendency similar to the previous one. The log phase was reached in 16-18 hours then undergoes a phase of stagnation or stationary phase before then experiencing a death phase. Therefore, for a continuous recycled fresh feed MFC process, bacteria were inoculated into the media and incubated for 13 hours to reach the initial log phase before mixing with molasse as the substrate. 13 hours were chosen considering the log phase.

*Shewanella oneidensis MR-1* was optimally working in acidity pH 6-7 to produce MFC with optimum electricity production [12]. Whereas indirectly, electricity production was influenced by population and bacterial activity. Therefore, the main factor in this study was the pH control of the anode compartment to maintain the stability of population conditions and bacterial activity. the pH of anode chamber was monitored every 12 hours. Before KOH addition, the pH of the anode compartment always decreased for all variables (curve (b)) in Figure 3 due to the metabolic reaction of the *Shewanella oneidensis MR-1* with molasses that produces protons or H⁺. The protons produced from this reaction should be transferred to the cathode chamber, but unfortunately, the protons did not completely be transferred. As a result, it caused a decrease in pH. Low pH (acid) in the anode compartment would deactivate microorganisms and reduce MFC performance [13].

**Figure 2.** Growth profile of *Shewanella oneidensis MR-1* from (a) the research of Jeong [11]; (b) in this research

**Figure 3.** pH of anode chamber before (b) and after (a) pH control using KOH
The use of buffer phosphate pH 7 was unable to anticipate pH reduction due to the production of H⁺. Salt bridge did not work optimally to balance the anion and cations. The H⁺ proton rate towards the cathode compartment was much lower than the rate of H⁺ production in the anode compartment. So, every 6 hours a strong base solution or KOH was added to the reactor to neutralize the reactor pH and maintain the bacterial population. So, it can be seen in the curve (a) of Figure 3, that after KOH addition, the pH rises to around 6-7.

3.3. The effect of bacteria concentration and electrode modification into power production

Systematically, the continuous system processed feeds and produced products continuously. In this process, the substrate fresh feed (anodes) and chromium solution (cathode) were injected continuously. Likewise, electricity should be produced stably. Electricity production was influenced by several factors such as the rate of substrate conversion, PEM (Proton Exchange Membrane), internal and external barriers in cells and the potential difference between the anode and cathode [14]. Biofilm formation around the electrode could also be a barrier to the maximum absorption of electrons from bacteria [15].

![Figure 4. Power production of MFC dual chamber](image)

Based on Figure 4, the increasing concentration of bacteria caused higher production of electricity. The concentration of bacteria $10^{11}$ cells/mL either without or with modification was able to produce a higher power than the concentration of $10^9$ cells/mL. The higher the concentration of bacteria, the more substrates were degraded by bacteria to produce electrons.

Electricity was generated every time unit by the system; therefore, an intergalactic calculation was carried out to find out all the electricity produced during the process. This refers to the area under each curve for each variable. For the variable concentration of $10^{11}$ cells/mL without modification, it produced a total of 446.06 mW/m² or 39.73% higher than the concentration of $10^9$ cells/mL without modification which was 319.23 mW/m². Likewise, for the variable with electrode modification, where the concentration of $10^{11}$ cells/mL was able to produce a total of 690.9 mW/m² or 66.95% higher than the concentration of $10^9$ cells/mL which was 413.84 mW/m².

Another factor affecting power production was anode modification. The electrodes used in the manufacture of MFC must have good electrical conductivity, a broad surface, low resistivity, non-corrosive, biocompatible, chemically and mechanically stable to obtain results that can be produced continuously [16]. Anode modification with strong acid-base and treatment of rice bran aimed to increase the surface area of the electrode. Silicon inside rice bran was expected to be able to attach to carbon electrodes so that it helps increase the contact area of electron transfer from bacteria. In this study, electrode modification was able to increase the profile of electricity production. For total electricity produced, the concentration of $10^{11}$ cells/mL bacteria with modification produced 54.89% more electricity than without modification. While the variable concentration of $10^9$ cells/mL produced 29.64% higher than without modification.
3.4. The effect of bacteria concentration and electrode modification into BOD removal
BOD or (Biological Oxygen Demand) was a measure of the amount of oxygen used by microbial populations contained in waters in response to the entry of decomposed organic matter [17]. BOD removal in this study was shown in the Figure 5.

![Figure 5. BOD removal of molasse substrate in MFC dual chamber](image)

BOD removal was strongly influenced by bacterial activity, while bacterial activity was influenced by bacterial concentration, bacterial population, and pH of the anode space. Based on bacterial concentration, $10^{11}$ cells/mL either without or with modification was able to produce BOD removal higher than the concentration of $10^9$ cells/mL.

3.5. The effect of bacteria concentration and electrode modification into $Cr^{6+}$ removal
$Cr^{6+}$ was one of the compounds that can put environment and water in danger. Therefore, the decrease in hexavalent chromium levels in this study was highly preferred. Hexavalent chromium metal reduction occurs by utilizing the metabolic results of *Shewanella oneidensis MR-1* in the form of protons and electrons in the anode chamber which moved to the cathode compartment due to potential differences and then reduces $Cr^{6+}$ to $Cr^{3+}$ in the cathode compartment.

![Diagram showing Cr6+ reduction](image)
Based on Figure 6, Cr$^{6+}$ concentration levels drastically decreased from the concentration of 8 ppm to almost touching the 0-ppm point in the first 12 hours. Then at the 24 to 96 hours, the levels were relatively stable between 0 and 1 ppm. This fluctuation of hexavalent chromium levels occurred because of the fresh feed entering the main chamber, this results in an increase in the amount of hexavalent chromium every time along with the presence of electric currents which caused the reduction of Cr$^{6+}$ to Cr$^{3+}$.

Based on these data, it showed that the higher the concentration or bacterial population the greater the chromium reduction that occurs. The large population of bacteria involved in anaerobic metabolic reactions affects the number of electrons formed. The higher the concentration of bacteria, the more bacteria that metabolize molasses so that more electrons were formed. Then, electrons would be transferred to the cathode chamber so that it can reduce Cr$^{6+}$. The modification of the electrode also affected the results of hexavalent chromium metal reduction. Electrode modification allowed higher removal than without modification due to the modification by using strong acids, strong bases and rice bran capable to increase the surface area of carbon rod electrodes. As a result, more electrons were transferred to the cathode chamber. So, the reduction of hexavalent chromium was higher.

The pH of the cathode compartment was maintained at pH 3 with the help of a citrate buffer. The optimum pH for Cr$^{6+}$ removal was in the range of pH 3-4 [18]. This was in line with the reduction of hexavalent chrome that carried out at acidic conditions.

Figure 6 (b) was the pH profile of the cathode compartment which has no significant fluctuation. Even though protons could not well transfer to the cathode, the available protons still able to reduce Cr$^{6+}$. The acid condition was chosen for the cathode chamber because Cr$^{6+}$ can be easily or spontaneously reduced to Cr$^{3+}$ [18]. So, it was easier for hexavalent chrome to be reduced in an acid solution than neutral or alkaline.

4. Conclusion
This research showed that dual-chamber microbial fuel cell (MFC) continuous recirculation system was capable to reduce metal Cr (VI), produce electrical energy and reduce BOD. The higher concentration of bacteria and modification of electrodes produce greater electricity and more efficient to reduce hexavalent chromium metal Cr$^{6+}$. Variable concentration of $10^{11}$ cell / mL bacteria with electrode modification produced more stable and the most optimum electricity with a total power density of 690.9 mW / m$^2$. It also reduced heavy metals Cr$^{6+}$ with a percent removal of 92.78% and BOD removal of 33.11%.
5. Acknowledgment
We would like to show our gratitude to Mr. Dimas Eko Prasetyo as Director of PT Energi Agro Nusantara for supplying molasses as the substrate of this research. We are also immensely grateful to Chemical Engineering Department, Institut Teknologi Sepuluh Nopember specially to Waste Water Treatment Laboratory for all chemical supplies and facilities to support the research.

6. References
[1] Watini 2009 Pengaruh waktu kontak eceng gondok (Eichornia crassipes) terhadap penurunan kadar Cd dan Cr pada air limbah industri batik (Home Industry Batik di Desa Sokaraja Lor) Kota Purwokerto Skripsi (Purwokerto: Universitas Jenderal Soedirman)
[2] Jain A 2010 Removal of Copper(II) from aqueous solution using spent tea leaves (STL) as potensial sorbent Water SA 36(3) ISSN 1816-7950
[3] Idaman N 2010 Metoda penghilangan logam berat (As, Cd, Cr, Ag, Cu, Pb, Ni Dan Zn) di dalam air limbah Industri Jurnal Air Indonesia 6(2) 1
[4] Li Z, Xingwanz L and Lechleg L 2008 Electricity production during the treatment of real electroplating containing Cr\(^{6+}\) using microbial fuel cell Process Biochemistry 43 1352
[5] Yakinudin A 2010 Bioetanol Singkong sebagai Sumber Bahan Bakar Terbarukan dan Solusi untuk Meningkatkan Penghasilan Petani Singkong (IPB : Bogor)
[6] Mohan, Lens S V and Nanchariaiah Y V 2015 Metal removal and recovery in bioelectrochemical system: A review Bioresource Technol 195(1) 96
[7] Rengeisen B, Ray R and Little B 2007 A miniature microbial fuel cell operating with an aerobic anode chamber Journal of Power Sources 165 591
[8] Agung F 2013 Ekstraksi silika dari abu sekam padi dengan pelarut KOH Jurnal Konversi 2(1)
[9] Novitasari D 2011 Optimalisasi Kinerja Microbial Fuel Cell (MFC) untuk Produksi Energi Listrik Menggunakan Bakteri Lactobacillus bulgaricus (Jakarta : Departemen Teknik Kimia FT Universitas Indonesia)
[10] Setyati, Wilis A, Erni M, Triyanto, Subagiyanto and Zainuddin M 2015 Kinetika pertumbuhan dan aktivitas protease isolat 36k dari sedimen ekosistem mangrove, Karimunjawa, Jepara Ilmu Kelautan 20(3) 163
[11] Jeong Y, Song S, Lee S and Hur B 2006 The growth and EPA synthesis of Shewanella oneidensis MR-1 and expectation of EPA biosynthetic pathway Biotechnology and Bioprocess Engineering 11 127
[12] Biffinger J C, Pietron J, Bretschger O, Nadeau L J, Johnson G R, Williams C C, Nealson K H and Ringeisen B R 2008 The influence of acidity on microbial fuel cells containing Shewanella oneidensis Biosensors & bioelectronics 24 906-11 doi: 10 1016/j bios 2008 07 034
[13] Li Z, Xingwanz L and Lechleg L 2008 Electricity production during the treatment of real electroplating containing Cr\(^{6+}\) using microbial fuel cell Process Biochemistry 43 1352
[14] Jadhav G S, More T T and Ghangrekar M M 2008 Microbial Fuel Cell: Application in Wastewater Treatment (India: Indian Institute of Technology)
[15] Sevda S and Sreekrishnan 2012 Effect of salt concentration and mediator in salt bridge microbial fuel cell for electricity generation from synthetic wastewater Journal of Environmental Science and Health 47(6) 878
[16] Akbar N, Kirom M R and Iskandar R 2017 Analysis of the effect of metals as an electrode in microbial fuel cell to the electrical energy Production Proceeding of Engineering 4(2) ISSN : 2355 – 9365
[17] Erwin 2014 Tingkat pencemaran pada saat pasang dan surut di perairan pantai Kota Makassar Skripsi (Makassar: Universitas Hasanuddin)
[18] Silva B, Figueiredo H, Neves I C and Tavares T 2009 The role of pH on Cr (VI) reduction and removal by Arthrobacter Viscosus International Journal of Chemical and Biological Engineering 2(2) 100